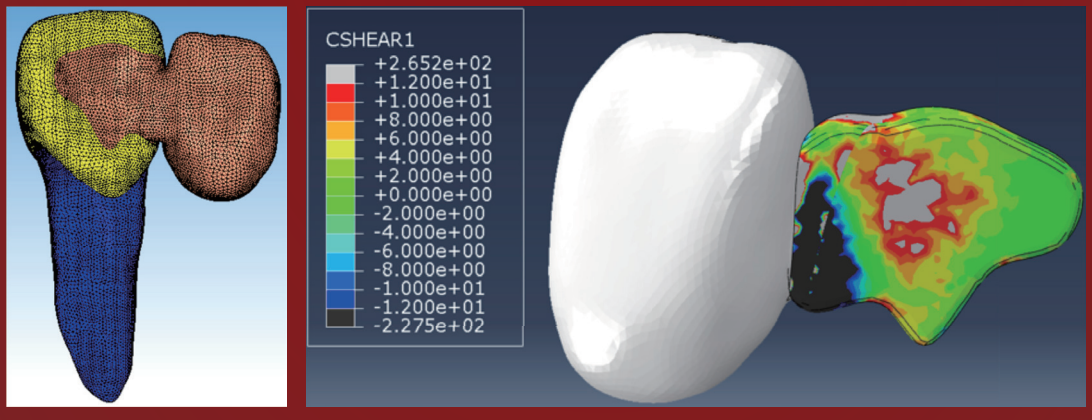
The Academy of Prosthetic Dentistry R.O.C., Taiwan

Journal of Prosthodontics and Implantology



Shape Optimization of Retainer Design for Maxillary Anterior Resin-Bonded Fixed Partial Dentures. Page 6 Fig. 2

Official Journal of
The Academy of Prosthetic Dentistry, R.O.C., Taiwan

Volume 7 Number 2
July 2018

Journal of Prosthodontics and Implantology

中華民國贋復牙科學會期刊

Vol.7, No.2
July 2018

Subscription：USD $100/ year　　訂價年費：NT $3000（四期）
創刊日期 ：2012年4月
出版者：中華民國贋復牙科學會　學會地址：105台北市復興北路465號2樓　學會電話：+886-2-2546-8834
學會傳真：+886-2-2546-9157　　學會官網：http://www.prosthod.org.tw
ISSN 2304-5418
承製編印：青田設計工作室　地址：235新北市中和區民富街64巷5-1號　電話：+886-2-2225-4014
E-mail：field.design@msa.hinet.net　傳真：+886-2-3234-5491

TABLE OF CONTENTS

Journal of Prosthodontics and Implantology

Editorial

To change or to be changed is a decision faced by everyone in this changing world. As the center of the world is moving from the West to the East in the coming year. Asia Pacific will be the global center in the next decade. With better economic condition, health becomes a noticeable issue in Asia Pacific region while dentistry plays an important role to keep people in good health.

We are now facing the challenge of the so-called Silver Tsunami. That is, the percentage of elderly people is increasing and we are all facing the challenges of an aging society. Dental education and research should be transformed to meet the needs of such a society. We have to change for the future demand. Under these circumstances, prosthetic dentistry becomes more and more important.

In order to achieve the goal of Journal of Prosthodontics and Implantology, which is to improve the knowledge of those interested in Prosthodontic dentistry by increased communication and exchange of scientific information as well as the stimulation from the professionals, we are working together to seek better solutions toward these goals and make the world a better place.

Allen Ming-Lun Hsu
Editor-in-Chief

Original Article

Shape Optimization of Retainer Design for Maxillary Anterior Resin-Bonded Fixed Partial Dentures

Yung-Chung Chen, DDS, MDsc, PhD
Department of Stomatology,
Division of Prosthodontics, National Cheng Kung University Hospital,
College of Medicine, National Cheng Kung University, Tainan, Taiwan
Institute of Oral Medicine,
College of Medicine, National Cheng Kung University, Tainan, Taiwan

Yuan-Ray Chang, MS
Department of Mechanical Engineering,
National Cheng Kung University,
Tainan, Taiwan

Chi-Lun Lin, MS, PhD
Department of Mechanical Engineering,
National Cheng Kung University,
Tainan, Taiwan

Corresponding author:
Chi-Lun Lin, MS, PhD
Assistant Professor, Department of Mechanical Engineering, National Cheng Kung University, Tainan, Taiwan R.O.C.
Tel: +886-6-2757575 ext. 62190
E-mail: linc@mail.ncku.edu.tw

Abstract

Objective

Given the unfavorable survival rates reported in previous studies, the improvement of resin-bonded fixed partial dentures (RBFPDs) is unquestionably necessary. Clinically, most failures of RBFPDs are attributed to interfacial debonding and inadequate retainer design. This paper presents an automated optimization approach that utilizes the framework of finite element analysis to obtain retainer designs for RBFPDs that can reduce the risk of debonding and further extend clinical service time.

Material and methods

The automated optimization process was developed and performed to determine the optimal RBFPD retainer designs in an iterative manner. The overall optimization process consisted of two steps: the manual building of models and the automated optimization process itself. Ten specimens for each group, the traditional group and optimal group, were fabricated for each design. The specimens were then loaded to failure in order to compare their mechanical performance.

Results

The in vitro mechanical tests indicated that the optimal design obtained was not able to outperform the traditional design. It was found that the assigned values simulating the adhesive's bonding strengths were set too high in the automated scripts. By lowering the adhesive's bonding strengths to reasonable values in the second set, the performance of the optimal design was experimentally validated. The optimal design outperformed the traditional design in terms of the amount of tooth preparation and debonding area. In the validation experiment, the optimal design was found to have a higher peak force and first load drop than the traditional design did.

Conclusion

The experimental results validated the automated optimization process and suggested that it can be used to generate optimized designs for various clinical conditions and to provide scientifically sound preparation guidelines for clinicians.

Key words: automated modeling, finite element analysis, resin-bonded fixed partial dentures, shape optimization

Introduction

During the past few decades, minimally invasive treatment protocols have become popular due to the development and improvement of dental adhesive materials, and hence, the philosophy for designing dental prostheses has also changed. When a patient needs a prosthetic restoration for missing anterior teeth, the fundamental rule is to restore function and esthetics at minimal biological cost[1]. Resin-bonded fixed partial dentures (RBFPDs), which are recognized as constituting a minimally invasive treatment, were first introduced by Ibsen[2] and Rochette[3]. The concept underlying their development and use was to decrease the amount of teeth preparation and to preserve healthy tooth tissue as much as possible[4].

RBFPDs can be made with different material systems. In early designs, metal alloys were used in the fabrication of the dental bridges. Recently, fiber-reinforced composites (FRCs) and ceramic materials have been increasingly adopted in fabricating RBFPDs due to the growing esthetic demands of patients. However, the application of ceramic materials is significantly limited because their brittleness leads to more tooth tissue removal[5, 6]. Consequently, FRC systems have drawn increasing attention for use in RBFPDs because they can provide a tooth-colored appearance and retain the advantages of minimal invasion. Moreover, FRC FPDs can be fabricated directly in a patient's mouth or indirectly by a dental technician. If slight damage to an FRC FPD occurs while the patient is wearing it, the denture can be restored directly in the patient's mouth[7]. Hence, FRC FPDs can provide better convenience than metal and ceramic FPDs. In addition, the lower cost of FRC FPDs makes them a more affordable option for patients. However, there are also some disadvantages to FRC FPDs. The strength of FRC is inferior to the strength of metal and ceramic, and the surfaces of FRC can become worn more easily and accumulate more plaque[8, 9].

For the main scope of the present study, the authors reviewed the success rates of FRC FPDs with thin wing-shaped retainers only. An FRC FPD was defined as successful if it exhibited or resulted in no pontic fracture, glass fiber fracture, abutment tooth fracture, chipping of the composite resin, or retainer debonding when worn. Frese and his colleagues reported a 72.6% success rate for anterior FRC FPDs at 54 months[10], while Malmstrom and her colleagues reported that 89.4% of anterior FRC FPDs that were all fabricated directly in patients' mouths remained successful at two years[11]. Spinas and his colleagues reported a 93.8% success rate for anterior FRC FPDs given to teenage patients after five years of observation[12], while Kumbuloglu and her colleagues also observed a relatively high success rate of 92.6% for anterior FRC FPDs at 58 months[13]. However, lower success rates for anterior FRC FPDs have also been noted. For example, Heumen and his colleagues reported that only 45% of anterior FRC FPDs survived at 5 years[7], and Wolff and his colleagues reported an overall survival rate of 53.0% and a functional survival rate of 73.5% for FRC FPDs over the 48-month observation period of a clinical trial that they conducted[14].

According to the aforementioned studies, the most common failure mode of FRC FPDs is chipping of the composite resin, followed by debonding of the retainer and fracture of the glass fiber. As such, FRC FPDs can be viewed as a feasible short-term to medium-term treatment option, but there is still much room for improvement in terms of their success rate. Relatedly, research supporting the long-term usage of FRC FPDs is currently lacking[15], but the long-term success rate for such dentures is certainly expected to be lower than the short-term and medium-term success rates.

At present, the traditional way to design FPD retainers is to create a covering area for bonding on the lingual surface of a tooth that is as large as possible[16, 17]. The most common way to test the mechanical performance of an FRC FPD is to use a universal testing machine to apply load to the FRC FPD until it fractures. However, there are many drawbacks to this load-to-fracture method, such as the expensive material costs, time-consuming specimen preparation, and difficulties in controlling specimen dimensions. Furthermore, the associated experimental results do not allow for a straightforward determination of how any new FPDs should be designed. Therefore, a number of studies have utilized the finite element method and observed the stress distribution in FPD structures in order to effectively optimize FRC FPD designs[18-21]. However, while this approach is very convenient for comparing stress distributions among different designs, it is still a very time-consuming approach to use when modifying designs repeatedly. Moreover, it is difficult to determine when an optimal design has been achieved with this approach.

After a comprehensive literature review, we found that the consideration of biomechanical issues during the clinical design process used for anterior cantilever FRC FPDs is insufficient. To reduce the risk of a retainer debonding, its optimal shape for minimizing interfacial stresses should be determined. Therefore, the present study was aimed at developing an automated optimization process that utilizes the framework of finite element analysis to solve the debonding problem and increase the success rate of anterior cantilever FRC FPDs.

