Original Article

Proliferative Effects of Hyaluronic Acid on Human Dental Pulp Stem Cells

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Abstract

Purpose

Stem cells have become increasingly important in the development of tissue engineering. Among them, dental pulp stem cells can be obtained in a non-invasive manner. In recent years, advances in technology have enabled dental pulp stem cells to be successfully applied to periodontal diseases and the regeneration of the lower jaw bone, in addition to creating the potential for more applications due to the self-regeneration and multi-differentiation capabilities of stem cells. Hyaluronic acid (HA) is a natural biological polymer that has important physiological functions in vertebrates and even bacteria. Past studies have indicated that HA, in addition to being an important material in tissue engineering, is related to the physiological activities of specific cells or even stem cells. Therefore, this study sought to explore the effect of HA on dental pulp stem cells.

Material and methods

Dental pulp stem cells were cultured with 20 KGy or 60 KGy γ-ray treated HA. Their cell viability was then tested by MTT assay.

Results

Our results showed that there was no significant difference between the control group and experimental groups.

Conclusion

That is, the results suggested that HA does not exhibit the ability to improve the viability of dental pulp stem cells. However, further experimental studies with various HA concentrations are still needed to determine whether HA has any positive effects on dental pulp stem cells.

Key words: **dental pulp stem cell, hyaluronic acid, tissue engineering, x-ray**

Introduction

Stem cell research is a rapidly emerging field of study that offers new options for cell therapy and tissue engineering. In the past, the use of differentiated mature cells, which can be obtained from autologous tissues, reduced the problem of immune rejection in tissue engineering applications, but the number of such cells that can be obtained has proven to be insufficient for some purposes.

Specifically, it is difficult to obtain the number of subsequent implantable stents required when using differentiated mature cells, and because such cells are in the mature stage of differentiation, their in vitro growth rate is relatively limited.

Stem cells are unspecified initial cells that are capable of dividing for proliferation, self-renewal, differentiation, and regeneration, and are further divided into embryonic stem cells and adult stem cells. Embryonic stem cells are derived from the inner cell mass of the post-fertilized blastocyst[1]. Although these cells cannot develop into a single individual by themselves, they can differentiate into the various cells of the endoderm, mesoderm, and ectoderm, and so are also known as pluripotent stem cells.

Dental pulp stem cells, which are a type of adult stem cells derived from pulp tissue, were successfully isolated and cultured in vitro by Gronthos et al. in 2000[2]. Although the amount of dental pulp tissue is small, such tissue can yield an abundant amount of stem cells, and the isolated dental pulp stem cells have high differentiation ability, with the ability to differentiate into dentin mother cells, osteoblasts, chondrocytes, and adipocytes. Also, unlike embryonic stem cells, the use of dental pulp stem cells does not involve embryo invasion and destruction, and therefore is less morally controversial.

Studies have shown that dental pulp stem cells are genetically similar to bone marrow stem cells[3]. However, a given volume of pulp tissue contains a higher proportion of stem cells than the same volume of bone marrow tissue[4], making it much more convenient to obtain dental pulp stem cells from dental pulp tissue than it is to obtain bone marrow stems cells via bone marrow aspiration. The genes involved in cell division are also much more abundant in dental pulp stem cells than in bone marrow stem cells[5].

Hyaluronic acid (HA) is found in almost all vertebrates, especially in the extracellular matrix of soft tissues. In fact, it is one of the main components of the extracellular matrix and can also be found in synovial fluid and intraocular lenses. HA molecules have excellent hydrophilic properties. Specifically, they can absorb 500 times their own volume in water and are therefore considered a natural moisturizer. Because of its good water absorption, biocompatibility, and low immune response, HA is widely used in the medical, pharmaceutical, and cosmetic industries in wide range of products and processes including moisturizers, arthritis treatments, ophthalmic surgery, and wound healing ointments. It has also been reported that HA could potentially be used in releasing agents for drugs with anti-inflammatory, anti-swelling, and antibacterial functions that are used to treat periodontal diseases[6].

HA has been shown to affect the physiological activities of cells either directly or indirectly, especially in certain cells and even stem cells. However, no previous studies have investigated the effect of HA on the growth of dental pulp stem cells. In this study, therefore, HA was added to culture medium used to grow dental pulp stem cells. The effect of the HA on the dental pulp stem cells was then tested.

