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# Journal of Contemporary Dental Sciences

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July, 2013

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## **Journal of Contemporary Dental Sciences (JCDS)**

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3. Sayeed MA, Hussain MZ, Banu A, Rumi MAK, Azad AK. Prevalence of diabetes in a suburban population of Bangladesh Diab Res Clin Prac 1997; 34: 149-155
4. Jarett RJ. Insulin and hypertension (Letter). Lancet 1987; 2: 748- 749
5. Banerji MA, Faridi N, Atulri R, Chiken RI, Lebovitz HE. Body composition, visceral fat, leptin and insulin resistance in Asian Indian men. J Clin Endocrinol Metab 1999; 84: 137-144 (Abstract)
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## Editorial

It is our utmost pleasure that we could deliver the 2nd issue of Journal of Contemporary Dental Sciences (JCDS) to the readers, albeit late. Teachers-examination intricate bond was a major factor in this unintentional delay. As teachers are the main contributors in this category of publications, their

involvement with examination is also inseparable.

As the 1st issue of JCDS was cordially accepted by the honorable readership, so we felt encouraged to bring out the 2nd issue as early as possible, without compromising its quality. This issue is enriched by contributions from researchers both from home and abroad as usual. First of all I would like to introduce an interesting review article that envisions future manufacture technology using "3-D Printing or Additive manufacturing" which is defined as a process of making a three-dimensional (3-D) object of virtually any shape from a digital model (data). 3-D printing is achieved using an additive process, where successive layers of material are laid down in different shapes. 3-D printing is also considered distinct from traditional 'machining' technique which mostly rely on the removal of material by methods such as cutting, drilling (subtractive processes). 3-D printing is new to most of us. The authors raised questions regarding the fate of conventional Dental Technicians of Japan who rely on manual pattern making and converting it to a restoration by casting, using lost-wax-technique. The most pertinent question that demands immediate attention is where do we (Bangladesh) stand now in this transition period?

One article evaluates the effects of sprue design on the castability of commercially pure Titanium (cpTi). While sprue design had no statistically significant influence on the castings, shape of the pattern had, and v-shaped patterns showed unpredictable result. One original article studied the acellular cementum formation in the mesial root of rodent molar tooth. Yet another original article studied detection of Hepatitis B Virus DNA in seronegative patients by polymerase chain reaction (PCR). Apart from these, interesting articles regarding the pre-surgical assessment of impacted lower third molar teeth and a case report describes how the impacted both permanent central incisors was brought to functional occlusion.

Prof. MU Chowdhury BDS, DDS, PhD  
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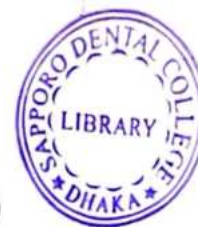
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## The effects of sprue design on castability of commercially pure titanium in different pattern shapes and sizes

KM Shahidullah,<sup>1</sup> PS Won<sup>2</sup>

### Abstract

**Purpose:** This study evaluated the effects of curved and straight sprue on castability of commercially pure titanium (CP-Ti) different pattern shapes and sizes. **Materials and Methods:** Curved and Straight sprues were made in this experiment with equal length (24mm) and diameter (5mm). Curved sprues were fabricated by using index to maintain same curvature. Two sizes of pattern were used. Each size consisted of four patterns and these were Rectangular; Semicircular; Horse shoe and 'V'-shaped. Small patterns were of same length (61mm) and height (17mm) consisting of 138 squares. Large patterns were of same length (61mm) and height (24mm) consisting of 207 squares. All patterns were fabricated by using commercially available wax mesh. Twenty (20) mesh patterns were for each sprue design was fabricated. The castings were performed in Ti-cast super R, casting machine. Casting completeness of each specimen was determined by visual examination. Statistical analysis was performed by using ANOVA and Kruskal Wallis test to identify statistically significant difference. **Result:** Castability of CP-Ti was determined by visual examination. Curved and straight sprues showed almost similar castability in both the small and large patterns with no statistically significant difference. V-shaped pattern yielded worst casting among rectangular, semicircular and h-shoe shaped patterns in both size of specimens. Although, One-way ANOVA and Kruskal Wallis test revealed no statistically significant difference among the pattern shapes. **Conclusion:** There was no statistical significant difference between straight and curved sprue in all the pattern shapes. But the rectangular, semicircular and horse-shoe shaped patterns showed the predictable castability and V-shaped pattern showed unpredictable results and produced worst castings regardless of sprue design and pattern size in this experiment.

**Key Words:** CP-Ti; castability; sprue design; pattern shape

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### Introduction

Commercially pure titanium (CP-Ti) could be important for making removable and fixed dental prostheses due to its good mechanical properties, high corrosion resistance, light weight and outstanding biocompatibility. However, several problems associated with poor titanium castability prevent it from being widely used. The problems in titanium casting are poor castability, high melting point, highly reactive with investment material; and vast difference between molten titanium and mold temperature. Inadequate mold filling and porosity are well-known defects often observed during pure titanium casting. The main factors leading to these defects are the casting force exerted on the molten metal; temperature of the melt and mold; the permeability of the investment and the spruing configuration.<sup>1</sup> One of the requisites for casting success is adequate spruing. The flow of molten titanium in molds of complicated shape is far from being completely understood; consequently, definite spruing criteria are not yet available for titanium removable partial denture (RPD) framework.<sup>2</sup>

The property, Castability is generally described as the ability of molten metal or alloy to flow through and fill a mold under given condition.<sup>3</sup> The prime requirement of a dental casting metal or alloy is a superior castability to fill a mold completely and no porosities will be there. The problem of casting pure titanium can be counteracted by higher rotational speed with centrifugal casting machine or by using special sprue designs. Theoretically, the curved bar will minimize turbulence and allow for unimpeded metal flow and reduced casting impact forces on the investment, yielding more complete and smoother castings.<sup>4</sup>

The results of previous studies influenced the specific sprue design used in this study to resolve the mold filling problem.<sup>5</sup> Curved individual sprues were used in this experiment with the conventional straight direct sprues. Some studies stated that a curved sprue design produced significantly less porosity in the circumferential clasp arms of a cast titanium removable partial denture than the conventional straight design.<sup>2,3</sup> Tsong-I. et al and Robert S. et al.<sup>6</sup> also stated that a modified sprue design is imperative to ensure proper mold filling with titanium.

To investigate the problem of CP-Ti castability, sieve pattern, fluidity spirals, and vacuum fluidity methods are often used to test how well a metal fills cavities. Majority of the above castability tests found that many dental alloys filled the molds well if appropriate

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casting conditions were followed. In order to make distinctions the mold filling property between the sprue designs tested, it is necessary to make a mold in such a way that the molten metal / alloy cannot easily fill its total volume. Studies mentioning<sup>7,8</sup> Vaidyanathan T. et al. and Penugonda B. et al compared two castability monitors and stated that measurement of castability was more easily accomplished with mesh grid. More recently, Watanabe et al. evaluated the castability of CP-Ti and traditional dental alloys using a mesh pattern (rounded grids) and also a perforated disc pattern.<sup>3</sup>

Porosities may easily develop during casting process if the spruing, pattern thickness and amounts of melting metal are inadequate. Although overall porosity is a valuable indicator of casting quality, size, shape and thickness of various parts of the framework may be more useful. Cast titanium clasps may also break because of internal porosity. Thus, many casting factors have been investigated in an effort to decrease the porosity. A curved sprue design produced significantly less porosity in the circumferential clasp arms of a cast titanium removable partial denture than the conventional straight design.<sup>9</sup>

Porosity in meshwork may not restrict clinical use of denture, and small and isolated pores may not be important in major connectors. But even a small pore can expose clasp arms and occlusal rests to a high risk of fracture in RPD framework. Therefore, to overcome inherent casting problem, two sprue designs were

studied such as curved and straight designs with three specifically designed mesh patterns i.e. semicircular, horse- shoe shaped and 'V'-shaped and one common rectangular shaped were used to prepare mold for investigating castability of pure titanium. These pattern shapes have been selected in order to simulate the complex patterns which are often designed in dental laboratories except the rectangular. These molds were also effective at differentiating the influence of two sprues on titanium flow behavior investigated. The objective of this study was to evaluate the ability of curved and straight sprue to produce complete and porosity less casting made of CP-Ti in the different patterns.

### Materials and Methods

Curved and straight sprue designs were used to investigate the completeness and porosity in commercially pure titanium casting. Curved sprues were fabricated by using commercial sprue wax (Roll-wax, Dae-dong industry, Korea). A custom made index was used to fabricate the curved sprue with a same curvature. To evaluate castability, specifically patterns were fabricated with commercial wax-mesh (Grid RN II, Dentaaurum, Germany). All patterns were fabricated on three custom made indices and the indices were made by using dental stone (Neo model plaster, Pumstone; Mutsumi chemical industries co. ltd, Japan) to maintain same curvature. Five samples for each pattern design were used in the experimental group. Total 80 specimens were cast for investigating the effect of curved and straight sprue on castability of CP-Ti.

**Table1 : Shows the distribution of specimens in the experimental groups.**

Experimental groups		Pattern design				CODE
Sprue design	Straight	Small	No.	Large	No.	
		1.Rectangular	5	1.Rectangular	5	SR
		2. Semicircular	5	2. Semicircular	5	SS
		3. Horse-shoe	5	3. Horse-shoe	5	SH
		4. V-shape	5	4. V-shape	5	SV
		Samples	20	Samples	20	
	Curved	1.Rectangular	5	1.Rectangular	5	CR
		2. Semicircular	5	2. Semicircular	5	CS
		3. Horse-shoe	5	3. Horse-shoe	5	CH
		4. V-shape	5	4. V-shape	5	CV
		Samples	20	Samples	20	
	Total		40	Total		40



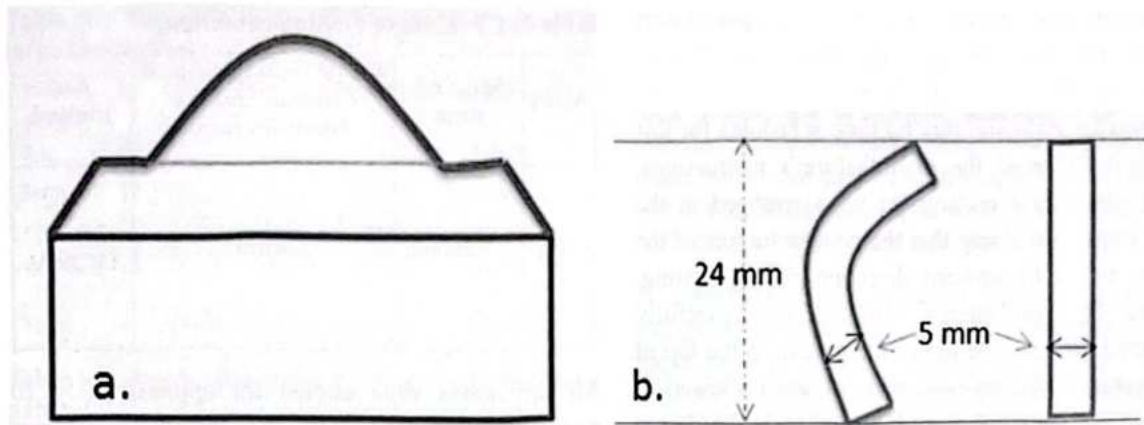


Fig.1 Shows schematic representation of a. sprue former ; b. curved and straight sprue.