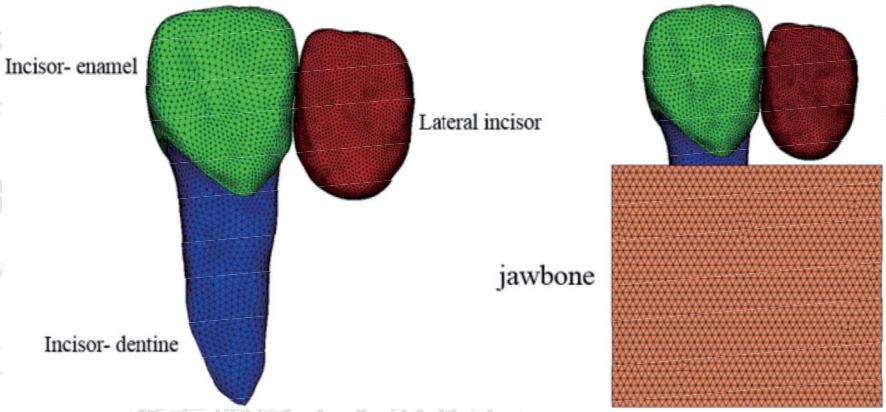


Fig. 1. The geometric parts of the finite element mode

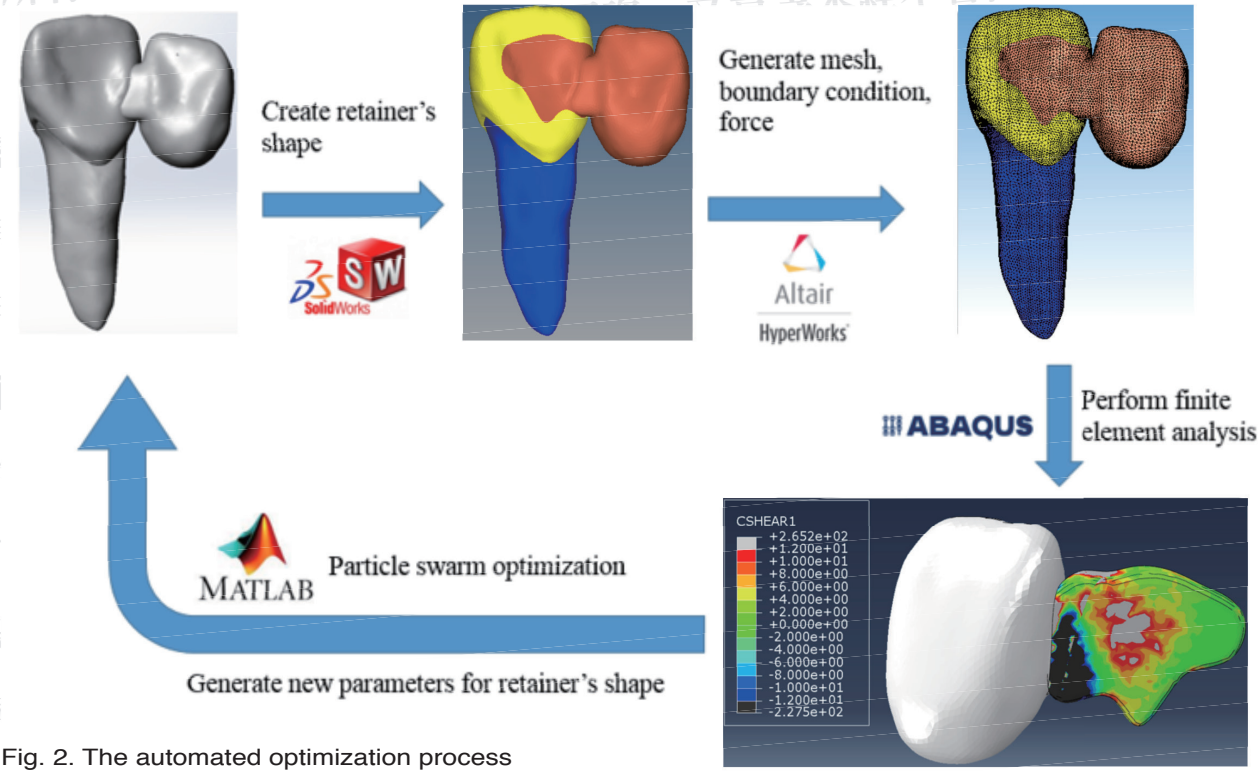


Fig. 2. The automated optimization process

Material and methods

To attain the optimal designs for anterior cantilever FRC FPDs, a numerical optimization process and in vitro experiment for validation were included in the present study. The study focused on replacing a missing maxillary lateral incisor with an RBFPD, and the retainer was designed to be bonded to a central incisor. The overall optimization process consisted of two steps: the manual building of models and the automated optimization process itself. The physical model of a central incisor and the cantilevered pontic was manually constructed using the software SolidWorks (Solidworks 2018, Dassault Systèmes SolidWorks Corporation, MA, United States).

Numerical optimization process

In this study, the geometric models used contained a central incisor, a cantilevered pontic for the lateral incisor, and a rectangular bone block (see Fig. 1). Both incisors' contours were obtained from real teeth scanned by a micro-CT scanner (Skyscan 1076, Bruker, Belgium). The material sections of the incisor model including enamel, dentine, and pulp were segmented.

The automated optimization process was conducted as follows. First, the shape of the retainer shape was devised using the software SolidWorks. Second, meshes of great quality were generated using the software Hypermesh (Hyperworks, Altair, MI, United States). The element sizes were 0.2 mm

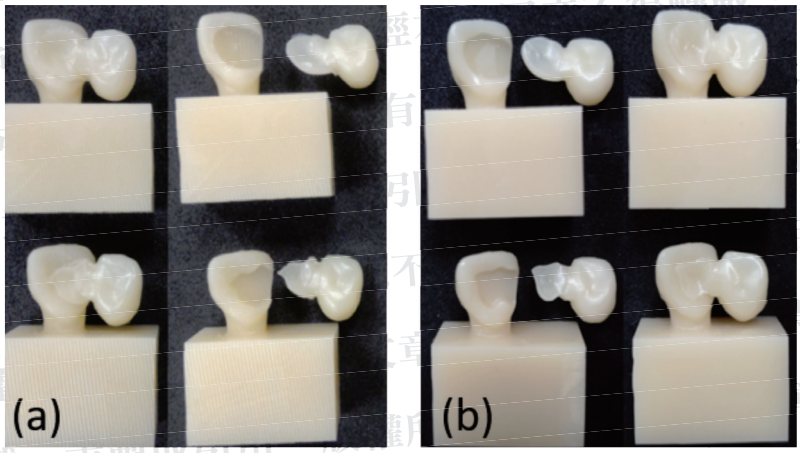


Fig. 3. Specimens of traditional and optimized designs: (a) 1st set (b) 2nd set

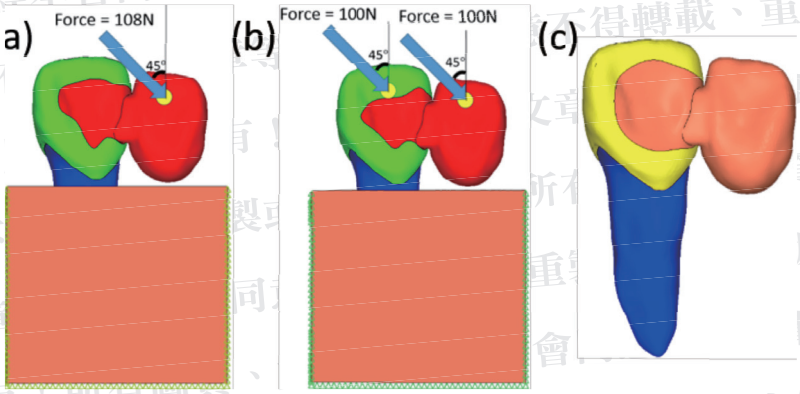


Fig. 5. The optimization results of the realistic RBFPD retainer: (a) Optimal design for the first loading type,; (b) optimal design for the second loading type,; (c) traditional design

Fig. 4. The configuration of in vitro mechanical test

for the RBFPD and the incisor and 0.2 ~ 0.8 mm for the bone block. Using ABAQUS element type C3D4, about 400,000 elements and 80,000 nodes were generated over the entire model. Next, a distributed load was applied at the lingual surface of the pontic of the RBFPD, and both sides of the bone block were fixed as the boundary condition. Two types of loading situation were simulated, including applying a load of 108N at the pontic only and applying a load of 100N at both the central incisor and pontic. Third, the finite element model was solved by using the software ABAQUS (ABAQUS 14-2, Dassault Systèmes SolidWorks Corporation, MA, United States) to retrieve the stresses at the interface between the RBFPD and central incisor. Last, the tensile stress and shear stress values at the interface between the retainer and prepared tooth were collected. The areas with higher stresses than the assumed adhesive's bonding strength were summed up as the total debonding area. The particle swarm optimization (PSO) algorithm was implemented to minimize the total debonding area. This study used ten vectors as the design variables to describe the shape of the retainer. The initial design (defined as the traditional design) was designed according to current clinical practices, in which the retainer is designed to cover the lingual side of the tooth as much as possible in order to maximize the contact area, as shown in Fig. 5(c). In each iteration, the PSO algorithm generated new design variables for the retainer's shape and automatically repeated the aforementioned steps until reaching convergence. The maximum number of iterations was set at 30. The whole process is shown in Fig. 2. Finally, the mechanical performance of the optimal design was compared with that of the traditional design.

In vitro experiment for validation

To ensure that the optimal design obtained from the numerical optimization process was valid,

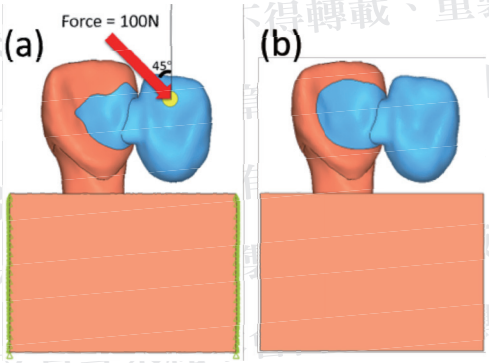


Fig. 6. The optimization results of the simplified RBFPD retainer for the first set of validation: (a) optimal design,; (b) traditional design

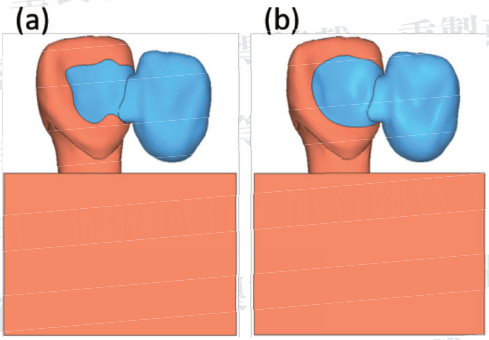


Fig. 8. The optimization results of the simplified RBFPD retainer for the second set of validation: (a) optimal design,; (b) traditional design

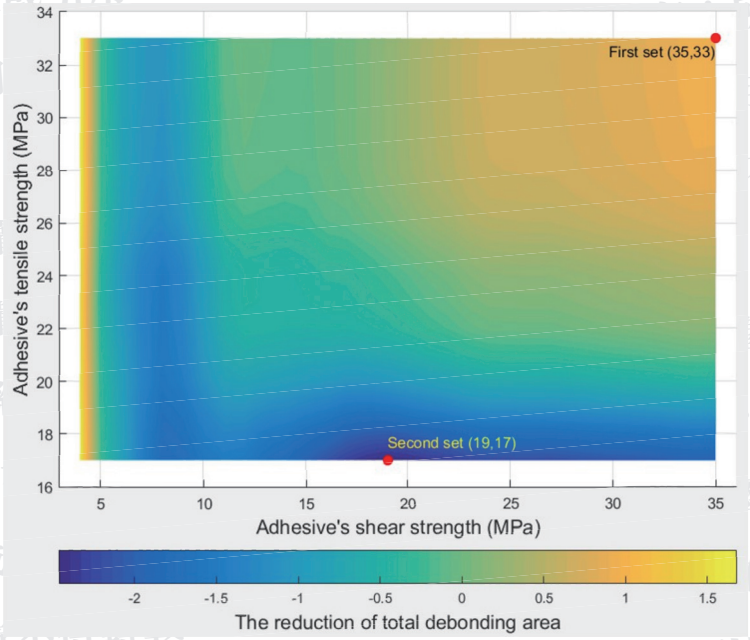


Fig. 7. The comparison of traditional and optimal designs in different adhesive's bonding

a simplified model was used to determine the optimal design so that the same settings could be exactly duplicated in both the finite element simulation and the experiment. The mechanical performance of the traditional and optimized designs was then compared.