Material and methods

The minimum essential medium alpha medium (11900-024), antibiotic-anrimycotic (15240-062), and fetal bovine serum (26140-079) used in this study were purchased from Gibco (USA). NaHCO3 and L-ascorbic acid 2-phosphate5 (49752-10G) were purchased from Sigma-Aldrich (San Louis, MO, USA).

Culture solution preparation

10.2 g of powder-packed α-MEM was added to 1 liter of secondary filtered water. Then 2.2 g of sodium hydrogencarbonate, 10 ml of antibiotic-anrimycotic, 5 ml of L-ascorbic acid 2-phosphate, and 150 ml of fetal bovine serum were added to the α-MEM solution. After the medium was uniformly mixed and filtered through a filter membrane (0.22 μm), the prepared medium was stored in a refrigerator at 4℃. To prepare α-MEM with HA, HA powder (0905281, GINKGO, USA) was dispensed and sterilized by γ-ray (20 or 60 KGy) for subsequent experiments with dental pulp stem cell culture. After being sterilized, the HA powder was added to the α-MEM culture solution containing fetal bovine serum.

The culture method for dental pulp stem cells

The dental pulp stem cells were cultured in a 10-cm culture dish using α-MEM culture medium, and a subculture was required when the cells had grown to a saturated state of eight to 90%. In the subculture, the culture solution in the culture plate was aspirated, and the culture plate was washed twice with PBS buffer, and the remaining culture solution was washed away. For passage, 1 ml of 0.1% Trypsin-EDTA was added to the culture medium, and the culture plate was put into the incubator for 5 to 10 minutes. After adding the appropriate amount of trypsin-EDTA, it was confirmed that the cells had detached from the culture plate. Then the cells were collected through centrifugation at 500 rpm for 5 minutes. After centrifugation, the supernatant was removed, and new culture solution was uniformly mixed with the cells. The cells were then cultured in a new culture plate for subsequent experiments.

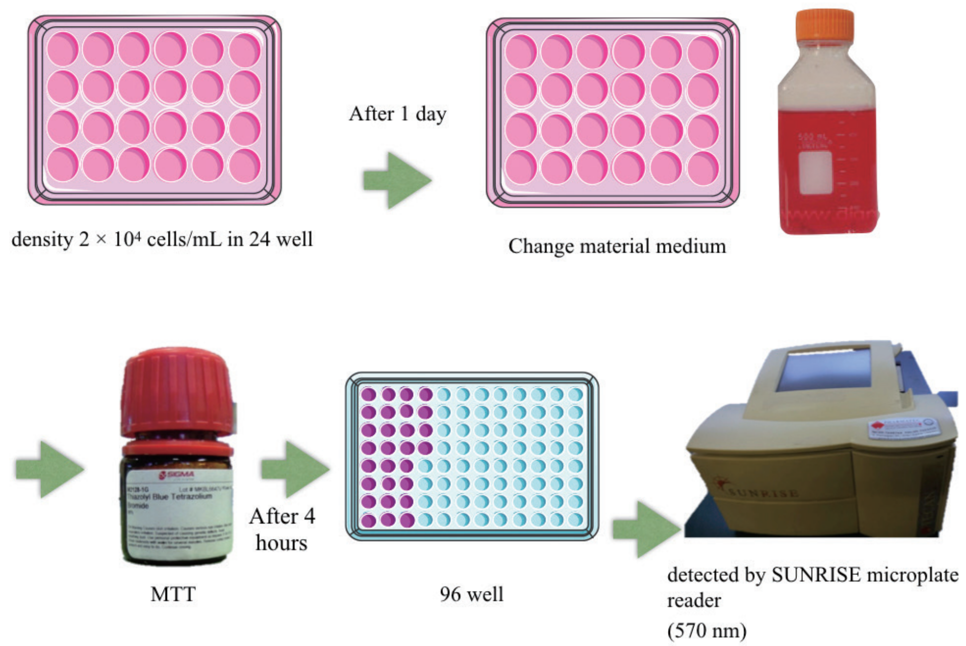

Fig. 1. The process diagram of the method

Cell culture experiment

The experiment was divided into two groups: a control group and an experimental group. The cell culture with normal α-MEM was defined as the control group, and the cell culture with α-MEM and different particle sizes of HA was defined as the experimental group. The dental pulp stem cells were cultured in a 24-well plate (cell concentration: 2 × 104 /ml) in α-MEM culture medium, and after the cells were attached for 24 hours, they were washed with PBS buffer, and the culture solution of the experimental group was replaced by the medium with HA (serum α-MEM with 1 mg/ml HA), with the day of this replacement being defined as day 0. MTT experiments were performed every 24 hours to observe the proliferation of the cells. The experimental group was further divided into two subgroups, which included cells treated with 20 KGy γ-ray treated HA or the cells treated with 60 KGy γ-ray treated HA.