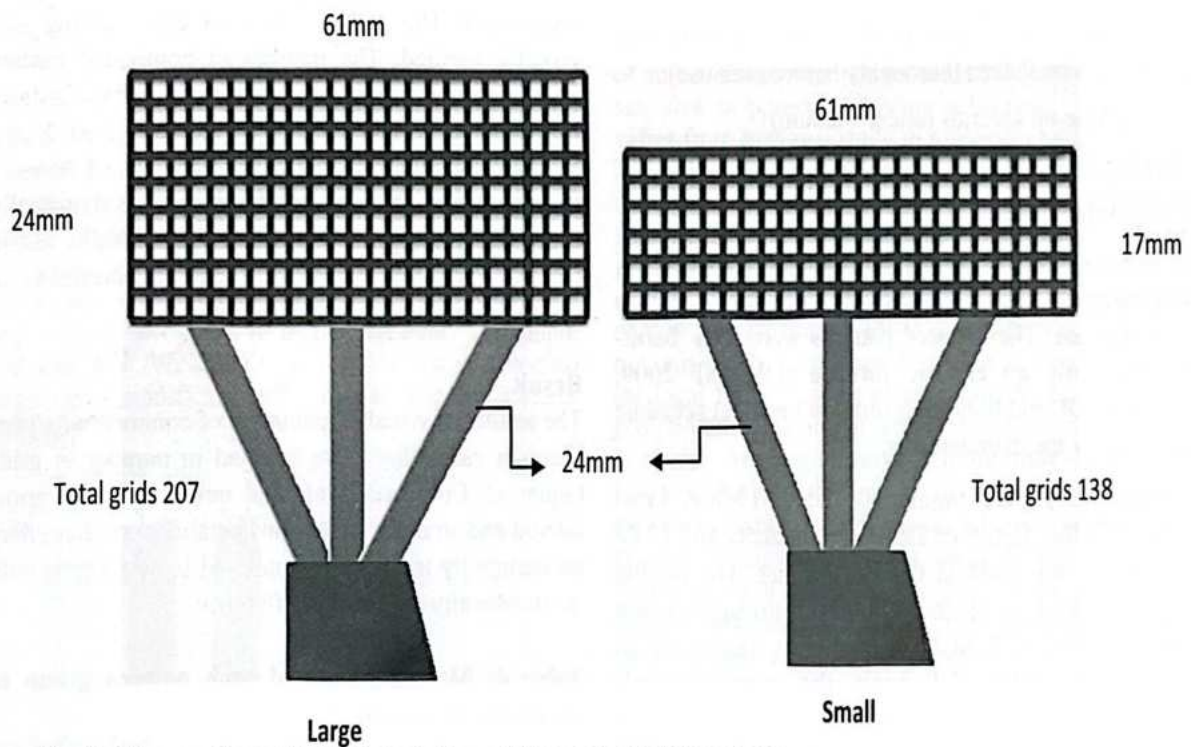


Fig. 2 Shows schematic representation of the rectangular pattern.

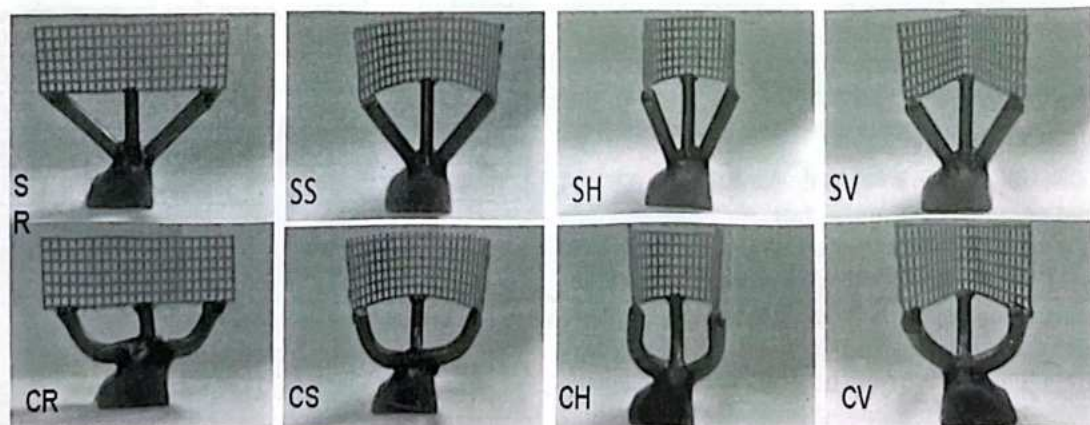


Fig. 3 Wax patterns of the specimens were used in the experiment.

The pattern was invested with precision investment material for titanium casting (Rematitan Plus®, Dentaurem, Germany) at room temperature. The liquid/powder ratio and mixing time schedules for this investment followed the manufacturer's instructions. All patterns except rectangular were arranged in the casting rings such a way that the convex surface of the patterns would be upward direction during casting. Four to five millimeter distance was carefully maintained between the top of the pattern to the top of the investment. All invested patterns were allowed to bench set for 1 hour at room temperature before being placed in a cool furnace.

#### **Investing condition:**

Mixing ratio-250mg powder: 40 ml liquid (the mixing liquid must be shaken thoroughly before each use).

Mixing time-60 seconds (under vacuum)

Working time-3 to 4 minutes (approximately)

Setting time-1 hour.

All invested patterns were allowed to bench set at room temperature before being placed in a cool (25°C to 33°C) furnace. The invested patterns were then burnt-out by using an electric furnace (OVMAT 2009, MANFREDI, and Italy) according to burn-out schedule provided by the manufacturer.

Commercially pure titanium, grade-2 (Selec, Titan ingot, JS2 40g, Japan) of 30.10 mm diameter and 12.85 mm height was used in this experiment. The casting was performed in a high rotational centrifugal casting machine (TICAST SUPER R, Selec, Japan) in an argon atmosphere following the manufacturer's instructions at the temperature of 430°C (mold temperature). Castings for total 80 specimens were done in random order.

#### **Casting condition:**

-Mold temp. 430°C

-Melting temp. 1668°C

-An argon atmosphere

-An electric high rotational centrifugal casting machine.

**Table 3: CP-Ti ingot melting condition.**

Alloy	Melting time	Space between tip of cathode and the titanium ingot	Arc melted
40g	58 sec	3mm	250A, DC45V

All the molds were cooled for approximately 20 minutes at room temperature after casting. The castings were divested manually and sandblasted lightly with aluminum oxide (50µm) abrasives to remove residual investment. The completeness of each casting was visually verified. The number of completed casting grids counted and tabulated with verification. Castings were also examined radio graphically (Easyti X-ray, MANFREDI, Italy) to detect the presence of porosity in the castings. Data of completeness were statistically analyzed by using ANOVA and Kruskal Wallis test to identify statistically significant differences in castability between two sprue design.

#### **Result**

The results of visual examination of commercially pure titanium castability were counted in number of grids (squares). Comparison of total number of cast grids, curved and straight sprues showed almost similar effect on castability in both the small and large patterns with no statistically significant difference.

**Table 4: Mean and SD of each pattern group in small size specimen.**

Pattern design	N	Mean	SD	Minimum	Maximum
Rect.	10	135.9	4.15	125.00	138.00
Semi.	10	136.1	2.23	132.00	138.00
H-shoe	10	134.9	4.28	124.00	138.00
V-shape	10	131.2	8.17	115.00	138.00
	40	134.52	5.36	115.00	138.00

**Table 2: Shows the burn-out schedule for all molds.**

Heating speed		6°C/minute	6°C/minute	6°C/minute	Casting temperature
Temperature	25°-33°C	150°C	250°C	1000°C	430°C
Holding time	4 minutes	90 minutes	90 minutes	90 minutes	3 hours



The pattern was invested with precision investment material for titanium casting (Rematitan Plus®, Dentaaurum, Germany) at room temperature. The liquid/powder ratio and mixing time schedules for this investment followed the manufacturer's instructions. All patterns except rectangular were arranged in the casting rings such a way that the convex surface of the patterns would be upward direction during casting. Four to five millimeter distance was carefully maintained between the top of the pattern to the top of the investment. All invested patterns were allowed to bench set for 1 hour at room temperature before being placed in a cool furnace.

#### **Investing condition:**

Mixing ratio-250mg powder: 40 ml liquid (the mixing liquid must be shaken thoroughly before each use).

Mixing time-60 seconds (under vacuum)

Working time-3 to 4 minutes (approximately)

Setting time-1 hour.

All invested patterns were allowed to bench set at room temperature before being placed in a cool (25°C to 33°C) furnace. The invested patterns were then burnt-out by using an electric furnace (OVMAT 2009, MANFREDI, and Italy) according to burn-out schedule provided by the manufacturer.

Commercially pure titanium, grade-2 (Selec, Titan ingot, JS2 40g, Japan) of 30.10 mm diameter and 12.85 mm height was used in this experiment. The casting was performed in a high rotational centrifugal casting machine (TICAST SUPER R, Selec, Japan) in an argon atmosphere following the manufacturer's instructions at the temperature of 430°C (mold temperature). Castings for total 80 specimens were done in random order.

#### **Casting condition:**

-Mold temp. 430°C

-Melting temp. 1668°C

-An argon atmosphere

-An electric high rotational centrifugal casting machine.

**Table 3: CP-Ti ingot melting condition.**

Alloy	Melting time	Space between tip of cathode and the titanium ingot	Arc melted
40g	58 sec	3mm	250A, DC45V

All the molds were cooled for approximately 20 minutes at room temperature after casting. The castings were divested manually and sandblasted lightly with aluminum oxide (50µm) abrasives to remove residual investment. The completeness of each casting was visually verified. The number of completed casting grids counted and tabulated with verification. Castings were also examined radio graphically (Easyti X-ray, MANFREDI, Italy) to detect the presence of porosity in the castings. Data of completeness were statistically analyzed by using ANOVA and Kruskal Wallis test to identify statistically significant differences in castability between two sprue design.