In the experiment, the entire model and the RBFPDs were made of PMMA (see Fig. 3). Ten specimens were fabricated for each design. The fabricated traditional and optimized RBFPDs were adhered to the central incisor using resin cement (RelyX U200, 3M ESPE, MN, United States). Both sides of the rectangular PMMA block were clamped by a fixture. In addition, an acoustic emission sensor was glued onto the rectangular PMMA block to monitor the development of cracks in the specimens during loading. A universal testing machine (JSV-H1000, Japan Instrumentation system, Narashi, Japan) was used to apply a compressive load to the pontic of the given RBFPD. The force was applied at 2 mm below the incisal edge using a steel ball indenter with a diameter of 2 mm and a constant loading speed of one millimeter per minute. The load was applied until the RBFPD debonded from the central incisor. The configuration of the experiment is shown in Fig. 4.

Results

The first part of the results presented here are the results of the numerical optimization process. A more realistic RBFPD design was simulated along with the automated optimization process under two loading conditions. The second part of the results presented here are the results for the validation of the numerical optimization process, in which a simplified RBFPD design was optimized and its results were compared with those of an in vitro mechanical experiment.

1. Deriving designs of RBFPDs for two loading conditions

As previously mentioned, the retainer designs under two types of loading situations, that is, loading at the pontic only or loading at both incisors, were simulated iteratively for optimization. The optimal designs for the first and the second loading types are shown in Figs. 5a and 5b. The traditional design is shown in Fig. 5c. The debonding areas and tooth preparation amounts were compared and are listed in Table 1. The debonding area of the RBFPD was found to be smaller when the load was applied at the enamel rather than at the retainer. It was further found that both optimal designs required lower tooth preparation amounts than the traditional

Table 1. Comparison of traditional and optimal designs for realistic RBFPD retainer

	Loads at central incisor and pontic (100N each)		Load only at pontic (108N)	
	Traditional	Optimal	Traditional	Optimal
Debonding area (mm^2)	15.69	10.97	12.26	10.63
Preparation amount (mm^3)	37.18	26.44	37.18	30.25

Table 2. First set - comparison of traditional and optimal designs for validation

	Debonding area (mm^2)	Preparation amount (mm^3)
Traditional	2.99	36.64
Optimized	2.07	26.04

Table 3. First set – in vitro mechanical test results for the traditional design

	Peak force	Slope	Number of AE events
T1	116.4	62.35	25
T2	47.8	45.8	83
T3	77.7	52.86	15
T4	122.7	55.28	23
T5	83.4	44.7	127
T6	51.6	42.5	39
T7	158.3	55.37	43
T8	107.4	44.82	44
T9	133.3	47.85	26
T10	114.5	41.27	15
Average	101.31	49.28	44
SD	33.73	6.49	33.53

Table 4. First set – in vitro mechanical test results for the optimal design

	Peak force	Slope	Number of AE events
O1	52.4	46.83	85
O2	43.3	30.15	5
O3	28.8	29.72	9
O4	66	46.3	13
O5	53.9	41.06	28
O6	61.3	39.19	16
O7	25.3	24.7	4
O8	59	44.03	13
O9	61.1	41.8	29
O10	28	34.19	64
Average	47.91	37.8	26.6
SD	14.68	7.28	25.66

design and that the debonding areas for both optimized designs were smaller.

2. Validation of the optimization process

2.1. Optimization process (1st set)

A distribution load of 100N was applied at the pontic, and both sides of the cuboid were fixed (see Fig. 6a). The bonding strengths, 33 MPa for tensile strength and 35 MPa for shear strength, were assumed to be the same as for the enamel bonded to the adhesive. The optimal design and the traditional design are shown in Figs. 6a and 6b, respectively. Compared to the traditional design, the optimal design had better performance in terms of both the amount of tooth preparation and the total debonding area (see Table 2).

2.2. In vitro validation (1st set)

After indentation, the force-displacement curve and AE signal were obtained. Nineteen specimens were debonded and only one specimen was fractured at the connector. The peak force, slope of the force-displacement curve, and number of AE events for the traditional and optimal designs are shown in Table 3 and Table 4, respectively. The traditional designs had higher peak forces, slopes, and numbers of AE events than the optimal designs did. This sug-

Table 5. Second set - comparison of traditional and optimal designs for validation

	Debonding area (mm^2)	Preparation amount (mm^3)
Traditional	7.92	36.64
Optimized	6.36	27.87

Table 6. Second set - in vitro mechanical test results for the traditional design

	Peak force	First force drop	Number of force drops	Number of AE events
T1	13.63	13.14	46	80
T2	20.50	11.38	43	86
T3	28.05	12.65	85	210
T4	21.28	11.67	55	202
T5	10.49	8.53	66	210
T6	36.58	13.04	28	81
T7	32.17	27.85	39	138
T8	19.42	19.42	68	185
T9	25.30	14.02	28	72
T10	22.16	13.24	43	82
Average	22.96	14.49	50.1	134.6
SD	7.52	5.15	17.42	57.76

gests that the traditional designs outperformed the optimal ones.

Thus, the optimal design obtained was shown to have poorer performance in the experiment. This poorer performance was most likely caused by the settings of the adhesive's bonding strengths being wrong. The bonding strengths were set as 35 and 33 MPa for shear stress and tensile stress, respectively. However, according to the literature, the ranges of bonding strengths for shear stress and tensile stress are 4-35 and 17-33 MPa, respectively. Hence, the debonding areas of the traditional and optimal designs were reevaluated with different bonding strengths for the adhesive. The resulting comparison of the total debonding area of the traditional and optimal designs for the different adhesive bonding strengths is shown in Fig. 7. The cases in which the optimal design had a lower total debonding area were marked with a triangular sign, while the cases in which the traditional design had a lower total debonding area were marked with a circular sign. If the bonding strengths between the adhesive and the PMMA were actually located at the region of the circular sign, then it would make sense that the traditional design was better than the optimal design.

2.3. Optimization process (2^{nd} set)

The finite element simulation of the second set was the same as that for the first set. The only difference was in the adhesive's bonding strengths. Specifically, strengths of 19 and 17 MPa for the shear stress and tensile stress, respectively, were assumed. Based on the evaluation of the parametrical study shown in Fig. 7, this selected configuration of bonding strengths would produce the largest discrepancy in the total debonding area between the traditional design and optimal design.

The resulting optimal design and the traditional design are shown in Figs. 8a and 8b, respectively. Compared to the traditional design, the optimal

Table 7. Second set - in vitro mechanical test results for the optimal design

	Peak force	First force drop	Number of force drops	Number of AE events
O1	54.03	15.00	9	102
O2	56.00	21.08	13	76
O3	34.03	24.91	31	146
O4	32.26	13.14	14	75
O5	36.58	16.28	12	53
O6	39.91	12.65	10	54
O7	44.52	19.02	10	46
O8	61.49	20.79	11	44
O9	10.40	10.40	71	177
O10	53.45	17.95	6	29
Average	42.27	17.12	18.7	80.2
SD	14.31	4.25	18.58	45.55

design had better performance in terms of both the amount of tooth preparation and the total debonding area (see Table 5).

2.4. In vitro validation (2nd set)

After indentation, the force-displacement curve and AE signal were obtained. All the models were debonded. The peak force, first force drop, number of force drops, and number of AE events of the traditional and optimal designs are shown in Table 6 and Table 7, respectively. All the optimal designs had higher peak forces and first force drops than the traditional designs, which suggested that the optimal designs outperformed the traditional ones. Thus, the optimization process was validated by the experiment performed with the adjusted adhesive bonding strengths.

Discussion

The optimal solution achieved a lower amount of tooth preparation and smaller debonding area at the same time. This result suggests that a larger retainer does not always provide better mechanical performance. Rather, the shape of the retainer plays a more important role in this regard. It was observed during the iteration process that the shape of the retainer tended to evolve to avoid the direct load, such that the load was finally applied on the enamel. Interestingly, this result matched the objective of current clinical practice.

Unexpected failure occurred in three of the twenty samples in the first set of the validation experiment. The O1 and O4 samples showed cracks and the connector of the T5 sample broke into two parts at a peak force of 83.4 N, which was much lower than the forces that the other samples with the traditional design were able to sustain. We suspected that the manufacturing process was not carefully performed so that local sharp edges were produced and concentrations of stress arose at these locations. Furthermore, two types of surface finish were observed among the twenty samples. As a result, one type of the samples had a smoother surface finish, while on the surface of the others a clear direction of lay was seen. A significant difference in the peak force between these two groups of samples was found in the experimental results (Tables 3 and 4). In order to obtain a more consistent sample condition for all the samples, the aforementioned issues were fixed by communicating with the dental technician before manufacturing the samples for the second validation experiment.

Previous studies have reported that the values of the tensile and shear strengths for an adhesive are distributed in wide ranges of 17-33 MPa and 4-35 MPa, respectively. The optimal design obtained in

the first set of optimization was compared with the traditional design in the finite element simulations using all combinations of the strength values in the ranges. Fig. 7 shows the results of this parametric study as a contour plot, where the reduction of the total debonding area was the improvement achieved by the optimization. Thus, the colors with positive values indicate that the optimal design outperformed the traditional design. The contour plot suggested that the current optimal design would outperform the traditional design to a greater degree when both the tensile and shear strengths of the adhesive are higher. However, the validation experiment obtained an opposite result, which proved that the bonding strengths of the adhesive selected in the optimization process were too optimistic. According to the contour plot, the traditional design would be most likely to outperform the optimal design when the tensile and shear strengths were set as 17 and 19 MPa, respectively (i.e., the poorest optimal design with the most negative reduction of total debonding area). Thus, this configuration was selected to conduct the second set of optimization and the validation experiment, and the results did prove that our guess was correct.