Cell viability tests

During the experiment, 50 μl of MTT solution was added to each culture well, and the wells were maintained in the incubator for three to four hours. Then the MTT solution and culture solution were aspirated, and 500 μl of DMSO was added to the well to dissolve the formazan crystals to make the DMSO solution purple. The cell viability was read by spectrophotometer. The absorbance values (570 nm - 690 nm) were recorded for use in subsequent analysis.

The dental pulp stem cells were cultured in a 24-well plate (cell concentration: 2 × 104 /ml) in α-MEM culture medium, and after the cells were attached for 1 day (24 hours), they were washed with PBS buffer, and the culture solutions of the experimental sub-groups were replaced with HA. MTT experiments were performed every 24 hours to observe the proliferation of the cells in a 96-well plate with the microplate reader.

Results

In Fig. 2, the blue group indicates the control group, and the orange and gray groups indicate the dental pulp stem cells grown in 20 KGy γ-ray treated HA and 60 KGy γ-ray treated HA, respectively. Regardless of the group, the dental pulp stem cells had a normal cell growth trend. Relatedly, the results revealed no significant difference between the control group and the experimental groups. That is, the results suggested that the HA does not exhibit any ability to improve the cell viability of such stem cells.

Discussion

HA is involved in a variety of cellular physiological functions which are also associated with the proliferation of stem cells. In past experiments, Zou and his team found that high concentrations (4 mg/ml) of HA promoted the release of growth