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V-shape	10	131.2	8.17	115.00	138.00
	40	134.52	5.36	115.00	138.00

**Table 2: Shows the burn-out schedule for all molds.**

Heating speed		6°C/minute	6°C/minute	6°C/minute	Casting temperature
Temperature	25°-33°C	150°C	250°C	1000°C	430°C
Holding time	4 minutes	90 minutes	90 minutes	90 minutes	3 hours



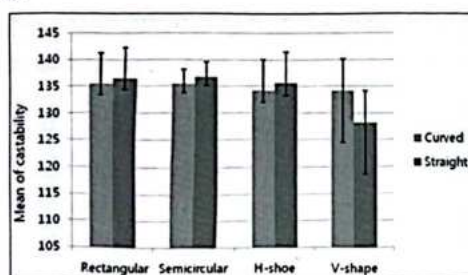
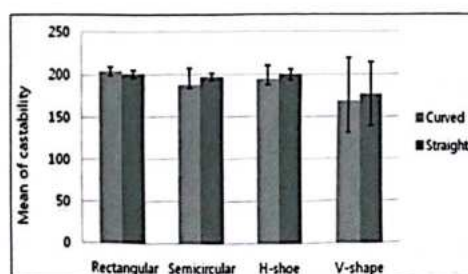
**Table 5 : Mean and SD of each pattern group in large size pattern**

Pattern design	N	Mean	SD	Minimum	Maximum
Rec.	10	202.4	5.10	194.00	207.00
Semi.	10	192.2	13.85	165.00	207.00
H-shoe	10	197.0	11.86	172.00	207.00
V- shape	10	172.5	42.33	107.00	207.00
Total	40	191.0	25.04	107.00	207.00

**Table 6 : Mean and SD of each sprue in the large pattern.**

Sprue design	N	Mean	SD	Minimum	Maximum
Curved	20	188.4	29.39	107.00	207.00
Straight	20	193.6	20.22	127.00	207.00
Total	40	191.0	25.04	107.00	207.0

The semicircular pattern showed the maximum castability and v-shaped pattern showed the minimum in small size specimen. On the otherhand, rectangular pattern exhibited maximum castability while v-shaped pattern demonstrated minimum in the large size specimen. V-shaped pattern yielded worst casting than rectangular, semicircular and h-shoe shaped patterns in both size of specimens in this experiment. Although, One-way ANOVA and Kruskal Wallis test revealed no statistically significant difference among the pattern shapes.

**Fig.4** Bar chart represents the castability mean of each casting pattern group in curved and straight sprue for small size pattern. The vertical T-bars indicate the standard deviation of each mean.**Fig.5** Bar chart represents the castability mean of each casting pattern group in curved and straight sprue for large size pattern. The vertical T-bars indicate the standard deviation of each mean.

## Discussion

The principal considerations in sprue design relate to their number and dimension, attachment site and the intended direction of metal flow. Criteria in relation to these factors can only be appreciated and adhered to if there is an understanding as to how they relate to the production of satisfactory castings.<sup>10</sup>

To investigate CP-Ti castability problem, curved and straight sprue designs were tested in the present study. Sieve pattern, fluidity spirals, and vacuum fluidity methods are often used to test how well a metal fills cavities. Therefore, this experiment evaluated the effect of curved and straight sprue on the castability of CP-Ti by using specifically made wax-mesh patterns (square grids), i.e. rectangular, semicircular, v-shaped and common rectangular patterns. However, the influence of sprue design was not so evident because it is very sensitive to particular casting geometry.<sup>11</sup> This study varies from previous study in respect to patterns shape. The sample's patterns were intentionally designed to distinguish the mold filling capability of curved and straight sprue in CP-Ti casting. These pattern's shapes might have slightly affected titanium flow due to change of direction of molten titanium within the thin narrow mold cavities. Flow direction was controlled not only by the centrifugal force but also by changes in the local fluidity in the channels of the cavity.<sup>3</sup> Sprue position and attachment design were kept similar in all samples. Although, curved sprue may change its position without formation of an acute angle and provides adequate metal supply where necessary. It is possible to attach the Curve sprue to the clasp arm/connector at 0° angulations. The lowest porosity in clasp arms was obtained with the 0° sprue direction for any of the investigated sprue diameters.<sup>2</sup>

The straight and flared sprue attachment was used in fabrication of the samples. All samples were carefully made with a target to produce smooth surface of the mold cavity. Therefore, it is presumed that molten CP-Ti may flow throughout the mold cavity with minimizing turbulent flow. Sprue attachment geometry may produce different castability and porosity effects for lower density non-noble alloy.<sup>8</sup>

In general, the prime criteria of a sprue are short and direct as possible. But when a complex pattern has been cast i.e. RPD frameworks consisting of thin clasps, rests, minor and major connector needs special attention on spruing method. Because, the molten titanium has to fill thin parts of the pattern, and the function of the sprue is to create a path through which molten metal can reach the entire mold cavity to ensure



its proper filling. In those cases, curved sprue may provide adequate metal supply avoiding acute angles instead of straight direct sprue. In the present study, large multiple sprues have been used with a view yielding voids free complete castings. Large and multiple sprues were found to reduce the porosity of titanium crowns and fixed partial dentures.<sup>2,4,6</sup> It is assumed that a longer sprue with an increased diameter acts as reservoir and reduces the porosity during titanium casting. Although, there were no porosities found at the sprue attachment site of the samples in the present study. For traditional alloys, the solidification starts after complete filling of the mold; for titanium, it occurs during mold filling. Consequently, later titanium inflows compensate for the shrinkage of earlier inflows.<sup>9</sup> Successful titanium casting requires a sprue diameter (6mm) greater than that commonly used with cobalt chromium dentures (4mm).<sup>12</sup> The curved and straight sprue used in this study exhibited roughly similar effect on castability of CP-Ti. Even though, there was no statistically significant difference between curved and straight sprue in respect of total number of grids.

The position of the samples during casting may need particular consideration which will be effectual for getting smoother and completeness of the CP-Ti casting. All samples pattern except rectangular were arranged in the casting rings such a way that the convex surface of all samples would be directed upward during casting. The surface of the castings were convex everywhere. These facts indicate that the flow was laminar and that sufficient pressure was maintained in the fluid until the moment of solidification. Results of visual examination indicate that long curved and straight sprues produced predictable castings of specifically design patterns used in this experiment except V-shaped pattern.

Eventually, V-shaped pattern demonstrated the worst castability in respect to rectangular, semicircular and horse-shoe shaped pattern in both the small and large pattern samples. Due to nature of V-shaped pattern, it can be difficult to cast. Because it contains an area where the casting solidification is slower. The metal around the hot spots, which will undergo solidification first, will cut off the hot spots from the flow of the molten metal.<sup>13-14</sup>

The mold temperature is one of the factors that can be considered a limitation of this experiment. Time requires approximately 3 minutes in transferring the

mold from burn out furnace to casting machine and casting completion. Therefore, it is very difficult to determine the real temperature of the mold at the moment of casting.<sup>15</sup> The aim of these sprue designs was to provide a simple sprue which will be versatile to be effective with a variety of pattern design and reduce time for laboratory procedure.

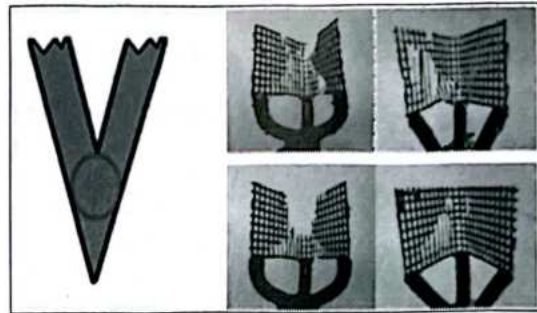


Figure 6: V shaped pattern

### Conclusion

Within the limitations of this study, the results indicate that the effect of curved and straight sprue on castability of CP-Ti in both the small and large patterns was similar.

There was no statistical significant difference between straight and curved sprue in all the pattern shapes. However, the rectangular, semicircular and horse-shoe shaped patterns showed the predictable castability and V-shaped pattern showed unpredictable results. V-shaped pattern produced the worst castings regardless of sprue design and pattern size in this experiment, although, statistical analysis found no statistical significant difference among the pattern shapes.

The result also showed acceptable sound density in all titanium casting specimens.

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## Mineralization process of acellular cementum in mesial root of rat molar

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### Abstract

**Purpose:** This study was designed to understand the mineralization process during acellular cementogenesis and also to detect the presence of bone sialoprotein (BSP) and osteopontin (OPN) by immunoperoxidase staining in developing rat molars. **Methods:** 21-day-old Wistar rats were examined, routine histological staining (hematoxylin and eosin), immunohistochemical staining for bone sialoprotein (BSP), osteopontin (OPN), were conducted. **Results:** In the established cementum, the cemento-dentinal junction appeared as an intensely hematoxylin-stained, fibril-poor layer, which was immunoreactive for BSP and OPN, with the onset of dentin mineralization. **Conclusion:** During the initial acellular cementogenesis, mineralization advances along collagen fibrils after principal fiber attachment subsequently, cemento-dentinal junction appears which is rich in non-collagenous proteins.

**Key words :** Acellular cementum; Mineralization; bone sialoprotein (BSP), osteopontin(OPN)

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### Introduction

A tooth consists of three layers of mineralized tissue. Among the mineralized structures (Enamel, Dentin, Cementum) of the tooth our concerning structure is cementum (acellular variety) and its formation. Cementum is a hard, avascular tissue, which covers the root surface and plays an important role for the tooth support in cooperation with the principal fibers and the alveolar bone proper. Cementum can be classified<sup>1</sup> on the basis of time of formation, on the basis of presence or absence of cells within its matrix and on the basis of distribution of extrinsic and intrinsic fibers. The cementum consists of organic component, inorganic component and cell. Organic components are the collagen fibers. Inorganic components are the interfibrillar matrices. As cell cementum contains cementoblast.<sup>1</sup>

Regarding noncollagenous interfibrillar matrices of the cementum, recent histological studies have focused on the immunolocalization of bone sialoprotein (BSP), osteopontin (OPN).<sup>2-7</sup> BSP and OPN are noncollagenous phosphorylated glycoproteins with

with Arg-Gly-Asp domains and are closely associated with mineralization, cell differentiation, matrix matrix attachment, and cell to cell/matrix attachment in collagen based hard tissue.<sup>8,9</sup>

The cemento-dentinal junction, the interface between cementum and dentin, stains intensely with hematoxylin in many species. For rat molars, it is generally accepted that the cemento-dentinal junction is fibril-poor and contains more highly accumulated BSP and OPN than other parts of cementum.<sup>10-16</sup>

The study here used histochemistry H/E, and immunohistochemistry (BSP and OPN) in developing rat molars, to explore the mineralization process during acellular cementogenesis. The knowledge of normal cementogenesis would be helpful in understanding other types of cementogenesis seen during clinical dental treatment.

### Materials and methods

Twenty 21-day-old male Wistar rats weighing about 50 gm were used in the study. The animals and tissue specimens were treated in accordance with the Guidelines of the Experimental Animal Committee, Hokkaido University Graduate School of Dental Medicine.

After anesthesia by an intraperitoneal injection of sodium pentobarbital, the animals were perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 15 minutes at room temperature. The molars together with the surrounding alveolar bone and covering tissues were dissected out and immersed in the same fixative overnight. Specimens were then

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demineralized in ethanolic trimethylammonium EDTA for 7 weeks at room temperature.<sup>17</sup> Then the specimens were dehydrated in a graded series of ethanols. Before immersion in 90% ethanol the mesial root of the molars were cut and embedded in paraffin. Next, 7 $\mu$ m thick serial longitudinal sections of the mesial root of the molars were cut for general histology and immunohistochemistry.