The design variables considered in this research consisted of ten length vectors that defined ten points to form the outline of the retainer. The retainer contacted the tooth at a plane, but in practice, it can have a curved surface or other 3D shapes. In addition, other types of designs have been used in other research studies, such as designs featuring holes and ditches, in order to enhance the bonding strength at the interface[29, 33, 34, 52-54]. For future improvements, more design variables can be included in the optimization process to consider more types of retainer designs.

With regard to the patient-specific modeling, the shape of the teeth for a given patient can be parameterized. That is, measurements of different patients' teeth can be performed to define proper geometric parameters. The approach of using many vectors to define the retainer can also be applied to define a given tooth.

Conclusion

In this study, an automated optimization process was developed and performed to determine the optimal RBFPD retainer designs under different load conditions. However, the optimal design obtained was shown to have poorer performance than the traditional design in the first round of the validation experiment. It was found that the adhesive's bonding strengths were set too high. By lowering the adhesive's bonding strengths to reasonable values in the second set, the obtained optimal design was successfully validated, as the optimal design outperformed the traditional design in terms of the amount of tooth preparation and the debonding area. In the validation experiment, the optimal design had a higher peak force and first force drop than the traditional design did. The results of the experiment suggested that the automated optimization process can be used to generate optimal designs for various clinical conditions and to provide scientifically sound preparation guidelines for clinicians.

References

1. Edelhoff D, Sorensen JA. Tooth structure removal associated with various preparation designs for anterior teeth. J Prosthet Dent 2002;87:503-9.
2. Ibsen RL. Fixed prosthetics with a natural crown pontic using an adhesive composite. Case history. J South Calif Dent Assoc 1973;41:100-2.
3. Rochette AL. Attachment of a splint to enamel of lower anterior teeth. J Prosthet Dent 1973;30:418-23.
4. Murdoch-Kinch CA, McLean ME. Minimally invasive dentistry. J Am Dent Assoc 2003;134:87-95.
5. P P, P R, G G, W G. All-ceramic resin-boned bridges. A 3-dimensional finite-element analysis study. European Journal of Oral Sciences 1996;104:390-5.
6. C K, M R, M B, R L, G H. In vitro examination of the fracture strength of 3 different fiber-reinforced composite and 1 all-ceramic posterior inlay fixed partial denture systems. Journal of Prosthodontics 2002;11:248-53.
7. van Heumen CCM, van Dijken JWV, Tanner J, Pikaar R, Lassila LVJ, Creugers NHJ, et al. Five-year survival of 3-unit fiber-reinforced composite fixed partial dentures in the anterior area. Dent Mater 2009;25:820-7.
8. Behr M, Rosentritt M, Handel G. Fiber-reinforced composite crowns and FPDs: A clinical report. Int J Prosthodont 2003;16:239-43.
9. Bollen CML, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: A review of the literature. Dent Mater 1997;13:258-69.
10. Frese C, Schiller P, Staehle HJ, Wolff D. Fiber-reinforced composite fixed dental prostheses in the anterior area: a 4.5-year follow-up. J Prosthet Dent 2014;112:143-9.
11. Malmstrom H, Dellanzo-Savu A, Xiao J, Feng C, Jabeen A, Romero M, et al. Success, clinical performance and patient satisfaction of direct fibre-reinforced composite fixed partial dentures - a two-year clinical study. J Oral Rehabil 2015;42:906-13.
12. Spinas E, Aresu M, Canargiu F. Prosthetic rehabilitation interventions in adolescents with fixed bridges: a 5-year observational study. Eur J Paediatr Dent 2013;14:59-62.
13. Kumbuloglu O, Ozcan M. Clinical survival of indirect, anterior 3-unit surface-retained fibre-reinforced composite fixed dental prosthesis: Up to 7.5-years follow-up. J Dent 2015;43:656-63.
14. Wolff D, Wohlrab T, Saure D, Krisam J, Frese C. Fiber-

reinforced composite fixed dental prostheses: A 4-year prospective clinical trial evaluating survival, quality, and effects on surrounding periodontal tissues. J Prosthet Dent 2017.

15. Ahmed KE, Li KY, Murray CA. Longevity of fiber-reinforced composite fixed partial dentures (FRC FPD) Systematic review. J Dent 2017;61:1-11.
16. Botelho M. Design principles for cantilevered resin-bonded fixed partial dentures. Quintessence Int 2000;31:613-9.
17. Stephen R, Martin L, Junhei F. Contemporary Fixed Prosthodontics. 2015:694-712.
18. Nakamura T, Ohyama T, Waki T, Kinuta S, Wakabayashi K, Takano N, et al. Finite element analysis of fiber-reinforced fixed partial dentures. Dent Mater J 2005;24:275-9.
19. Yokoyama D, Shinya A, Lassila LVJ, Gomi H, Nakasone Y, Vallittu PK, et al. Framework Design of an Anterior Fiber-Reinforced Hybrid Composite Fixed Partial Denture: A 3D Finite Element Study. Int J Prosthodont 2009;22:405-12.
20. Ootaki M, Shin-Ya A, Gomi H, Shin-Ya A, Nakasone Y. Optimum design for fixed partial dentures made of hybrid resin with glass fiber reinforcement by finite element analysis: Effect of vertical reinforced thickness on fiber frame. Dent Mater J 2007;26:280-9.
21. Magne P, Perakis N, Belser UC, Krejci I. Stress distribution of inlay-anchored adhesive fixed partial dentures: a finite element analysis of the influence of restorative materials and abutment preparation design. The Journal of prosthetic dentistry 2002;87:516-27.

Original Article

Proliferative Effects of Hyaluronic Acid on Human Dental Pulp Stem Cells

Shiau-Ting Shiu, DDS
School of Dentistry, College of Oral Medicine, Taipei Medical University, Taipei, Taiwan
Taipei Medical University-Shuang Ho Hospital, Ministry of Health and Welfare, New Taipei City, Taiwan

Sheng-Wei Feng, DDS, MS, PhD
School of Oral Hygiene, College of Oral Medicine, Taipei Medical University, Taipei, Taiwan

Haw-Ming Huang, PhD
Graduate Institute of Biomedical Optomechatronics, College of Biomedical Engineering, Taipei Medical University, Taipei, Taiwan
School of Dentistry, College of Oral Medicine, Taipei Medical University, Taipei, Taiwan

Corresponding author:
Haw-Ming Huang, PhD
School of Dentistry,
Taipei Medical University,
250 Wu-Hsing Street,
Taipei, Taiwan R.O.C.
Tel: +886-2-2736-1661 ext. 5128
E-mail: hhm@tmu.edu.tw

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°C. 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.

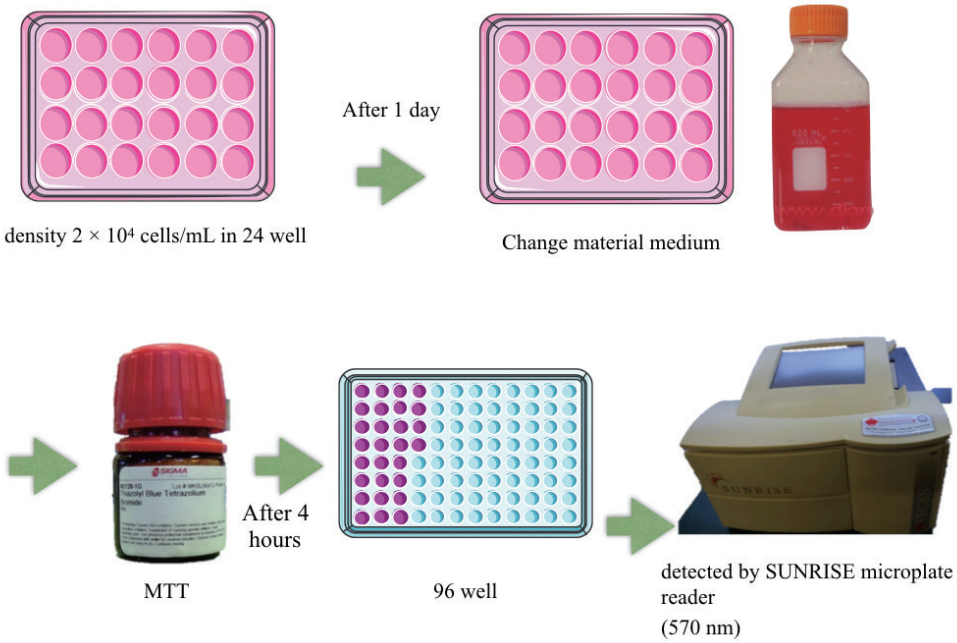


Fig. 1. The process diagram of the method

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

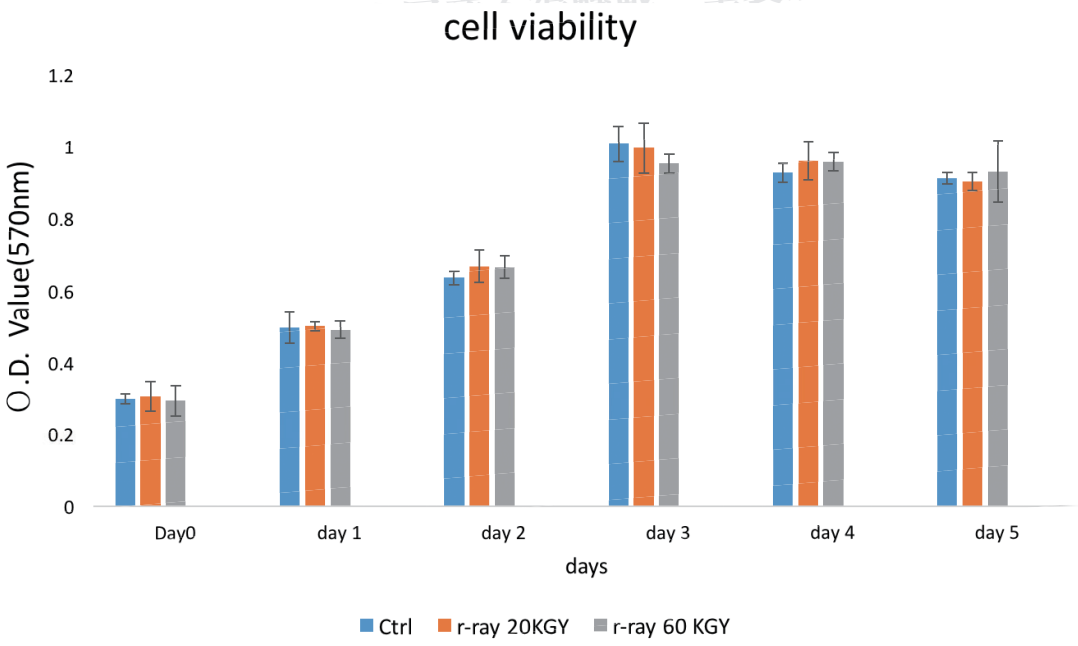


Fig. 2. MTT assay cell viability results

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.