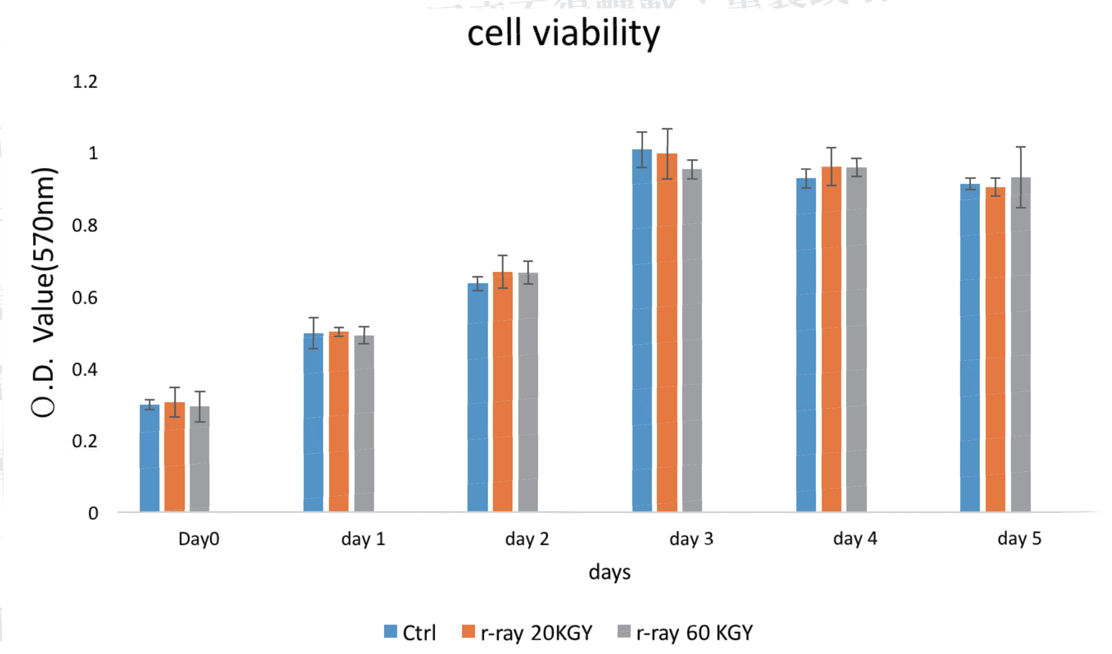

Fig. 2. MTT assay cell viability results

factors from cells, stimulating cell-cell interactions and increasing the rate of cell proliferation[7]. In this study, the concentration of HA we used was 1 mg/ml, which was different from the concentration used in previous studies. As such, further studies may be required in order to determine the concentration of HA that it most suitable for spurring dental pulp stem cell hyperplasia. In addition, it should also be noted that different stem cells have different sensitivities, so more research will also be needed to determine whether or not HA has any positive effects in bone tissue engineering involving stem cells of various kinds. Meanwhile, the current study did show that HA is not harmful to dental pulp stem cells. The results of the MTT assays showed that the amounts of dental pulp stem cells in the experimental sub-groups were not decreased. This proved that HA is harmless to these human cells. However, more research is needed to identify any mechanism underlying a proliferative effect of HA on dental pulp stem cells.

Conclusion

The results of this study suggested that the HA did not have any positive effects on the development of the dental pulp stem cells. However, since there is still much that remains unknown about HA, more research will be needed in the future to reveal the effects of HA on tissue engineering and stem cells.

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Case Report

Complete Denture Fabrication for a Patient with Limited Mouth Opening – A Case Report

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Abstract

Temporomandibular joint (TMJ) ankylosis is a severe disease that results in hypomobility of the TMJ and thus affects patients' daily functions of speech, mastication, and oral hygiene. For prosthodontists, restricted mouth opening commonly leads to challenging clinical scenarios. The following case report describes the case of a 43-year-old female with a history of TMJ ankylosis. The patient's maximum mouth opening from ridge to ridge was about 31 mm after left gap arthroplasty with temporalis muscle flap reconstruction. Complete dentures were fabricated through careful clinical procedures. As a result, the dentures improved the patient's appearance, chewing function, and maximum mouth opening. In conclusion, when treating a patient with limited mouth opening, a set of well-made dentures can improve the patient's functional abilities and esthetics.

Key words: complete dentures, gap arthroplasty, limited mouth opening, Temporomandibular joint (TMJ) ankylosis

Introduction

Temporomandibular joint (TMJ) ankylosis is a severe disease that affects the patient's TMJ, causing hypomobility of the jaw and, in turn, affecting the daily functions of speech, mastication, and oral hygiene[1]. The etiologies of TMJ ankylosis include trauma, arthritis, infection, previous TMJ surgery, congenital defects, and idiopathic factors[2-4]. According to the literature, trauma is the most common etiology of TMJ ankylosis, accounting for 75% to 98% of cases[3,5-8]. Epidemiological investigations have shown that the type of trauma and the patient's age are the most important factors related to ankylosis[9-11]. Children can develop TMJ ankylosis very easily by overreacting to trauma because they have more growth potential than adults[1]. Intracapsular condylar fracture destroys both the condylar head and the surrounding soft tissue more severely than other types of condylar fractures. Therefore, it is the most dangerous cause of ankylosis[1]. The management of TMJ ankylosis is difficult and is mainly conducted through surgical intervention, even as there are no standard surgical procedures for treating it.

For prosthodontists, restricted mouth opening commonly leads to challenging clinical scenarios. Special attention should thus be paid in performing impression procedures and vertical dimension determinations, and in making jaw relation records and

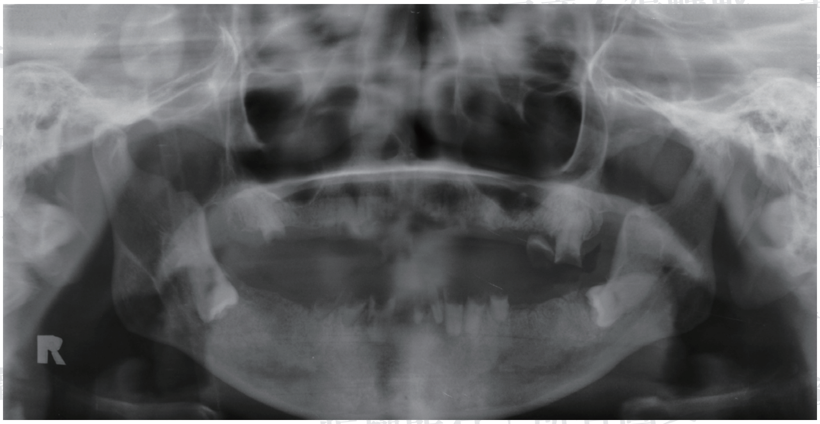

Fig. 1. Panoramic x-ray showing multiple residual roots, impacted lower third molars, and resected bilateral coronoid process.