### General histology

#### Histochemistry

Deparaffinized sections were stained with hematoxylin and eosin. Some of them were used for immunohistochemistry as described below

#### Immunohistochemistry

For immunodetection of BSP and OPN, anti-human BSP rabbit polyclonal antibody (Alexis) and anti-mouse OPN rabbit polyclonal antibody (LSL) were used as primary antibodies. The authenticity of these antibodies to rats has been confirmed.<sup>18</sup> After inhibition of endogenous peroxidase, deparaffinized sections were pretreated with 2.5% testicular hyaluronidase (Sigma) at 37°C. They were then incubated with the primary antibodies, biotinylated anti-rabbit swine polyclonal antibody (DAKO), and streptavidin-biotin-horseradish peroxidase complex. Immunostaining was visualized as described above. Normal rabbit serum was substituted for the primary antibodies in the negative controls.

### Results

The mesial root of the maxillary first molars of 3-week-old rats was examined in this study (Figure 1a and 1b). The mesial root displays the full range of stages of acellular cementogenesis.

#### General histology

At the apical end, Hertwig's epithelial root sheath consisted of two or three cell layers (Figure 1c). In the cervical direction, the epithelial sheath cells became smaller and darker and the epithelial sheath disintegrated. Dental papilla cells facing the epithelial sheath differentiated into columnar odontoblasts and formed predentin (Figure 1c). Dental follicle cells approached the exposed predentin and formed dense cell population. With the onset of dentin mineralization, a hematoxylin stained layer appeared as the initial cementum on the mineralized dentin (Figure 1d). At this point, dental follicle cells were at a short

distance from the root surface, and on the basis of the location and cell shape these cells could be identified as cementoblasts.

More cervically, the cementum increased in thickness, but in the mid-root region the cemento-dentinal junction was still indiscernible (Figure 1e). Where the cementum had developed to a maximum thickness of about 3 mm in the cervical region, the cemento-dentinal junction became discernible as the most intensely hematoxylin-stained line in the cementum (Figure 1f). The periodontal ligament cells including cementoblasts became elongated and arranged in regular direction.

#### Immunohistochemistry

Intense immunoreactivity for BSP (Figure 2a) and OPN (Figure 2b) was detected in the acellular cementum. Other tissues, viz., dental follicle, epithelial sheath, dentin, periodontal ligament, and dental pulp stained weakly or did not stain. The initial cementum started to form with the onset of dentin mineralization (Figure 2c), and as the cementum increased in thickness, the cemento-dentinal junction appeared as the most intensely stained line in the cementum (Figure 2d).

### Discussion

The mineralization process of the rat acellular cementum proceeds as follows. The initial cementum is deficient in collagen fibrils.<sup>18</sup> Under this condition, successive mineralization spreading from the mineralized dentin may not occur, and the initial cementum is mineralized independent of collagen fibrils, and principal fibers induce the mineralization of the initial cementum. The initial cementum requires a dense accumulation of BSP and OPN for its mineralization, probably due to the lack of stable mineralization media (collagen fibrils). Once the mineralization extends to the principal fibers, the mineralization advances in the known fashion dependent on collagen fibrils.<sup>10-16</sup>

Earlier, we have also studied the structure and development of the acellular cementum in rat molars by light and electron microscopy<sup>10-16</sup> and found the following:



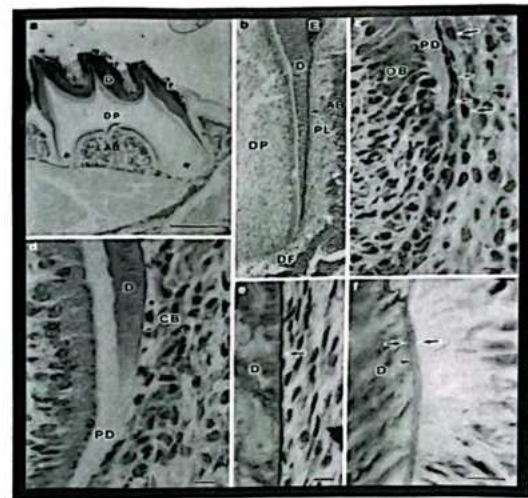
- (a) The cemento-dentinal junction is a fibril-poor layer (1–2  $\mu\text{m}$  thick) and contains more densely accumulated BSP and OPN than the successively formed acellular cementum with extrinsic fibers.
- (b) Developmentally, prior to the principal fiber attachment to the root surface, BSP and OPN are densely deposited as the initial cementum, which results in the fibril-poor cemento-dentinal junction
- (c) During the initial cementogenesis, matrix vesicles and calciferous spherules appear on the root surface. After the acellular cementum is established, neither of the matrix vesicles nor calciferous spherules are observed and the mineralization advances along the attached principal fibers.

In addition to the adhesive function, BSP and OPN are very important for mineralization as mineralization-initiating and mineralization-inhibiting factors, respectively<sup>11,12</sup>. The acellular cementum appeared to be fully mineralized in rat incisors. Although BSP and OPN may be involved in the mineralization through all stages of the acellular cementogenesis, the results suggest that they are particularly necessary in the earliest stage. The initial cementum is deficient in collagen fibrils, which generally act as mineralization media in collagen-based hard tissues. Under such conditions, successive mineralization from mineralized dentin to cementum may not occur. Therefore, the initial cementum may require a dense accumulation of BSP and OPN, to induce or initiate the mineralization under collagen fibril-deficient conditions<sup>8</sup>. This is supported by Bosshardt et al.<sup>19</sup> and Bosshardt and Nanci<sup>20</sup>, who immunodetected densely accumulated BSP and OPN in collagen-free, mineralized cementicle-like structures and acellular afibrillar cementum. After the principal fiber attachment, the mineralization may advance along the principal fibers.

## Conclusion

This study reveals that cemento-dentinal junction is a fibril-poor layer (1–2  $\mu\text{m}$  thick) and contains more densely accumulated BSP and OPN. Developmentally, prior to the principal fiber attachment to the root surface, BSP and OPN are densely deposited in the

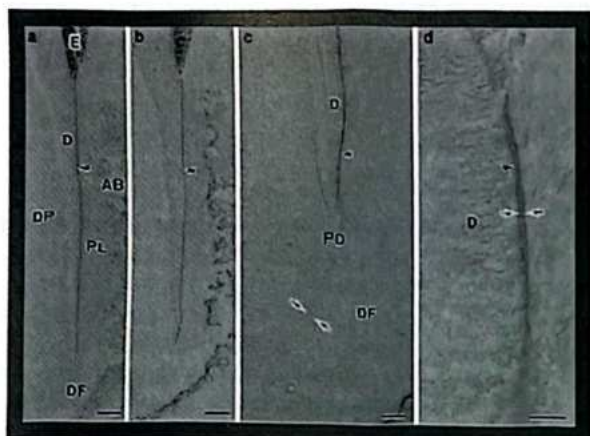
initial cementum, which results in the fibril-poor cemento-dentinal junction. The mineralization advances along the attached principal fibers.



**Figure 1.** General histology of the mesial root of the maxillary first molar of 3-week-old rats in hematoxylin-stained sections:

- a) Overall view of the maxillary first molar (AB, alveolar bone; D, dentin; DP, dental pulp; E, enamel). Apical foramen of mesial (right) and distal (left) roots are still widely open (asterisks). Bar 1  $\mu\text{m}$ .
- b) Overall view of the mesial root (AB, alveolar bone; D, dentin; DF, dental follicle; DP, dental pulp; E, enamel; PL, periodontal ligament). Thin acellular cementum (arrowhead) covers the root dentin. Bar 100  $\mu\text{m}$ .
- c) At the apical end Hertwig's epithelial root sheath (asterisk) consists of two or three cell layers. More cervically, columnar odontoblasts (OB) form predentin (PD). Where the epithelial sheath disintegrates, the epithelial cells (small arrows) become smaller and darker. Dental follicle cells (large arrows) approach the predentin surface. Bar 10  $\mu\text{m}$ .
- d) With the onset of dentin (D) mineralization, the hematoxylin-stained, initial cementum (arrowhead) appears on the mineralized dentin. PD predentin. Bar 10  $\mu\text{m}$ .
- e) In the mid-root region the cemento-dentinal junction is indiscernible in the cementum (arrow). D, dentin. Bar 10  $\mu\text{m}$ .
- f) In the most cervical region the cemento-dentinal junction (arrowhead) appears as the most intensely stained line in the cementum (between arrows). D, dentin. Bar 10  $\mu\text{m}$ .





**Figure 2:** Sections stained with anti-BSP (a, c, d) and anti-OPN (b) antibodies.

- (a & b) Overall view of mesial roots (AB, alveolar bone; D, root dentin; DF, dental follicle; DP, dental pulp; E, enamel; PL, periodontal ligament). The acellular cementum (arrowheads) stains intensely for BSP (a) and OPN (b). Bars 100  $\mu$ m.
- (c) With the onset of dentin (D) mineralization, the BSP- and OPN (not shown)-immunoreactive layer (arrowhead) appears as the initial cementum. The epithelial sheath is demarcated between arrows. DF, dental follicle; PD, predentin. Bar 20  $\mu$ m.
- (d) In the most cervical region the cemento-dentinal junction (arrowhead) shows the most intense immunoreactivity for BSP and OPN (not shown) in the cementum (between arrows). D, dentin. Bar 10  $\mu$ m

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## Effects of dairy products on plaque pH: an Experimental Study

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## Abstract

**Purpose:** Milk and dairy products have been associated with caries preventing effects. The inhibitory property of milk products with respect to dental caries has been attributed to the presence of minerals such as phosphate and calcium and peptides such as caseinophosphopeptides (CPP). An experimental study investigated the changes in plaque pH after rinsing with a control 10% sucrose solution and four different milk products supplied by the New Zealand Dairy Research Institute (NZDRI). The objective of the study was to compare the effects of control and experimental products on plaque pH. **Methods:** Nine volunteers, after abstaining from brushing their teeth, rinsed with either the control sucrose solution or a milk sample in five separate tests. On each occasion, a plaque sample was collected just before the rinse to determine the baseline pH of the plaque and then subsequent plaque samples were collected at 1, 3, 7, 11, 15, 19, 23 and 27 minutes post-rinse and the plaque pH at these times was measured. **Result:** The average change in plaque pH values due to the 10% sucrose solution was statistically significantly different ( $p < 0.0001$ ) from the change with any of the dairy products. With sucrose treatment, there was a drop in plaque pH, as expected, although this was not as large as that observed in some reports. With each dairy product, there was little drop in plaque pH, and a tendency for the pH to increase above baseline at some time intervals. The plaque pH values due to the dairy products generally ended higher than the starting values. However, although different trends were observed with each product these did not achieve statistical significance due to the large inter-individual variations between subjects. **Conclusion:** It was concluded that further testing of possible differences between such milk products would be better carried out in experiments in vitro where variables could be better controlled.