References

1. Tuch BE. Stem cells: A clinical update. Australian Family Physician. 2006; 35(9): 719-21.
2. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proceedings of the National Academy of Sciences. 2000; 97(25): 13625-30.
3. Shi S, Robey PG, Gronthos S. Comparison of human dental pulp and bone marrow stromal stem cells by cDNA microarray analysis. Bone. 2001; 29(6): 532-9.
4. Carinci F, Papaccio G, Laino G, Palmieri A, Brunelli G, D'Aquino R, Pezzetti F. Comparison between genetic portraits of osteoblasts derived from primary cultures and osteoblasts obtained from human pulpar stem cells. Journal of Craniofacial Surgery. 2008; 19(3): 616-25.
5. Nakamura K, Yokohama S, Yoneda M, Okamoto S, Tamaki Y, Ito T, Okada M, Aso K, Makino I. High, but not low, molecular weight hyaluronan prevents T-cell-mediated liver injury by reducing proinflammatory cytokines in mice. Journal of gastroenterology. 2004; 39(4): 346-54.
6. Dahiya P, Reet K. Hyaluronic acid: A boon in periodontal therapy. North American Journal of Medical Sciences. 2013; 5(5): 309-15.
7. Zou X, Li H, Chen L, Baatrup A, Bünger C, Lind M. Stimulation of porcine bone marrow stromal cells by hyaluronan, dexamethasone and rhBMP-2. Biomaterials. 2004; 25(23): 5375-85.

Case Report

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

Shao-Chun Wang, DDS
Attending Doctor of Prosthodontics,
Division of Prosthodontics,
Department of Stomatology,
National Cheng Kung University
Hospital, Tainan, Taiwan

Ju-Chun FanChiang, DDS, MS
Part-time faculty of Prosthodontics
Division of Prosthodontics,
Department of Stomatology,
National Cheng Kung University
Hospital, Tainan, Taiwan

Corresponding author:
Ju-Chun FanChiang, DDS, MS
Part-time faculty of Prosthodontics
Division of Prosthodontics,
Department of Stomatology,
National Cheng Kung University
Hospital, Tainan, Taiwan R.O.C.
Tel: +886-2-6223-2789
E-mail: jcfanchiang2001@yahoo.com.tw

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

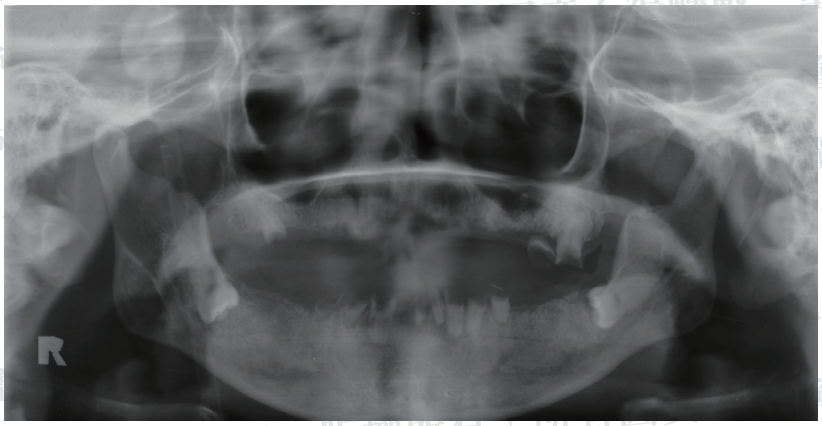


Fig. 1. Panoramic x-ray showing multiple residual roots, impacted lower third molars, and resected bilateral coronoid process.

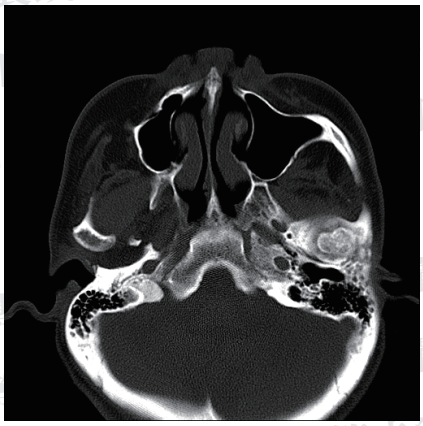


Fig. 2. CT scan showing abnormal left TMJ with suspected TMJ ankylosis.

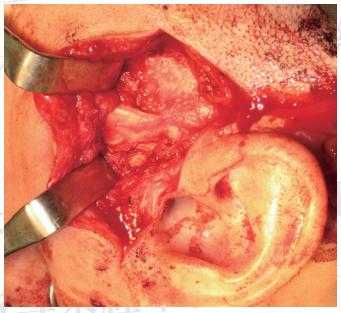


Fig. 3. Left gap arthroplasty with temporalis muscle flap for the treatment of left TMJ ankylosis.

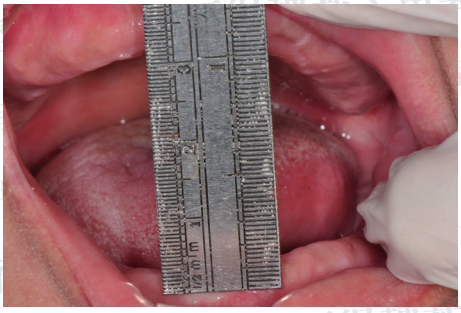


Fig. 4. The maximum mouth opening was only 31 mm (from ridge to ridge).

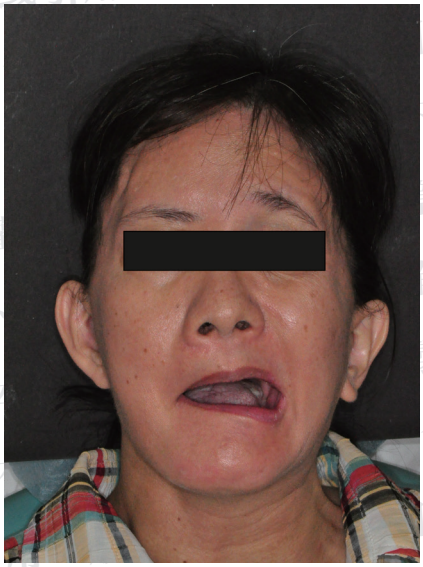


Fig. 5. The best "smile". The patient's facial muscle control was poor.

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.

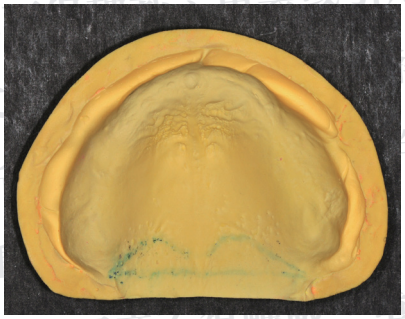


Fig. 6. The upper working cast.

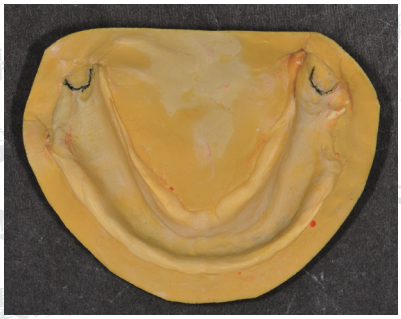


Fig. 7. The lower working cast.

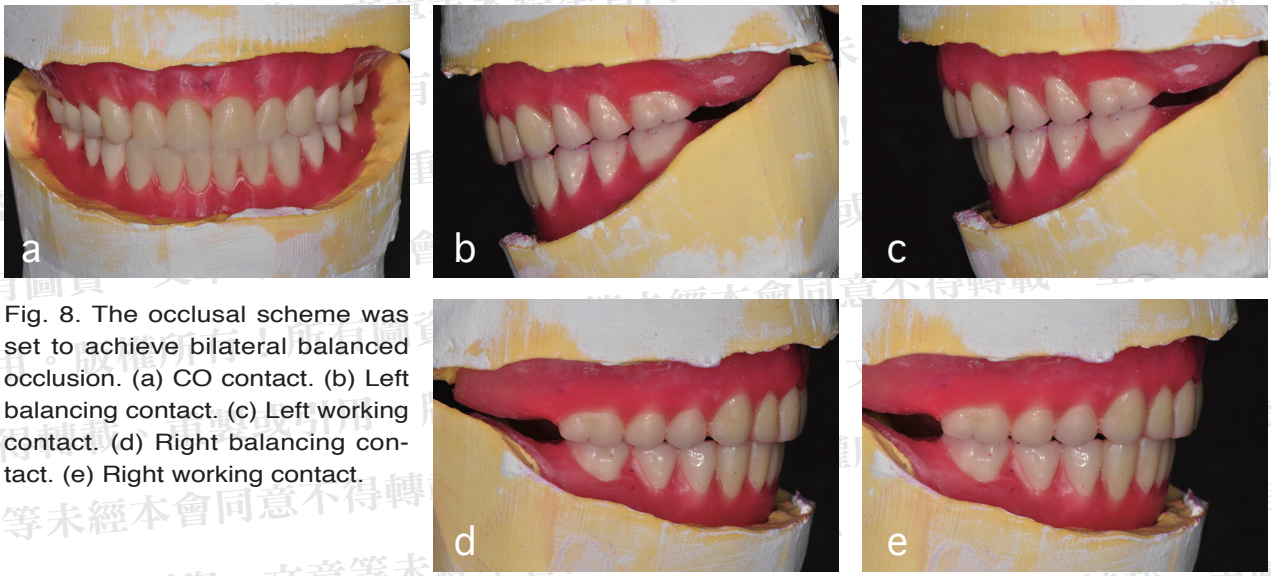


Fig. 8. The occlusal scheme was set to achieve bilateral balanced occlusion. (a) CO contact. (b) Left balancing contact. (c) Left working contact. (d) Right balancing contact. (e) Right working contact.

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

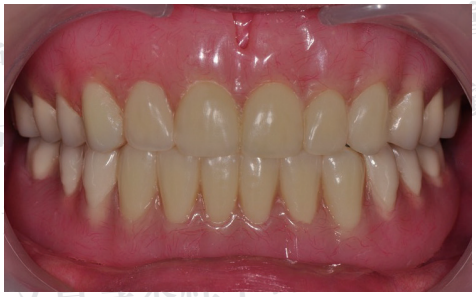
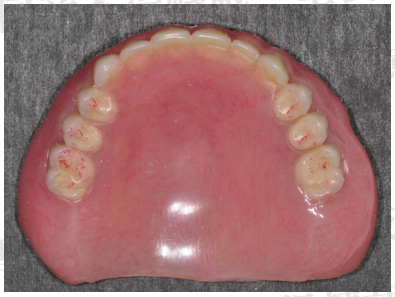


Fig. 9. 6-month follow-up image. Stable occlusion was noted.