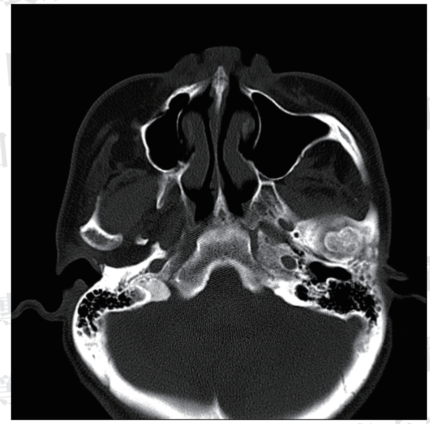

Fig. 2. CT scan showing abnormal left TMJ with suspected TMJ ankylosis.

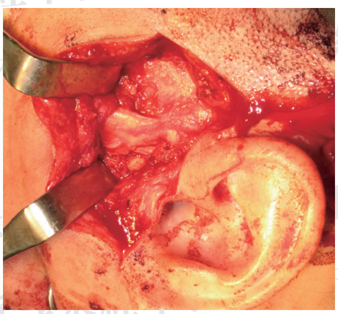

Fig. 3. Left gap arthroplasty with temporalis muscle flap for the treatment of left TMJ ankylosis.

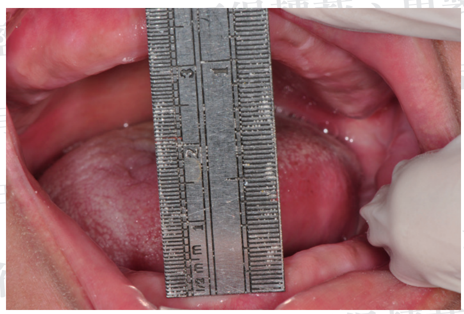

Fig. 4. The maximum mouth opening was only 31 mm (from ridge to ridge).

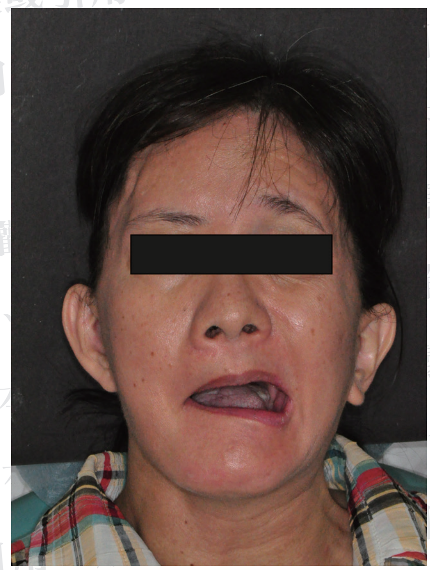

Fig. 5. The best "smile". The patient's facial muscle control was poor.

determining prosthetic teeth arrangements. The following case report describes the case of a denture-rehabilitated patient with a history of TMJ ankylosis.

Case report

A 43-year-old female experienced a head injury due to traffic accident in December of 1994. She was diagnosed with an epidural hematoma (EDH) and a subdural hematoma (SDH) and underwent two craniotomies. However, her vegetative status persisted for more than two months. She was then referred to the NCKUH department of physical medicine and rehabilitation. Fortunately, the patient ultimately regained conscious and was discharged from NCKUH, although she still suffered from some sequelae of the brain injury, including mouth opening limitation. The patient was subsequently brought to the NCKUH department of stomatology for oral rehabilitation in 1998. Her maximum mouth opening at that time was only 7 mm. Mouth opening exercise was suggested at that time, but the patient was uncooperative because of the brain injury. A surgical intervention including left interpositional arthroplasty and bilateral coronoidectomy under general anesthesia was then performed. However, severe mouth opening limitation and deviation to the left could still be observed after the surgery. The patient was then lost to follow-up for a long period of time (1998~2012). Recently, however, she returned to the NCKUH department of stomatology with her parents and asked for further management for her mouth opening limitation and dental rehabilitation. Although the patient had regained consciousness after a two-month period of vegetative status, her mental status after the head injury was child-like and she had forgotten everything, including her parents and even who she was. Moreover, her ability to control her facial muscles was poor, and her left eye was almost blind.