**Key Words:** Dental caries, Plaque pH, Caseinophosphopeptide (CPP), Dairy Products

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## Introduction

Caries is a multifactorial disease of tooth in which fermentable carbohydrate and cariogenic bacteria play major roles in lowering the pH of plaque and saliva.<sup>1</sup> The Classical Stephen Curve demonstrated cariogenic potential of dietary sucrose by initiating a sharp fall in plaque and salivary pH.<sup>2</sup> The lowered pH of oral milieu begins a cascade of chemical reactions resulting in decalcification and destruction of the tooth structure. A plaque pH below 6 produces an increased risk for demineralization of teeth. Studies have demonstrated a critical plaque pH of 5.5 at which minerals of teeth start to disintegrate.<sup>3,4</sup> Some studies on the contrary have associated consumption of milk and dairy products with anticariogenic activities.<sup>5-8</sup>

Dairy foods eaten alone, before or after fermentable carbohydrates or in combination with other foods have demonstrated protective effect against dental caries.<sup>8</sup> The beneficial effects milk and milk products include : buffering of bacterial acids, acceleration of the pH rise.

after eating, bacterial inhibition, decrease in demineralisation with casein phosphoproteins and/ or calcium phosphates, and low cariogenicity of lactose (milk sugar) compared to other sugars.<sup>6-9</sup> Milk and milk products are excellent sources for calcium and caseinophosphopeptide (CPP) and CPP has an ability to stabilize calcium phosphate in solution and can substantially increase the level of calcium phosphate in dental plaque decreasing plaque pH.<sup>5,10</sup> Results of a number of experiments have demonstrated that lower levels of soluble caseinate could produce significant reduction in caries activities.<sup>11-15</sup>

With a view to evaluate the pH altering property of dairy products, an experimental study was designed to investigate the effects of sucrose and four different enriched-milk products on dental plaque involving human volunteers tested in-vitro.

The main objective of the study was to observe the change in plaque pH from a baseline pH after rinsing with the control sucrose and the four other experimental milk products.

## Methods And Materials

This experimental study involving human volunteers was carried out in the Post Graduate Human Physiology performance Laboratory at Massey

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University, New Zealand, after obtaining approval from Massey University Ethics committee and Manawatu-Whanganui Ethics committee (PN Protocol 00/64, 1/01). The method adopted for this study is known as 'Plaque pH monitoring by the Sampling Method.'<sup>15</sup>

Volunteers were invited through advertisements. The purpose of the experiment was explained to them and a written informed consent was obtained. Because of the nature of the experiment which required the participants to abstain from brushing for 48 hours before each session of sample collection, only 9 volunteers agreed to participate in the experiment. It was explained to the volunteers that any harmful after effect of the experiment would be compensated for by New Zealand Dairy Research Institute (NZDRI), who sponsored the experiment without any gain or benefits from the experiment. The participants were also assured of confidentiality and privacy of the collected data.

During the experiment volunteers rinsed with either the control 10% sucrose solution or a milk sample in five separate tests. On each occasion, plaque samples were collected from teeth with two-headed standard curettes (supplied by Shafoon Ltd, Auckland, New Zealand) just before the rinse to determine the baseline pH of the plaque and then subsequent plaque samples were collected at 1, 3, 7, 11, 15, 19, 23 and 27 minutes post-rinse. The pH of the pooled plaque for each occasion were measured outside the mouth after diluting the collected plaque in 200 $\mu$ L of Deionized water in small test tubes with Eppendohrf tubes by a digital plaque pH

meter (Sentron 3001, made by Sentron Inc. Roden, Netherlands).

For each subject, and the solutions with which they rinsed were in the following order:

- Day 1:** Control (10%) sucrose solution,
- Day 2:** Caseinphosphopeptide (CPP)enriched milk product,
- Day 3:** Calcium enriched milk product,
- Day 4:** Calcium and caseinphosphopeptide (CPP) enriched milk product,
- Day 5:** Skim milk.

The results were collated by Microsoft Excel and SAS statistics.

### Discussion

The results of the experiment clearly indicated that the drop in pH caused by the sucrose solution was much more dramatic than with the dairy products (Table1-5). Although the average values of plaque pH after rinsing with sucrose, do not give the expected shape of the classical Stephan curve it was clear, however, that in general the pH values are on average lower after rinsing with sucrose solution than with any of the dairy products. There was an observed trend that each of the dairy products individually compared to the sucrose solution showed an increase in the plaque pH over time and the end pH values were considerably higher than the starting (baseline) pH values.

### Results

**Table 1: Plaque pH Values for 10% sucrose solution (Day One)**

Time (min)	Volunteer Number									Mean pH	Std. Dev.
	1	2	3	4	5	6	7	8	9		
0	7.14	6.62	6.63	6.50	6.56	6.21	6.36	6.58	6.12	6.52	0.29
1	6.94	6.14	6.21	6.18	6.14	5.98	6.16	6.33	4.96	6.11	0.51
3	6.81	6.87	5.20	6.10	6.70	5.80	6.24	6.53	5.40	6.18	0.61
7	6.73	6.97	6.49	6.20	6.43	6.35	6.55	6.63	5.33	6.40	0.46
11	6.28	6.50	7.19	6.09	6.26	6.01	7.13	6.76	5.86	6.45	0.48
15	6.43	6.78	5.68	6.27	6.34	5.86	5.91	6.70	5.50	6.16	0.44
19	6.52	6.48	6.62	6.38	6.40	5.92	7.01	6.70	5.50	6.39	0.44
23	6.46	5.87	7.48	6.22	6.43	6.45	6.77	6.75	5.30	6.41	0.60
27	6.17	6.96	7.50	6.50	6.08	6.31	6.32	6.86	5.40	6.45	0.60

**Table 1:** shows a general trend to decrease in plaque pH with 10% sucrose rinse



**Table 2: Plaque pH Values for CPP enriched milk (Day Two)**

Time (min)	Volunteer Number									Mean pH	Std. Dev.
	1	2	3	4	5	6	7	8	9		
0	6.51	6.67	6.60	6.73	5.66	6.56	6.61	6.57	6.50	6.49	0.31
1	6.15	6.97	6.48	6.31	5.60	6.20	6.48	6.55	6.82	6.39	0.40
3	6.22	6.60	6.55	6.51	5.40	6.44	6.62	6.97	6.07	6.37	0.44
7	6.33	6.48	6.53	6.41	6.00	6.80	7.00	6.66	6.39	6.51	0.28
11	6.38	6.60	6.75	6.41	5.90	6.57	6.50	6.82	6.18	6.45	0.28
15	6.82	6.67	6.73	6.58	5.60	6.61	6.20	7.02	6.40	6.51	0.41
19	6.50	6.73	6.70	6.65	6.30	6.56	6.20	6.44	6.30	6.48	0.19
23	6.53	6.98	6.80	6.70	6.90	6.40	6.20	6.24	7.00	6.63	0.30
27	6.55	6.97	6.78	6.77	6.90	6.84	6.17	6.31	7.00	6.69	0.29

**Table 2 :** shows a general tendency to rise in plaque pH above baseline after CPP-enriched milk rinse particularly in volunteer number 9

**Table 3: Plaque pH Values for Calcium enriched milk (Day Three)**

Time (min)	Volunteer Number									Mean pH	Std. Dev.
	1	2	3	4	5	6	7	8	9		
0	6.64	6.53	6.05	5.79	7.02	6.31	6.83	6.46	6.59	6.46	0.37
1	6.42	6.09	6.18	5.76	6.96	6.20	6.50	6.44	6.41	6.32	0.33
3	6.98	6.61	6.62	5.84	6.80	6.63	6.45	6.61	6.60	6.57	0.31
7	6.77	6.85	6.70	5.88	6.41	6.00	6.70	6.59	6.65	6.57	0.28
11	6.61	6.65	6.80	5.86	6.40	6.78	6.77	6.67	6.54	6.56	0.29
15	6.60	6.54	6.81	6.23	6.23	6.53	6.90	6.81	6.45	6.56	0.24
19	6.67	6.66	6.60	6.07	6.70	6.19	6.56	6.84	6.40	6.52	0.25
23	6.61	6.75	6.38	6.29	6.98	6.51	6.37	6.60	6.43	6.54	0.21
27	6.68	6.70	6.35	6.10	6.74	5.92	6.30	6.71	6.24	6.41	0.30

**Table 3** shows rise in plaque pH particularly in volunteer no.7 and 9

**Table 4: Plaque pH Values for CPP and Calcium enriched milk (Day Four)**

Time (min)	Volunteer Number									Mean pH	Std. Dev.
	1	2	3	4	5	6	7	8	9		
0	6.55	6.44	6.69	6.80	6.77	6.64	5.52	6.60	5.27	6.36	0.56
1	6.70	6.62	6.40	6.13	6.49	6.17	5.80	6.81	4.80	6.21	0.61
3	6.65	6.79	6.19	6.52	6.33	6.39	5.98	6.39	5.78	6.33	0.31
7	6.80	6.26	6.15	6.41	6.29	6.63	5.99	6.69	5.66	6.32	0.36
11	6.66	6.29	6.48	6.80	6.80	6.50	6.23	6.76	5.86	6.48	0.31
15	6.78	6.65	6.07	6.47	6.50	6.11	6.12	6.65	5.71	6.34	0.35
19	6.58	6.31	6.51	6.50	6.46	6.59	6.67	6.73	5.69	6.44	0.30
23	6.53	6.61	6.76	6.70	6.14	6.91	6.64	6.50	5.86	6.51	0.32
27	6.76	6.47	6.66	6.98	6.79	6.56	5.93	6.42	5.70	6.47	0.41