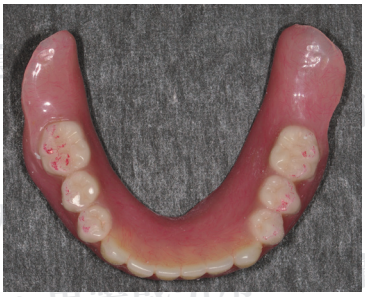


Fig. 10. 6-month follow-up image. Note the bilateral and even CO contact and the zero-degree non-anatomic posterior prosthetic teeth.

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).

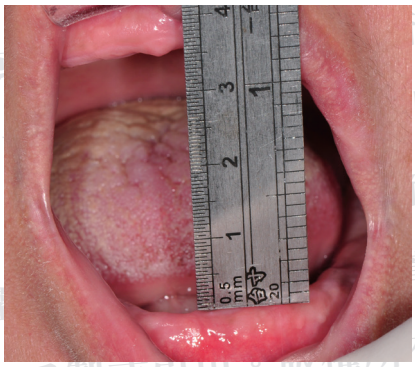


Fig. 11. 6-month follow-up image. The maximum mouth opening was increased by 4.5 mm (from 31 mm to 35.5 mm).

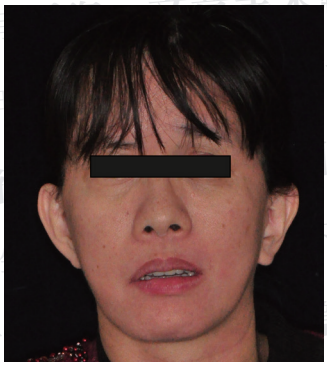


Fig. 12. The frontal view after delivery of the dentures.

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.

References

1. He D, Yang C, et al. Traumatic temporomandibular joint ankylosis: our classification and treatment experience. J Oral Maxillofac Surg 2011 Jun; 69(6): 1600-7.
2. Loveless TP, Bjornland T, et al. Efficacy of Temporomandibular Joint Ankylosis Surgical Treatment. J Oral Maxillofac Surg 2010; 68(6): 1276-82.
3. Roychoudhury A, Parkash H, et al. Functional restoration by gap arthroplasty in temporomandibular joint ankylosis: A report of 50 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999; 87:166-9.
4. Erol B, Tanrikulu R, et al. A clinical study on ankylosis of the temporomandibular joint. J Craniomaxillofac Surg 2006; 34:100-6.
5. Wen JQ, Jian XC, et al. Etiological analysis of temporomandibular joint ankylosis. J Mod Stomatol (Chin) 2002; 16:565.
6. Ya ZM, Tan NH, et al. Titanium condyle prosthesis for the treatment of temporomandibular joint ankylosis: a 71 cases report. J Plast Reconstruct Surg (Chin) 2004; 1:74-7.
7. el-Sheikh MM. Temporomandibular joint ankylosis: the Egyptian experience. Ann R Coll Surg Engl 1999 Jan; 81(1):12-8.
8. Valentini V, Vetrano S, et al. Surgical treatment of TMJ ankylosis: our experience (60 cases). J Craniofac Surg 2002 Jan; 13(1):59-67.
9. Ellis E 3rd. Complications of mandibular condyle fractures. Int J Oral Maxillofac Surg 1998 Aug; 27(4): 255-7.
10. Daniels JS, Ali I. Post-traumatic bifid condyle associated with temporomandibular joint ankylosis: report of a case and review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005 Jun; 99(6): 682-8.
11. Sales MA, Oliveira JX, et al. Computed tomography imaging findings of simultaneous bifid mandibular condyle and temporomandibular joint ankylosis: case report. Braz Dent J 2007; 18(1): 74-7.
12. Bayat M, Badri A, et al. Treatment of temporomandibular joint ankylosis : gap and interpositional arthroplasty with temporalis muscle flap. Oral Maxillofac Surg 2009; 13(4): 207-12.
13. Guruprasad Y, Chauhan DS, et al. A Retrospective Study of Temporalis Muscle and Fascia Flap in Treatment of TMJ Ankylosis. J Maxillofac Oral Surg 2010; 9(4): 363-8.
14. Kwon TG, Park HS, et al. Staged surgical treatment for temporomandibular joint ankylosis: intraoral distraction after temporalis muscle flap reconstruction. J Oral Maxillofac Surg 2006; 64(11): 1680-3.
15. Manfredini D, Bucci MB, et al. Temporomandibular joint bilateral post-traumatic ankylosis: a report of a case treated with inter positional arthroplasty. Minerva Stomatol 2008; 58(1-2): 35-42.
16. Schliephake H, Schmelzesien R, et al. Long-term results of the use of silicone sheets after discectomy in the temporomandibular joint: clinical, radiographic and histopathologic findings. Int J Oral Maxillofac Surg 1999; 28(5): 323-9.
17. Su-Gwan K. Treatment of temporomandibular joint ankylosis with temporalis muscle and fascia flap. Int J Oral Maxillofac Surg 2001; 30:189-93.
18. Baker PS, Brandt RL, et al. Impression procedure for patients with severely limited mouth opening. J Prosthet Dent 2000; 84(2): 241-4.
19. Whitsitt JA, Battle LW. Technique for making flexible impression trays for the microstomic patient. J Prosthet Dent 1984 Oct; 52(4): 608-9.
20. Cheng AC, Koticha TN, et al. Prosthodontic management of an irradiated maxillectomy patient with severe trismus using implant-supplrted prostheses: a clinical report. J Prosthet Dent 2008 May; 99(5): 344-50.
21. Cheng AC, Kwok-Seng L, et al. Prosthodontic management of edentulous patient with limited oral access using implant-supported prostheses: a clinical report. J Prosthet Dent 2006 Jul; 96(1): 1-6.
22. Zarb GA, Hobkirk J, et al. Prosthodontic Treatment for Edentulous Patients : Complete Dentures and Implant-Supported Prostheses 13th ed., Mosby Co, St Louis, 2013; pp190-2.

Case Report

Apical Surgery and Guided Bone Regeneration for Immediate Implant Placement and Provisionalization: A Case Report

Ching-Chieh Lin, DDS
Attending Doctor,
Department of Prosthodontics,
Chi Mei Medical Center,
Tainan, Taiwan

Hsiao-Chun Lai, DDS
Attending Doctor,
Department of Endodontics,
Chi Mei Medical Center,
Tainan, Taiwan

Lian-Ping Mau, DDS, MS
Attending Doctor,
Department of Periodontics,
Chi Mei Medical Center,
Tainan, Taiwan

Jui-Chung Chang, DDS, MSD
Director, Department of Prosthodontics
Chi Mei Medical Center,
Tainan, Taiwan

Corresponding author:
Jui-Chung Chang, DDS, MSD
Director,
Department of Prosthodontics,
Chi Mei Medical Center,
Tainan, Taiwan, R.O.C.
Tel: +886-6-281-2811 ext. 56058
E-mail: csg129@gmail.com

Abstract

When planning to place an implant after the extraction of a failing tooth with an apical lesion, it should be noted that the performance of a series of soft and hard tissue reconstruction efforts by the clinician can cause considerable patient distress. This case report describes an alternative strategic approach that combines apical surgery with guided bone regeneration for immediate implant placement and provisionalization. This approach can not only preserve the architecture of the hard and soft tissue, but can also achieve esthetic and functional outcomes and increase patient satisfaction.

Key words: apical surgery, guided bone regeneration, immediate implant placement, immediate provisionalization

Introduction

A tooth with a combined periodontal and periapical infection often results in defective buccal bone. Such infections also affect the success rate of implant and bone augmentation surgery, as the removal of the source of an infection of the teeth will cause horizontal and vertical bone loss followed by soft tissue atrophy. According to one previous study[1], the width of alveolar bone is reduced by an average of 50% within one year of losing a tooth, with two-thirds of the bone resorption occurring in the first three months. Meanwhile, the reconstruction of hard and soft tissue is often laborious, time-consuming, and unpredictable. With these issues in mind, this report presents a case in which a technique combining apical surgery with guided bone regeneration was used to achieve esthetic and functional outcomes in the presence of a failing infected tooth.

Case report

The present case involves a 35-year-old non-smoking male who denied having any systemic diseases or drug allergies. A clinical examination revealed no tooth attrition or other abnormal oral habits. However, the patient reported having suffered from gum swelling over the right upper posterior tooth for one month. A gumboil over his right maxillary second premolar was noted. The apical lesion measured approximately 9 mm × 9 mm in size according to the periapical film taken during the examination (Fig. 1.). No gingival swelling, inflammation, or deep probing above 3 mm were observed in the clinical examination. Furthermore, a bone sounding examina-

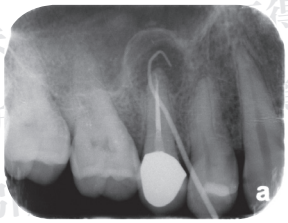


Fig. 1. Periapical film: GP tracing to apex of the right maxillary second premolar

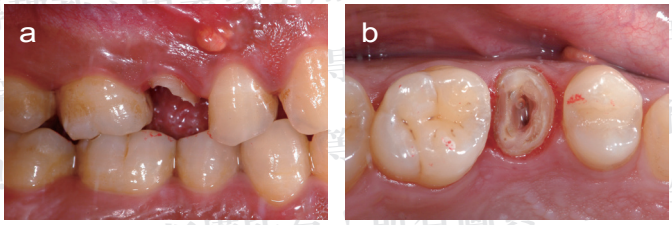


Fig. 2. Clinical examination after PFM crown removal. (a)buccal intra-oral view; (b) occlusal intra-oral view; (c)periapical film