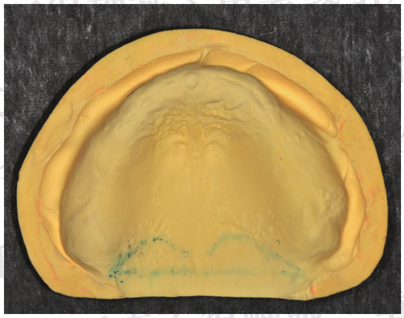

Fig. 6. The upper working cast.

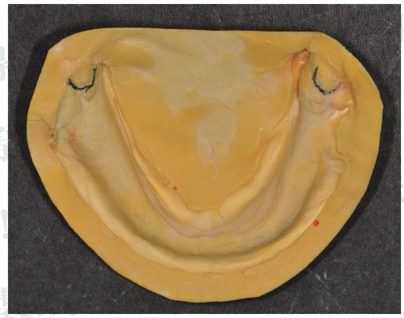

Fig. 7. The lower working cast.

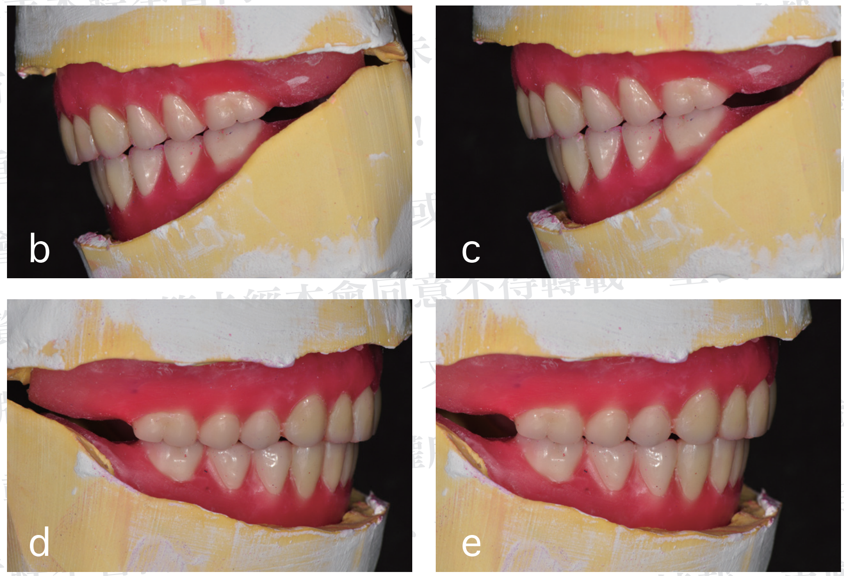

Fig. 8. The occlusal scheme was set to achieve bilateral balanced occlusion. (a) CO contact. (b) Left balancing contact. (c) Left working contact. (d) Right balancing contact. (e) Right working contact.

A panoramic x-ray was taken that showed multiple residual roots, impacted lower third molars, and the resected bilateral coronoid process (Fig. 1). A CT scan showed an abnormal left TMJ, which led to the suspicion of TMJ ankylosis (Fig. 2). The patient's maximum mouth opening at that time was only 15 mm, and chin deviation to left side during mouth opening was also noted. On November 9th, 2012, the patient underwent left TMJ surgery, and all the residual roots and the impacted tooth #38 were extracted under general anesthesia. After exposing the left TMJ region, no evidence of bony TMJ ankylosis was observed, but it was revealed that a silicon sheet placed 14 years prior to the surgery had become encompassed by fibrous tissue. The silicon sheet was then removed, and a central perforation at about 6 mm was observed. The fibrous ankylosis mass was trimmed to create adequate space for the condyle head, and the left temporalis muscle flap was used for interpositional material (Fig. 3).

In October of 2013, complete denture fabrication procedures were started. The patient's maximum mouth opening at that time was only 31 mm from ridge to ridge (Fig. 4). Figure 5 shows the best "smile" she could do. Upper and lower stock trays and alginate were used to make preliminary impressions. Then, upper and lower individual trays were made. After border molding, final impressions were made with additional silicone. After boxing, the working casts were poured (Figs. 6, 7).

Standard protocol was followed to determine the occlusal plane and vertical dimension with the occlusion rims first. Then, the vertical dimension was reduced by evenly reducing the height of the upper and lower occlusion rims. The rest position could not be used for reference due to the patient's poor control ability. Facial support and a phonetics test were used as guides to determine the vertical dimension.