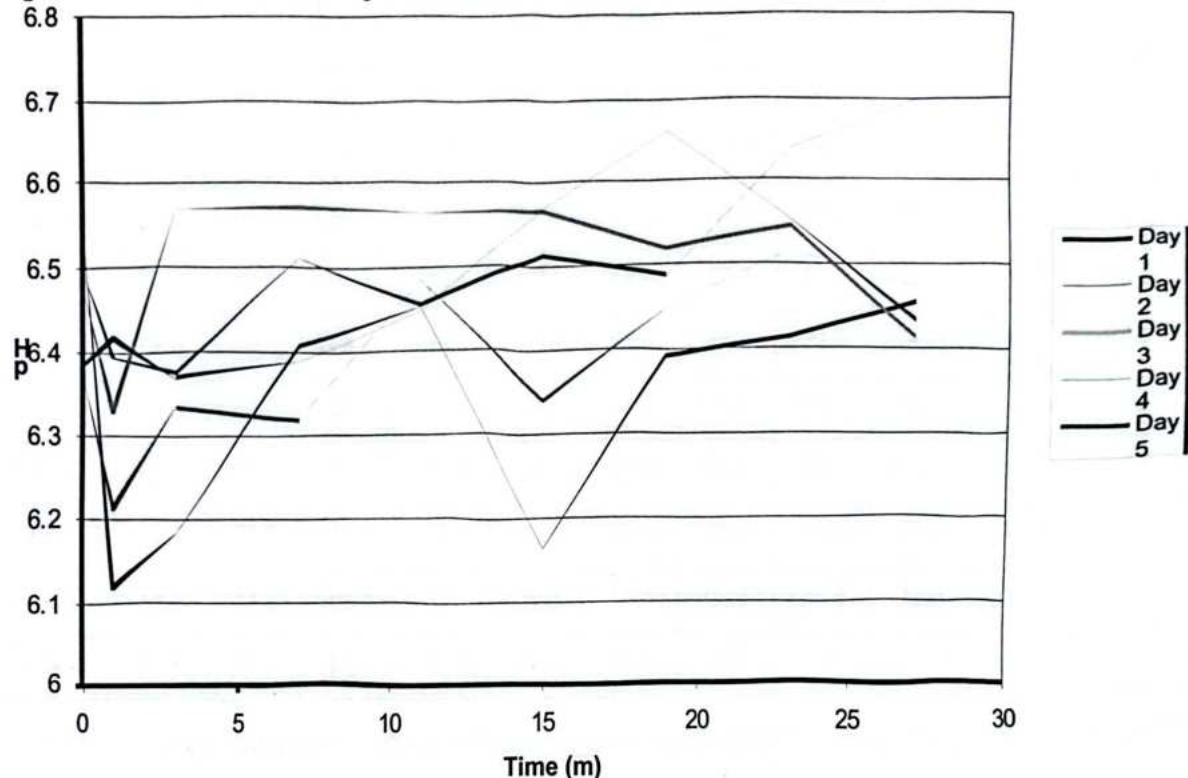
**Table 4** shows a rise in plaque pH at 27 minutes post-rising with CPP+Calcium enriched milk in all but two volunteers

**Table 5: Plaque pH Values for skim milk (Day Five)**

Time (min)	Volunteer Number									Mean pH	Std. Dev.
	1	2	3	4	5	6	7	8	9		
0	6.86	6.68	6.04	6.60	6.67	6.17	6.33	6.29	5.84	6.38	0.33
1	7.06	6.47	6.04	6.00	6.85	6.19	6.64	6.28	6.24	6.41	0.36
3	6.83	6.13	6.05	6.40	6.67	6.26	6.45	6.54	6.00	6.37	0.28
7	6.43	6.32	6.19	6.29	6.68	6.45	6.52	6.33	6.31	6.39	0.14
11	6.33	6.50	6.33	6.35	6.72	6.35	6.81	6.57	6.08	6.44	0.22
15	6.64	6.93	6.43	6.45	6.81	6.62	6.69	6.43	6.13	6.57	0.23
19	6.87	6.80	6.82	6.93	6.85	6.33	6.48	6.70	6.17	6.66	0.26
23	6.80	6.40	6.50	6.72	6.80	6.30	6.45	7.09	5.94	6.55	0.33
27	6.76	6.30	6.50	6.20	6.79	6.20	6.32	6.95	5.90	6.43	0.34

**Table 5** shows plaque pH reaching above 7 in volunteer 1 and volunteer 8 and a general rise in mean plaque pH 27 minutes post-rinse with skim milk



**Figure 1: Plot of mean values for pH overtime****Figure-1:** The average values for pH at each time interval for each experimental day shown in linear model ( $P < 0.0001$ )

The CPP enriched milk ended with the highest pH value, and all dairy products, with the exception of the calcium-enriched milk, showed an increase in the plaque pH over 3 to 19 minutes after rinsing.

All the volunteers in this study started with an initial plaque pH less than 7 before skim milk rinse. The final pH recorded at 27 minutes after the skim milk rinse was greater than the starting pH, reaching more than the baseline pH in most volunteers. The change in the pH curve with skim milk was very similar to the pH curve shown by DePaola et al.,<sup>16</sup> which reached a maximum pH of 7, slightly lower than the final pH value demonstrated by the current study. This was an interesting find, as the pH curve showed by DePaola et al. was a result of skim milk ingestion without any food being eaten the night before plaque sampling, whereas in the present study volunteers had eaten before plaque sampling, and started with pH values even lower than the earlier study. The results of this study suggest that dairy products may even be able to promote higher pH values than the baseline plaque pH values.

Some of the trends in change in plaque pH with individual dairy products are interesting, although not achieving statistical significance. In particular, the trend for a continuing rise in plaque pH with casein phosphopeptide-enriched products is intriguing. It should be noted, though, that the control skim milk product showed, overall, the most consistent elevation above baseline pH.

As a whole, the observed trends in pH changes with the dairy products were not significantly different for each product because of the large inter-individual variations in pH. However, the difference in change in pH from baseline between sucrose and any one of the dairy products was highly significant ( $p < 0.0001$ ) when a linear model with volunteer, treatment(experimental regimen) and time as fixed effects was fitted to the pH change data, using SAS (Fig1).

## Conclusion

Results of this study demonstrated that all the dairy products used show potential to protect teeth by initiating an increase in plaque pH above the baseline value. These results agree with previous similar reports of the effectiveness of milk products in increasing plaque pH.<sup>5-7, 11, 12, 13</sup> Even though the pH curves due to the dairy products generally ended with pH values higher than their starting pH values, in the final average curves this was not statistically significant due to the large variation amongst the subjects and the dairy products. It cannot be concluded that there were any differences between the dairy products tested in this study due to the variation between the volunteers. However, it is important to note that overall dairy products showed an increase in plaque pH compared with the sucrose rinse. The trend for the plaque pH to increase above the baseline value, which was most marked in the caseinphosphopeptide (CPP) enriched product, warrants more investigation in an experimental protocol with less inherent variability.

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# Seronegative hepatitis B virus DNA detection by Polymerase Chain Reaction

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## Abstract

**Purpose:** The purpose of the study was to detect Seronegative hepatitis B virus DNA by Polymerase Chain Reaction (PCR). **Methods:** HBsAg negative 401 sera were taken from non-professional and professional blood donors, clinically suspected chronic liver disease patients, injection drug users and commercial sex workers. Sera were tested for the presence of anti-HBc antibody and polymerase chain reaction (PCR) were done on all HBc antibody positive sera to detect HBV DNA. **Results:** Out of 401 HBsAg negative blood samples, 57 (14.21%) were positive for anti-HBc. Among anti-HBc positive 57 cases, 5 (8.77%) were positive for viral DNA. The highest rate (22.58%) of anti-HBc positivity was found in chronic liver disease patients and lowest rate (11.92%) was found in non-professional blood donors. HBV DNA was found in 8.77% of HBsAg negative and anti-HBc positive cases. The highest rate (14.29%) of HBV DNA was found in professional blood donors and clinically diagnosed chronic liver disease patients and lowest rate (6.45%) was found in non-professional blood donors. **Conclusion:** In spite of a sensitive assay for the detection of hepatitis B virus surface antigen (HBsAg) there are some cases who are seronegative (HBsAg) but found out to be positive (8.77%) when viral DNA is detected even in presence of anti-HBc. These DNA positive cases are assumed to be possible source of infection to susceptible people.

**Key words:** Seronegative, HBV, Polymerase chain reaction, Blood donor, Drug user

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## Introduction

Approximately 2 billion people in the world have been infected with hepatitis B virus and more than 350 million are chronic carriers.<sup>1</sup> Of them, approximately 15% - 40% of HBV infected persons develop cirrhosis of liver, liver failure or hepatocellular carcinoma.<sup>2</sup>

The prevalence of HBV infection varies markedly in different geographic areas of the world and can be categorized as high (>8%), intermediate (2-7%) and low endemicity (<2%).<sup>2</sup> Approximately 45% of the global population lives in areas of high chronic HBV prevalence.<sup>3</sup> Bangladesh is in the intermediate prevalence region for HBV infection.<sup>4</sup> Among blood donors, the prevalence of occult HBV infection varies between 7% - 19% in endemic region where 70% - 90% of the population are exposed to HBV. In Western countries, frequency of occult HBV infection ranges between 0% - 9%.<sup>5</sup>

HBV spreads through contact with infected body fluids. Blood is the most important vehicle for transmission, but other body fluids have also been implicated, including semen and saliva.<sup>2</sup> Other common routes of

transmission are mother to child transmission, via contaminated injection drug use and sexual contacts.<sup>6</sup>

The safety of blood products is one of the major issues in the area of transfusion medicine.<sup>7</sup> Hepatitis B surface antigen (HBsAg), in spite of a sensitive screening assay for the detection of HBV infection, occasional cases of post-transfusion hepatitis B virus infection are common. This is probably related to seronegative window period,<sup>8</sup> occult hepatitis B virus infection and blood donors infected with HBsAg mutants.<sup>7</sup> Anti-HBc appears in the acute phase of HBV infection and persists indefinitely after apparent antigen clearance.<sup>9</sup>

In Taiwan, 7% of negative HBsAg and positive anti-HBc blood samples have HBV DNA and in Iran, this rate is 8%.<sup>8</sup> For this reasons, some countries like USA and Japan have implemented anti-HBc testing in routine blood donor screening to prevent transfusion-transmitted HBV infection.<sup>10</sup> In Japan and Germany, mini pool HBV NAT (Nucleic Acid Testing) is performed for screening of blood donor.<sup>11</sup> The advent of molecular technology has enabled the sensitive detection of viral nucleic acid.<sup>12</sup>

In case of viral DNA detection, the polymerase chain reaction (PCR) is potentially a more sensitive assay than molecular hybridization.<sup>13</sup> HBV-DNA detection by PCR is important in certain situation, for example, in HBsAg negative and anti-HBc positive carrier where genetic variant of the virus may exist.<sup>14</sup>

The purpose of this study was to detect HBV DNA in the HBsAg negative and anti-HBc positive cases by PCR and to find out the rate of HBV carrier in those people. Using PCR as an additional tool in the diagnosis of HBV has also been assessed.

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### Materials and Methods:

This cross sectional study was carried out in the department of Microbiology, Bangladesh Disease Research Institute (BDRI) Lab, Dhaka, from January 2009 to December 2011.

A total of 401 HBsAg negative respondents between 9 - 65 years were taken as the study population. Of them, 299 were male and 102 were female. They were classified into low, middle and high income groups according to their monthly family income. Among these 401 HBsAg respondents 300 were blood donors, 31 were clinically diagnosed chronic liver disease patients, 40 were injection drug users and 30 were commercial sex workers. Among 300 blood donors, 260 were non-professional donors and 40 were professional donors.

### Procedure :

Five milliliter blood was collected from each volunteer in a dry, clean and sterile test tube. Sera were separated within 4 hours and stored at -20°C.

### Serological Tests :

Serum samples from all participants (401 cases) were tested for HBsAg and anti-HBc by commercially available enzyme immunoassays, following the JAJ International (USA) and Atlas Medical (UK) manufacturer's instructions respectively.

HBsAg negative and anti-HBc positive cases were distributed according to age, sex and monthly family income in different groups.