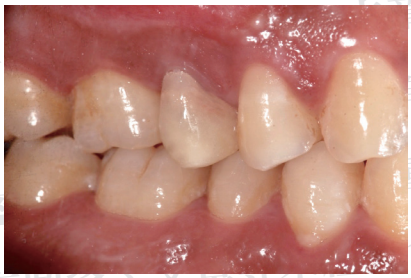


Fig. 3. Provisional crown fabrication

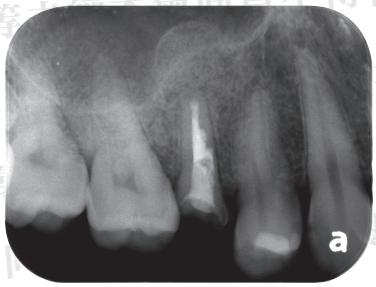


Fig. 4. Root canal retreatment

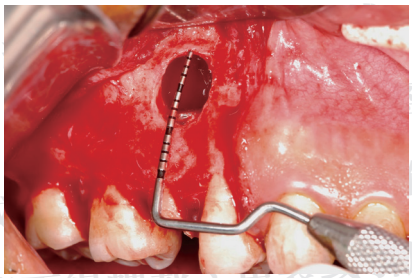


Fig. 5. Apicoectomy

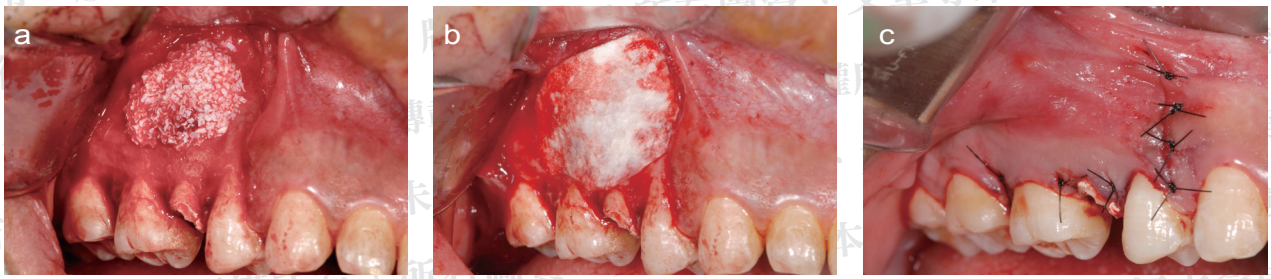


Fig. 6. Guided bone regeneration. (a)Bio-oss; (b)resorbable collagen membrane; (c)suture with 5-0 Nylon

tion indicated that the buccal bone crest remained intact. The clinical diagnosis was an apical cyst with a previously treated root canal. After removal of the PFM crown and dental caries, tooth extraction was indicated because there was a lack of sufficient tooth structure for tooth restoration (Fig. 2.). The suggested treatment plans called for a single implant replacement or 14×16 FDP. After discussion with the patient, a definitive treatment plan of implant placement was decided upon. To prevent collapse of the buccal bone after tooth extraction and subsequent patient embarrassment due to the missing tooth, the following treatment steps were proposed: (1) fixed provisional crown fabrication (Fig. 3.), (2) root canal retreatment (Fig. 4.), (3) apical surgery combined with guided bone regeneration, (4) tooth extraction, immediate implant placement, and provisionalization, and (5) final implant-supported crown delivery.

Apical surgery and guided bone regeneration

For the apical surgery, cyst enucleation, apicoectomy of 3 mm of root apex, retrograde preparation, and retrograde filling with MTA (mineral trioxide aggregate) were performed (Fig. 5.). At the same time, guided bone regeneration was performed in the apical bony defect with xenograft (Bio-Oss®, Geistlich) and resorbable collagen membrane (EZ Cure™, Biomatlante) (Fig. 6.).

Immediate implant placement and provisionalization (IIPP)

After 6 months of bone healing time (Fig. 7.), the right maxillary second premolar was extracted

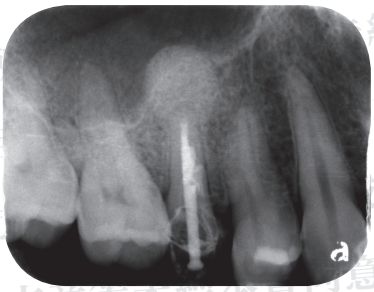


Fig. 7. Periapical film: apical surgery and GBR 6 months later

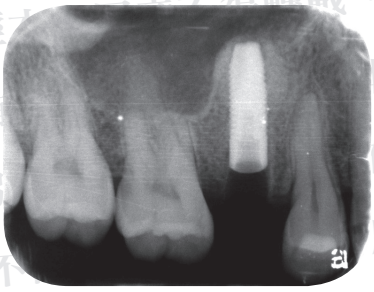


Fig. 8. Periapical film: immediate implant placement in fresh socket

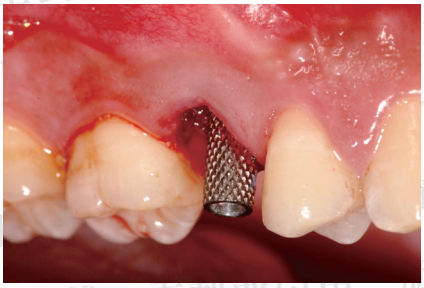


Fig. 9. Titanium temporary abutment was hand tightened onto the implant

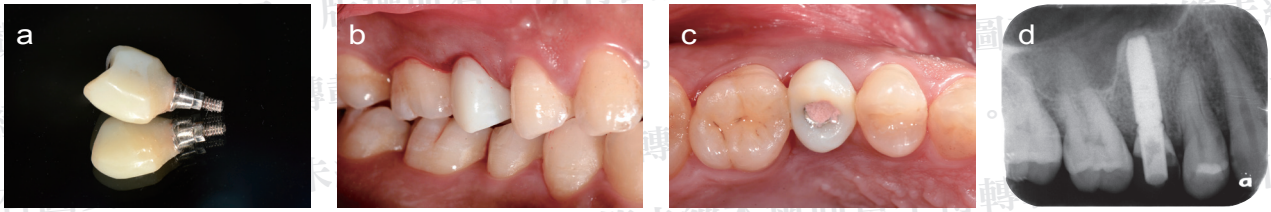


Fig. 10. (a)provisional implant supported crown; (b)buccal intra-oral view; (c)occlusal intraoral view; (d)periapical film

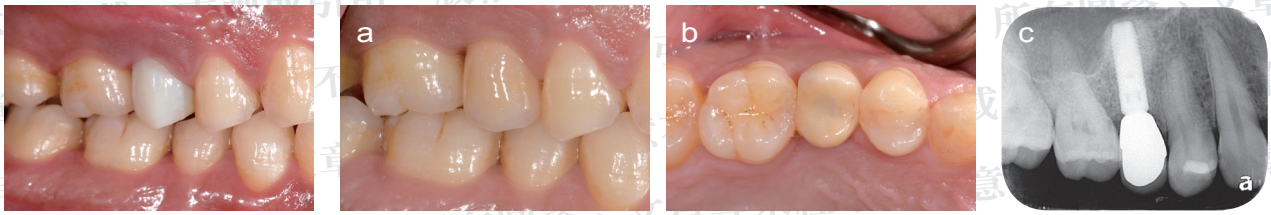


Fig. 11. Provisional implant supported crown after 3 months healing time

Fig. 12. Final restoration (a)buccal intra-oral view; (b)occlusal intra-oral view; (c)periapical film

with periotome in a less traumatic approach. The placement of a flapless implant (OsseoSpeed TX, 4.0 × 13 mm, Astra Tech AB; Mölndal, Sweden) was then performed (Fig. 8.). Due to the primary stability of the implant, a titanium temporary abutment was hand tightened onto the implant (Fig. 9.). The provisional crown was fabricated with light cured resin (Protemp™ 4, 3M ESPE) with the assistance of a surgical stent (Fig. 10.). The gap between the implant and the bony socket was filled with bone graft (FDBA, LifeNet®). The screw-retained implant-supported provisional crown was adjusted to clear all centric and eccentric functional contacts, and an appropriate antibiotic (amoxicillin 250 mg, 4 times daily) and analgesic (acetaminophen 500 mg, every 4 to 6 hours as needed for pain) were prescribed. The patient was instructed not to brush the surgical side, but rather to rinse with 0.2% chlorhexidine gluconate (Parmason, PBF). The patient was also asked to maintain a soft diet for 2 weeks. After 3 further months of bone healing time (Fig. 11.), a final impression was taken and the final screw-retained implant supported crown (monolithic zirconia occlusal surfaces: Ceramill Zi, Amann Girrbach AG; veneer procelain: Vita VM9; Vita Zahnfabrik, Bad Säckingen, Germany) was delivered (Fig. 12.). There were no biological or mechanical complications upon clinical and radiographic examinations at the 1-year follow-up visit (Fig. 13.).

Discussion

The standard approach is to eliminate the infection via tooth extraction when a hopeless tooth with an apical infection. Once the tooth is extracted, the buccal plate and bundle bone that had been adjacent to it prior to its removal will resorb. This bone resorption will then result in vertical and/or horizontal bone and soft tissue loss, and that loss, in

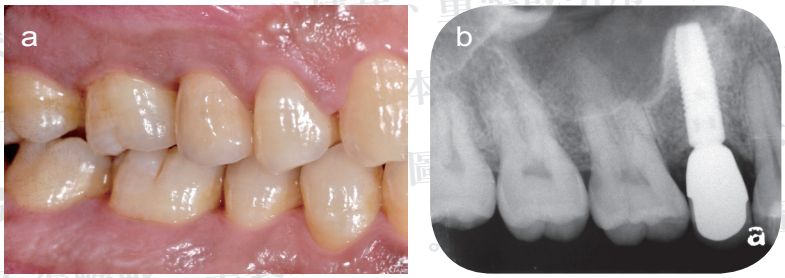


Fig. 13. 1-year follow (a)buccal intra-oral view; (b)periapical film

turn, will necessitate a combination of soft and hard tissue augmentation. However, these reconstruction procedures are usually strenuous and time-consuming, and yield unpredictable results. In this early implant placement approach, the patient is effectively required to experience a period time of during which the extracted tooth is simply missing or, alternatively, the patient wears a removable appliance that can possibly interfere with the masticatory function, alter the alignment of the dentition, and decrease the patient's quality of life. In contrast, the IIPP approach presented in the current case can provide better patient comfort, offer good esthetics[2], and eliminate the inconvenience of a second surgery for the placement of a healing abutment. This often leads to early soft tissue healing and stabilization of the peri-implant mucosa [3].

Recently, many studies have shown that an apical lesion is a risk factor for IIPP, but not a contraindication [4]. If chronic infection of the alveolar bone is treated with appropriate debridement, preoperative and postoperative antibiotic use, and the case is carefully selected, the survival rate can reach 92%~100% [5]. However, all of these studies excluded teeth without primary stability, whereas the key factor in IIPP is the primary stability of the implant. It is generally recommended that the implant should extend at least 4 to 5 mm into the root apex to obtain primary stability [2]. In this case, the patient was diagnosed with an apical cyst with a previously treated root canal, and the inflammation was confined only to the apical part with no involvement of the periodontal tissue. The periodontal pocket probing depth of the tooth was 3 mm. Therefore, considering the patient's desire not to experience an extended period of time with a missing tooth, we performed apical surgery to remove the apical infection, including the root tip up to 3 mm, in addition to performing guided bone regeneration surgery in the apical area to increase the bone volume. These actions in turn increases the primary stability of the implant that was then placed.

IIPP is a predictable surgery with a high success rate. According to a study by Kan et al. from 2011[6], an average of four years (2 to 8.2 years) prospective study of 35 single anterior teeth immediate implant placement and provisionalization, the cumulative success rate was 100%. The IIPP procedure is performed with a less traumatic form of tooth extraction and immediate placement of the implant in order to retain the alveolar crest and maintain the gingival architecture surrounding the implant [7,8,9]. This likewise provides early healing of the soft tissue surrounding the implant. In this case, we present an alternative treatment option that combined apical surgery with guided bone regeneration to replace an unrestorable and apically infected failing tooth with implantation in a staged approach. Although the overall treatment time for this approach is not faster than that for early implant placement, we propose that it constitutes a predictable method for not only maintaining the hard and soft tissue around the implant site but also results in high patient satisfaction while allowing the implant prosthesis to be effective in both esthetic and functional regards [10].