After the vertical dimension determination, bite registration and facebow transfer were performed, and then the working casts were mounted on a semi-adjustable articulator. The zero-degree non-anatomical posterior teeth were selected, and the occlusal scheme was set to achieve bilateral balanced occlusion (Fig. 8). After packing, the upper and lower

Fig. 9. 6-month follow-up image. Stable occlusion was noted.

Fig. 10. 6-month follow-up image. Note the bilateral and even CO contact and the zero-degree non-anatomic posterior prosthetic teeth.

Fig. 11. 6-month follow-up image. The maximum mouth opening was increased by 4.5 mm (from 31 mm to 35.5 mm).

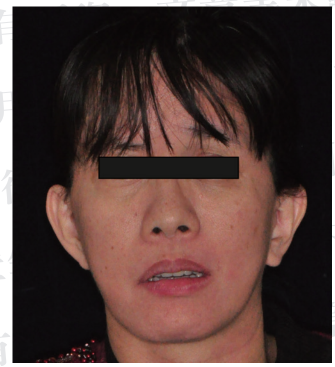

Fig. 12. The frontal view after delivery of the dentures.

complete dentures were delivered, and their stability and retention were good (Figs. 9, 10). At first, the patient could not eat while wearing the dentures. At the 2-week follow-up, however, she could eat soft foods while wearing the dentures. At the 1.5-month follow-up, there was no specific complaint, and the patient and her parents were satisfied with the dentures. At the 6-month follow-up, the patient's maximum mouth opening had increased by 4.5 mm (from 31 mm to 35.5 mm) (Figs. 11, 12).

Discussion

The management of TMJ ankylosis is difficult and is mainly conducted through surgical intervention, even as there are no standard surgical procedures for treating it. Rather, a variety of techniques and interpositional materials have been used. Today, gap arthroplasty with interpositional graft has come to be seen as one acceptable method for the primary surgical management of TMJ ankylosis[12]. A variety of interpositional materials have been used in this procedure, including the temporalis muscle and fascia, dermis, auricular cartilage, fascia lata, fat, lyodura, silicone, and various metals[13-16]. The temporalis muscle flap is the most commonly used interpositional material. The advantages of using this flap are as follows: (1) good blood supply; (2) close proximity to the surgical area, such that it can be easily accessed from the same incision; (3) easy preparation and harvesting; and (4) minimal cosmetic and functional morbidity at the donor site[12].

According to a study conducted by He et al.[1], placement of an interpositional material between the ramus and the skull base is usually advocated to reduce the recurrence rate. The temporalis myofascial flap is the most popular interpositional graft and typically achieves good results[17]. When the medially displaced condylar head and disc are intact, lateral arthroplasty with temporalis myofascial flap is a good procedure for treating TMJ ankylosis.

For prosthodontists, restricted maximum mouth opening commonly leads to compromised impressions and prostheses. Therefore, a modification of the standard fabrication procedures is often necessary. In some cases, the mouth opening is so limited that placement of even the smallest available stock tray into the mouth is not feasible. Trimming the flange lengths and adding compound as necessary are often helpful means of addressing this issue. Furthermore, if a 1-piece tray cannot be used, then the stock tray may be cut in half and the halves approximated to form the preliminary cast[18]. Procedures have been introduced in which putty silicone is used as flexible trays washed with light body silicone for more details. After pouring, the resultant preliminary casts can then be used for making rigid sectional trays for final impressions[19-21]. In the present case, the smallest available stock tray could be slowly placed in the patient's mouth and so was used for the preliminary impressions.

In patients with limited mouth opening, entrance of the bolus is more easily accomplished by increasing the interocclusal space. Therefore, reduced vertical dimension is sometimes needed for these patients. Some experimental research studies have shown that a rapid adaptation can take place after changing the vertical dimension, leading to another rest position and the creation of a new interocclusal distance[22]. This seems to occur even after an increase that was greater than the original interocclusal distance. Such findings indicate that the adaptive capacity of the masticatory system is usually substantial and that the rest position alone is not a reliable basis for the determination of maxillomandibular relations. Relatedly, small variations in the vertical dimension are not so critical[22]. Lingualized or monoplane occlusion is favored in patients with limited mouth opening. The non-anatomic occlusal schemes generate less horizontal force.

For patients with limited mouth opening, special attention should be paid to fabrication procedures. Moreover, practical prosthodontic treatment goals are crucial in patients with compromised oral function.

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