### DNA extraction from samples:

DNA was extracted from 200µl serum sample using the PureLink™ Genomic DNA Mini Kit according to the manufacturer's instruction (Invitrogen, USA) following standard protocol.<sup>15</sup> Briefly, serum samples were subjected to lysis at 55°C temperature with 200 µl lysis buffer (Genomic Lysis Buffer) and 20µl of protease reagent (Proteinase K). The DNA was extracted from the lysate using 96% ethanol and subsequently purified using spin columns. Finally, purified DNA was eluted from the spin columns using elution buffer (Genomic Elution Buffer) and stored at -20°C. The extracted DNA was subjected to amplification.

PCR and Gel Electrophoresis: PCR was carried out in a 25 µl mixture containing 10mM Tris-HCl (PH 8.3), 50 mM MgCl<sub>2</sub>, 2.5mM of each dNTPs, 0.5 M of each primer, 0.1 l(5U/µl) Taq DNA polymerase and 5 µl of DNA extracted sample.

Primers were designed on highly conserved HBV

surface gene (JOL43,sense,codon number 190-217 and JOL44, anti-sense, codon number 787-760) and were used to amplify a 597-bp region. PCR was performed using a standard protocol with the following cycling parameters: 94°C for 1 minute (denaturation), 55°C for 1 minute (annealing), and 72°C for 2 minutes (extension). Final extension was 10 minutes at 72°C.<sup>7,16</sup>

### Detection of PCR product:

Ten µl of reaction (8 µl PCR product + 2 µl loading dye) product was electrophoresed in a 1.5% agarose gel made in Tris-Borate-EDTA(TBE). The electrophoretic band was visualized by using UV transilluminator after ethidium bromide staining.

### Result

In this study, sera from 401 respondents who were HBsAg negative tested by ICT or by ELISA were taken. Sera of negative HBsAg were confirmed by ELISA. Of them, 299 were male and 102 were female. These 401 serum samples were tested for anti-HBc by ELISA. Of them, 57 serum samples were positive for anti-HBc. Among these 57 anti-HBc positive respondents, 43 were male and 14 were female. They were also distributed according to age groups and monthly family income groups. Among 57 anti-HBc positive serum samples, 5 were positive for HBV DNA by PCR. These 5 HBV DNA positive respondents were distributed according to socio-demographic characteristic.

**Table-I. Results of PCR test among anti-HBc positive samples in different groups.**

Group	Positive	Negative	Total
<b>Group-A:</b>			
I. Non-professional blood donors	2 (6.45%)	29 (93.55%)	31
II. Professional blood donors	1 (14.29%)	6 (85.71%)	7
<b>Group-B:</b>			
Clinically diagnosed chronic liver disease patients	1 (14.29%)	6 (85.71%)	7
<b>Group-C:</b>			
Injection drug users.	1 (11.11%)	8 (88.89%)	9
<b>Group-D:</b>			
Commercial sex workers	0 (0%)	3 (100%)	3
<b>Total</b>	<b>5 (8.77%)</b>	<b>52 (91.27%)</b>	<b>57</b>



Table-I shows the results of PCR for the detection of HBV-DNA among anti-HBc positive samples. Of the 57 anti-HBc positive samples, HBV DNA was detected in 5(8.77%) samples and 52(91.23%) were PCR negative. Within 31 non-professional blood donors 2(6.45%) were PCR positive and 29(93.55%) were PCR negative. Among 7 professional blood donors one(14.29%) was PCR positive and 6(85.71%) were PCR negative. Out of 7 clinically diagnosed chronic liver disease patients, one(14.29%) was PCR positive and 6(85.71%) were PCR negative. Among 9 injection drug users one(11.11%) was PCR positive and 8(88.89%) were PCR negative. Among 3 commercial sex workers none was PCR positive.

### Discussion

HBV is highly contagious and relatively easy to transmit from one infected individual to another, by blood to blood contact, during birth, unprotected sex and by sharing needles.<sup>17</sup> In the present study, it was observed that among 401 HBsAg negative samples, 57(14.21%) were positive for anti-HBc(total). The highest rate was found in chronic liver disease patients (22.58%) followed by injection drug users (22.50%), professional blood donors(17.50%), non-professional blood donors(11.92%) and commercial sex workers(10%). A study from West Bengal showed anti-HBc positivity rate was 18.3% in voluntary blood donors.<sup>18</sup> In a study in Italy, reported by Vitale et al.(2008), revealed that "anti-HBc alone" serological pattern was found in 10.2% in injection drug users and 21.5% in patients with HCC.<sup>9</sup>

In the present study, HBV DNA was detected by PCR among 8.77% of anti-HBc positive cases. The higher percentage (14.29%) of HBV DNA positivity was found in the professional blood donors and chronic liver disease patients. In injection drug users, the percentage was 11.11% and in non-professional blood donors, it was 6.45%. In a study in Spain, among 109 anti-HCV negative and HBsAg negative patients with increased liver enzymes with unknown etiology, HBV DNA was detected in 19% cases.<sup>19</sup> A study reported from Iran, the rate of HBV DNA positivity was 12.2% among anti-HBc positive donors.<sup>7</sup> In Taiwan, the rate was 7%.<sup>10</sup> The rate of occult HBV infection was 45% in injection drug users in Baltimore.<sup>20</sup>

In the present study, among 5 HBV DNA positive cases, 4 were male and one was female who was a non-

professional blood donor. Among 4 male, one was non-professional blood donor, one was professional blood donor, one was chronic liver disease patient and one was injection drug use. All of them come from low income families. In a study in Iran, 16 HBV DNA positive cases were found among 131 anti-HBc positive individuals and all (100%) were male.<sup>7</sup>

A study showed that upto 16% of anti-HBc and anti-HBs positive donors have circulating HBV DNA unbound to anti-HBs in their sera and thus in a potentially infective form.<sup>21</sup> Another study shows that persistence of HBV DNA in serum and peripheral blood mononuclear cells in patients can be found up to 70 months after complete clinical, biochemical and serological recovery from acute viral hepatitis.<sup>22</sup> HBV DNA is the earliest indicator of infectivity.<sup>23</sup> So, PCR assay has demonstrated that some HBsAg negative individuals and those positive for anti-HBc continue HBV replication.<sup>24</sup> HBV DNA is the earliest indicator of infectivity. In some countries, implementation of routine blood donor screening for anti-HBc has resulted in a decrease risk of post-transfusion HBV infection.

### Conclusion

In spite of a sensitive assay (HBsAg by ELISA) for the detection of Hepatitis B Virus surface antigen, alarmingly, there were more than 8% cases in this study who were positive for HBV DNA in presence of HBc antibody. This finding suggests an urgent need for the implementation of anti-HBc screening before any kind of transplantation or transfusion.

Regarding the possible source of HBV infection to a susceptible person HBsAg carrier alone should not be considered, rather a search can be done to detect viral DNA.

### Recommendation

- 1) Anti-HBc antibody should be tested routinely for blood donors or organ donors and be discarded if the test result becomes positive.
- 2) Anti-HBc positive sera can be tested for the presence of HBV DNA by PCR to find out occult HBV infection and surface mutation if the facility permits.



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## Pattern of smokeless tobacco use among the women in old Dhaka

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## Abstract

**Purpose:** This descriptive type of cross sectional study was carried out to find out the pattern of smokeless tobacco use among the women in old Dhaka. **Methods:** A total of 105 women using smokeless tobacco were selected by purposive sampling technique. Data was collected by a structured pretested questionnaire by face to face interview. **Results:** A total of 105 respondents reported smokeless tobacco consumption. Among them smokeless tobacco use was 36.2% in the age group of 31-40 years. Most of the smokeless tobacco users (73.3%) were illiterate. Regular use of smokeless tobacco was 74.3% and occasional use was 25.7%. About 63% were house wives and 41.9 % user had monthly family income of Taka 5000-10000. Among them 45.3% used zarda, 27.2% used Kahini, 17.1% used sada pata and 10.5% used gul. The initiation of smokeless tobacco use was from others members of the family 44.8%. The reason behind using smokeless tobacco was stated as fun by 40% respondents. Almost 68.6 % respondents think smokeless tobacco do not cause any harm in the oral cavity while only 31.4 % stated smokeless tobacco cause harm in the oral cavity. There was significant association between occupation and regular use of smokeless tobacco ( $p < 0.05$ ). **Conclusion:** From the study finding it may be concluded that zarda is mostly used by the women as smokeless tobacco which is harmful for general health and oral health as well. Mass media should be involved to address the injurious use of smokeless tobacco on oral health which though known is ignored by the general population.

**Keywords:** Smokeless tobacco, Oral Cavity, Women, Old Dhaka

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## Introduction

Smokeless tobacco products have been in existence for thousands of years among population in South America and South East Asia.<sup>1</sup> Smokeless tobacco is consumed without burning the product and can be used orally or nasally. Oral Smokeless tobacco products are placed in the mouth, Cheek or lip and sucked (dipped) or chewed. Tobacco paste or powder are used in the similar manner and placed on the gums or teeth. Fine tobacco powder mixture are usually inhaled and absorbed in the nasal passages.

According to WHO global epidemic Bangladesh consumption report (2012), approximately 43% of adults (age 15+) in Bangladesh use tobacco. Among youth (Age 13-15), 2% Smoke Cigarettes and 16% use tobacco products other than Cigarettes. Rate of smokeless tobacco use however are slightly higher among women than men (28%-26%).<sup>2</sup> In Bangladesh more than 57,000 people die each year from tobacco related disease.<sup>3</sup> Though dental health have been included as 9th element of primary health care since

1994, people living in old Dhaka are socially and culturally addicted to smokeless tobacco use from the ancient period. Interestingly, smokeless tobacco is traditionally popular among women of old Dhaka.<sup>4</sup> Studies related to smokeless tobacco use are limited. Developing countries like Bangladesh have few resources for a curative or restorative approach as well as little research is being conducted on this subject. The consequence of smokeless tobacco use needs to be spread amongst the general population through awareness programme for prevention of tobacco abuse. Thus this study would help the policy makers to take necessary steps for creating awareness among the people for avoiding the use of smokeless tobacco. Thus the primary objective of this study was to understand the pattern of smokeless tobacco use among the women in old Dhaka.

## Materials and Method

This descriptive type of cross sectional study was conducted in Wari in old Dhaka. Study population were women who are resident in Wari in old Dhaka. A purposive sampling technique was used to get a total of 105 respondents. A structured questionnaire was developed based on the objective, contents and variables of the study. Data was collected by using the questionnaire through face to face interview with the respondents. Collected data were processed manually, tabulated and analyzed based on the objectives of the study. Data analyzed by using statistical package for social science (SPSS)-version 17.