References

1. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. Int J Periodontics Restorative Dent. 2003 Aug;23(4):313-23.
2. Kan JY, Rungcharassaeng K, Lozada J. Immediate placement and provisionalization of maxillary anterior single implants: 1-year prospective study. Int J Oral Maxillofac Implants. 2003 Jan-Feb;18(1):31-9.
3. Tsuda H, Rungcharassaeng K, Kan JY, Roe P, Lozada JL, Zimmerman G. Peri-implant tissue response following connective tissue and bone grafting in conjunction with immediate single-tooth replacement in the esthetic zone: a case series. Int J Oral Maxillofac Implants. 2011 Mar-

Apr;26(2):427-36.

4. Ortega-Martínez J, Pérez-Pascual T, Mareque-Bueno S, Hernández-Alfaro F, Ferrés-Padró E. Immediate implants following tooth extraction. A systematic re-view. Med Oral Patol Oral Cir Bucal. 2012 Mar 1;17(2):e251-61.
5. Waasdorp JA, Evian CI, Mandracchia M. Immediate placement of implants into infected sites: a systematic review of the literature. J Periodontol. 2010 Jun;81(6):801-8.
6. Kan JY, Rungcharassaeng K, Lozada JL, Zimmerman G. Facial gingival tissue stability following immediate placement and provisionalization of maxillary anterior single implants: a 2- to 8-year follow-up. Int J Oral Maxillofac Implants. 2011 Jan-Feb;26(1):179-87.
7. Werbitt MJ, Goldberg PV. The immediate implant: bone preservation and bone regeneration. Int J Periodontics Restorative Dent. 1992;12(3):206-17.
8. Paolantonio M, Dolci M, Scarano A, d'Archivio D, di Placido G, Tumini V, Piattelli A. Immediate implantation in fresh extraction sockets. A controlled clinical and histological study in man. J Periodontol. 2001 Nov;72(11):1560-71.
9. Kan JY, Rungcharassaeng K. Site development for anterior single implant esthetics: the dentulous site. Compend Contin Educ Dent. 2001 Mar;22(3):221-6, 228, 230-1; quiz 232.
10. Kan JY, Rungcharassaeng K. Prophylactic root resection and periapical grafting for anterior implant aethetics: an integration of tissue-reconstruction and tissue-preservation concepts. Pract Proced Aesthet Dent. 2004 Jun;16(5):392-9; quiz 400.

Instructions for authors

The Journal of Prosthodontics and Implantology is an official publication of the Academy of Prosthetic Dentistry, ROC, published quarterly in April, July, November and January. Articles related to clinical and basic prosthodontics, implantology, implant related periodontology, periodontology and surgery as well as biological and material sciences related to prosthodontics and implantology are welcome. Articles may be categorized as original paper, case report, technical reports and literature review related to future research. Invited review articles are written by representative scholars on some important topics this journal wishes to emphasize.

Submitted papers will be evaluated by the editorial board and invited reviewers. Papers submitted shall not be accepted or published by any other journals. Papers published in this journal will become the copyright of this journal and shall not be subsequently published in any other publication in any language without prior written consent of the publisher.

Manuscript should be prepared according to the guidelines produced by the International Committee of Medical Journal Editors: Uniform Requirements for Manuscripts submitted to Biomedical Jopurnals. Further information can be found at http://www.icmje.org.

Manuscript should be submitted via e-mail at the address of: prosthor@ms48.hinet.net

The manuscript format

1. Title page: including title, names and institutions of each author. Corresponding author should also provide mailing address, phone and fax number and e-mail address. A running title no more than 40 words and no more than 4 keywords should also be provided.
2. Abstract: no more than 400 words, should briefly describe the purposes of the study, material and methods used, results and discussion with conclusion.
3. Text:
   A. Review articles: can be of author's preferred format.
   B. Original articles: should include introduction, materials and methods, results, discussion and conclusion.
   C. Case reports and technical reports: should include introduction, case or technique description and discussion.
4. References: All publications cited in the text should be presented in the references part. The order of references should follow the order cited in the text. Examples of the reference style of this journal are as follow:
   A. Journal reference style: List all authors if the number is six or less. If author number is more than six, list six authors and add et al for the remaining authors.
      (1) Journal article: Lin YT, Chang LC. Space changes after premature loss of the mandibular primary first molar: a longitudinal study. J Clin Pediatr Dent 1998; 22: 311-6.
      (2) Online journal article: Yavuz MS, Aras MH, Büyükkurt MC, Tozoglu S. Impacted mandibular canines. J Contemp Dent Pract 2007; 8: 78-85. Available at: http://www.thejedp.com/issue036/index.htm. Accessed November 20, 2007.
   B. Book reference style:
      (1) Complete book: McDonald RE, Avery DR. Dentistry for child and adolescent. 5th ed, Mosby Co, St Louis, 1988; pp339-41.
      (2) Chapter in book: Moore BK, Avery DR. Dental materials. In: McDonald RE, Avery DR (ed). Dentistry for child and adolescent. 6th ed., Mosby Co., St. Louis, 1994; pp349-72.
5. Tables and figures: should be kept in minimum number. Figures should be in JPG, EPS or TIF file and can be e-mailed to the editor. They should be clear enough and listed in separated pages. Scale marker should be provided for magnification. All abbreviations shown on the table and figure should be explained in the legend.

   Photos of patients should be obscured for identification. Otherwise a patient's consent form should be provided.
6. Further information can be found at http://www.prosthod.org.tw/

中華民國贋復牙科學會 學術期刊

Journal of Prosthodontics and Implantology

投 稿 須 知

一、 本期刊為季刊，每年四、七、十及一月出刊，以英文刊出。

二、 凡與基礎或臨床補綴學、顳顎關節或咀嚼功能、人工植體相關、牙科補綴之技工學有關之著作，均為刊載之對象。接受的稿件類型共有下列五種，來稿請註明類型：

1. 社論(editorial)
2. 學術綜論(review article，限邀稿)
3. 研究論文(original artic1e)
4. 病例報告(case report)
5. 技術報告(technical report)

三、 稿件撰寫一般格式:

1. 稿件必須是沒有刊出或將被接受刊於其他雜誌者。
2. 以英文投稿，文章內容以複行 (double spacing) 繕打，字體大小12以上，其每邊邊緣至少須留2.5公分，不須任何格式編排。
3. 全文總頁數 (包括摘要、圖、表、參考文獻) 以八頁為限，必要時可增至十二頁。
4. 請以電子文件投稿，文書處理程式建議以IBM-Microsoft Word為主。須標示使用程式、投稿題目、第一作者姓名，將電子文件e-mail至: prosthor@ms48.hinet.net。
5. 請同時附上致本誌主編簡短信函，表明投稿本誌之意願，並說明所有著者均曾過目並簽名同意。若為人體試驗須附人體試驗委員會之同意函。

四、 稿件內容詳細規格如下:

1. 首頁 (title page)：包括題目、作者姓名、服務單位、學歷；英文簡題(running title)長度在四十個字以內；稿件主要負責者(corresponding author)之英文姓名與地址、電話、傳真、e-mail。
2. 摘要 (abstract)：以400字為限，簡潔說明並標示研究目的、方法、結果、主要結論或新發現。若為原始著作則須說明並標示，並需附關鍵詞(key words) 5個以內。
3. 本文 (text)：
   A. 學術綜論(review article)—無一定格式。
   B. 原始著作(original article)—分前言、材料與方法、結果、討論、結論。
      (1) 前言 (introduction)：簡要說明研究由來。
      (2) 材料與方法 (material and methods)：敘述研究設計、對象、步驟。
      (3) 結果 (results)：研究結果以文字、表格或插圖表示之。
      (4) 討論 (discussion)：強調重要結果與論點，與前人論述作比較等。
      (5) 結論 (conclusion)：結論要簡要明確。
   C. 技術報告(technical report)—分前言、方法描述、討論。
   D. 病例報告(case report)—分前言、病例描述、討論。

4. 參考文獻 (references)：以出現於本文中之先後順序用阿拉伯數字(上標)排列之，書寫方式請參考Cumulated Index Medicus，內容含作者姓名(全部列出)、篇名、期刊名、年代、頁數。例如：(1)期刊之書寫：Lin YT, Chang LC. Space changes after premature loss of the mandibular primary first molar: a longitudinal study. J Clin Pediatr Dent 1998; 22: 311-6. (2)書籍之書寫：McDonald RE, Avery DR. Dentistry for child and adolescent. 6th ed., Mosby Co, St Louis, 1994; pp339-41. (3)有編輯者之書籍章節書寫：Moore BK, Avery DR. Dental materials. In: McDonald RE, Avery DR. Dentistry for child and adolescent. 6th ed., Mosby Co., St. Louis, 1994; pp349-72. (4)電子期刊之書寫：Yavuz MS, Aras MH, üyükkurt MC, Tozoglu S. Impacted mandibular canines. J Contemp Dent Pract 2007; 8:78-85. Available at: http://www.thejedp.com/issue036/index.htm. Accessed November 20, 2007.

5. 插圖與表格 (figures and tables)：
   (1) 插圖請勿放置於本文中，圖與表之數量盡量少，也不要編排，應儲存於另外的檔案夾。影像圖檔應以JPG、EPS或TIF 形式存檔。插圖以電子檔e-mail傳送投稿。
   (2) 插圖之標題及詳細說明，須另頁複行繕打。顯微照像須以比例尺(internal scale marker)標明放大倍數。
   (3) 病人臉部照片須遮蓋眼睛至無法辨認是何人的程度，否則須附病人之書面同意書。
   (4) 繪圖軟體應使用如Photoshop、Photoimpact、Illustrator等。彩色或灰階圖形須掃瞄至300 DPI，線條圖形則須至1200 DPI，並請標明圖檔名稱及所使用軟硬體名稱。
   (5) 圖或表中出現之字母或符號，均需於註解中詳細解釋。

五、 投稿清單

(1) 致主編簡短信函。
(2) 提供稿件主要負責者之姓名與地址(英文)、電話、傳真、e-mail、所有作者之服務機構(英文)。
(3) 附英文摘要(400字以內)，研究論文的摘要應分研究目的、方法、結果、主要結論。
(4) 附英文關鍵詞(5個以內)；附英文簡題(長度在40個字以內)。
(5) 確認所有參考文獻的格式、內容、引用順序皆完整無誤。
(6) 確認所有表格(標題、註腳)及插圖之標題及詳細說明，另紙複行繕打。
(7) 確認所有圖表皆符合格式。圖表皆儲存於另外的檔案夾，而未放置於本文中。
(8) 若為人體試驗須附人體試驗委員會之同意函。
(9) 全部作者同意簽名之證明函。

六、 稿件一經刊載，版權屬本誌所有；本誌將免費印贈第一作者抽印本20份。

TABLE OF CONTENTS

ISSN 2304-541-8
9 772304 541008