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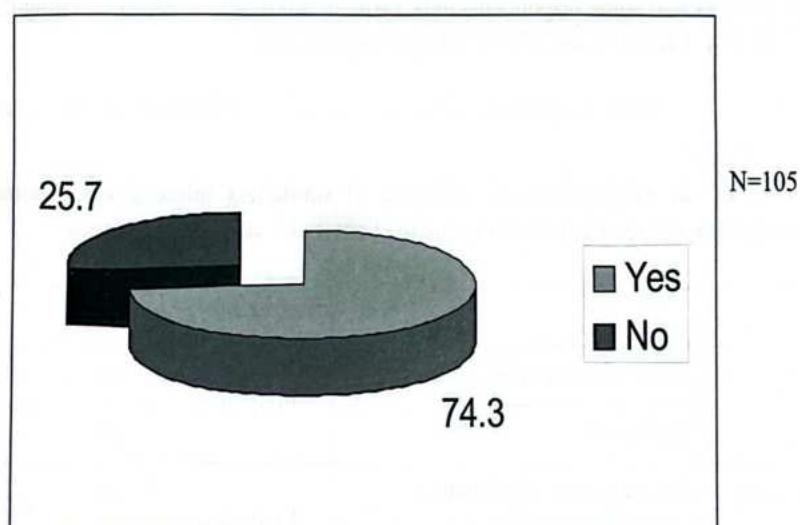
## Results

**Table-1: Distribution of the respondents by their age, educational qualification, monthly income in Taka**

Characteristics					
Age group	31-40 years	Frequency	38	Percent	36.2
	> 50 years		28		26.7
Educational qualification	Illiterate	Frequency	77	Percent	73.3
	Primary		20		19
Monthly income in Taka	Tk1000-5000	Frequency	41	Percent	39
	Tk5000-10000		44		41.9

Table-1: shows that out of 105 respondents, smokeless tobacco use was 36.2 % in the age group of 31-40 years, among the smokeless tobacco user 73.3 % were illiterate and 41.9 % had monthly family income of Taka 5000-10000.

**Figure-1: Distribution of the respondents by regular use of smokeless tobacco**

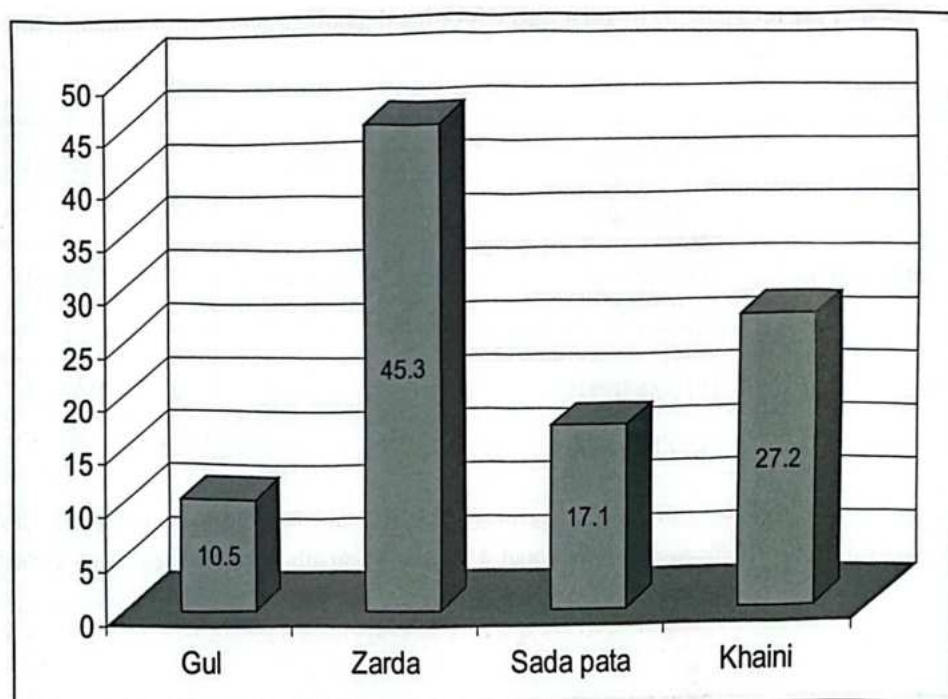


**Figure-1:** This figure shows that out of 105 respondents, 74.3% regularly used smokeless tobacco and 25.7% used occasionally.



**Figure-2: Distribution of the respondents by types of smokeless tobacco use**

N=105

**Figure-2:** The figure shows that some respondents took varieties smokeless tobacco . Among them 45.3 % used Zarda, 10.5 % Gul, 17.1 % Sada Pata and 27.2 % Khaini, respectively.**Table-2: Distribution of the respondents by initiation of smokeless tobacco use, reasons behind using smokeless tobacco, opinion cause of any harm in the oral cavity by smokeless tobacco**

N=105

Characteristics					
Initiation of smokeless tobacco use	From other members of the family	Frequency	47	Percent	44.8
	By myself		38		36.2
Reasons behind using smokeless tobacco	For addiction or habituation	Frequency	39	Percent	37.1
	For fun		42		40
Opinion cause of any harm in the oral cavity by smokeless tobacco use	Yes	Frequency	33	Percent	31.4
	No		72		68.6

**Table-2** reveal that out of 105 respondents, 44.8% had initiated smokeless tobacco use from other members of the family. The reasons behind using smokeless tobacco was stated as fun by 40 % respondents. About 68.6 % think smokeless tobacco did not cause any harm in the oral cavity while only 31.4 % stated smokeless tobacco could cause harm in the oral cavity.

**Table-3: Distribution of the respondents by type of diseases occurring in the mouth due to smokeless tobacco use**

N=33

Types of disease occurring in the mouth due to smokeless tobacco use	Frequency	Percent
Ulcer in the mouth	10	30.3
Stain in the teeth	9	27.3
Cancer in the oral cavity	1	3
Loss of appetite	4	12.1
Stain in the teeth, Loss of appetite	9	27.3
Total	33	100

**Table-3** show that 30.3% respondents answered ulcer occurs in the mouth occur due to smokeless tobacco use, 27.3 % think that it causes stain in the teeth.

**Table-4: Relationship between occupation and regular use of smokeless tobacco**

N=105

Occupation	Regular use of smokeless tobacco				Total
	Yes		No		
	Frequency	Percent	Frequency	Percent	
House wives	45	66.2	21	31.8	66
Others (business women, day laborer, service holder)	33	84.6	6	15.4	39
Total	78	74.3	27	25.7	105

$p < 0.05$

**Table-4** shows the distribution of the respondents by occupation and regular use of smokeless tobacco. Among the house wives 66.2 % used smokeless tobacco regularly and where as in 84.6% of other groups (business women, day laborer and service holder) used smokeless tobacco regularly. Study results shows there was significant association between occupation and regular use of smokeless tobacco ( $p < 0.05$ ).



## Discussion

According to WHO global status report (2005) tobacco usage is high among the Bangladeshi population and chewers are greater in proportion among women (40%) than among men (23%).<sup>3</sup> Out of the 105 respondents, 36.2% used smokeless tobacco in the age group of 31-40 years (Table-1). The finding is similar with this study conducted by MK Biswas, AK Biswas.<sup>4,5</sup> According to the level of education, 73.3 % tobacco users were illiterate. Majority of the respondents (41.9%) had family monthly within TK 5000-10000. Above finding contradicts the study conducted by Farazi MNI.<sup>6</sup>

The present study found that out of 105 respondents, regular use of smokeless tobacco was 74.3% and occasional use was 25.7% (Fig.-1). This finding is similar to the study conducted by Ahmed S, Rahman A, Hulls.<sup>8</sup> According to the initiation of smokeless tobacco use was from others members of the family 44.8% (Table-2). The reasons behind using smokeless tobacco were stated as fun by 40 % respondents. Above finding is not similar to the study conducted by Jalil MA.<sup>7</sup> Prevalence of tobacco consumption habits among the rural people in a selected Thana of Gazipur district, Dissertation, NIPSOM 1997 showed that about 49% respondents were motivated by friends to start tobacco consumption habits, 28.7% due to family influence and 20.5% of the respondents started due to curiosity.<sup>7</sup> In this study about 68.6 % respondents think smokeless tobacco do not cause harm in the oral cavity while only 31.4 % stated smokeless tobacco cause harm in the oral cavity. Among them one third (30.3%) answered ulcer occurs in the mouth due to smokeless tobacco use, 27.3 % thought that it causes stain in the teeth, 12.1 % think that it causes loss of appetite and 27.3 % think that it causes stain in the teeth & loss of appetite (Table-3). It was also found that nearly 79.9% of adult American men thought tobacco snuff increase the risk of cancer whereas 83.8% adult American men considered chewing tobacco increases cancer risk. Among those who chew tobacco leaves, 71.5% knew the risk associated with it as compared to those who did not chew tobacco.<sup>9</sup> A study on smokeless tobacco use among the rural kadazan women in sabah, Malaysia out of 472 women about 73.3% of smokeless tobacco users were unaware of any adverse health effect of this type of tobacco as compare to 53.9% of the non-tobacco users.<sup>10</sup>

## Conclusion

Tobacco being called a silent killer not *only* cause physical problem but also a burden to a *developing* country like Bangladesh. Tobacco consumption related diseases cause serious disability both in male and female. There was significant association between occupation and regular use of smokeless tobacco ( $p < 0.05$ ). Health educational intervention programme could be targeted among the tobacco users to prevent smokeless tobacco use. Further research could be done for risk assessment among smokeless tobacco Consumer.

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## Radiological assessment of impacted mandibular 3rd molar

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## Abstract

**Purpose:** The purpose of this study was to assess impacted mandibular 3rd molar radiologically in 33 Bangladeshi patients. **Methodology:** Radiographic evaluation (by orthopantomogram) of 53 impacted mandibular 3rd molar teeth of 33 Bangladeshi patients of ages ranged from 21 to 70 years was done who were conveniently selected from patients attended to the department of Oral & Maxillofacial Surgery, Dhaka dental College Hospital & Confidence Dental Surgery, Dhaka. **Result:** In the study of 33 patients, 21 (63.64%) were males and 12 (36.36%) were females. Out of 53 impacted teeth of 33 patients, highest number of mandibular third molars were in vertical position (39 teeth, i.e. 73.59%), followed by horizontal (8 teeth, i.e. 15.09%), mesio-angular (4 teeth, i.e. 7.54%), and disto-angular (1 tooth, i.e. 1.87%) position. About 55 percent (29 number of teeth, 54.71%) of third molars were at level A, followed by level B (18 teeth, i.e., 33.97%) and level C (06 teeth, i.e., 11.32%). 43 mandibular third molars were in class II relation (81.12%), followed by 8 teeth (15.09%) in class I relation and 2 teeth (3.78%) in class III relation. **Conclusion:** The pattern of Impaction of mandibular 3rd molar is characterized by high prevalence in male. The most common type is vertical, the most common position is position A and the most common class is Class-II.

**Key Words:** Impaction, Third molar, Orthopantomogram (OPG)

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## Introduction

The word "Impaction" is from Latin word "Impactio" that means pressing together. Impacted tooth may be defined as- the tooth that has failed to erupt completely or partially to its correct position in the arch and its eruption potential has been lost.<sup>1</sup>

The third molar continues to generate more controversy concerning eruption pattern and pathologic sequel than any other tooth in the oral cavity. Despite racial variation in eruption sequence and dates, it is universally accepted that third molars are the last teeth to erupt. This late eruption explains the fact that third molars are the most frequently impacted teeth.<sup>2</sup> Etiology of permanent teeth impaction includes several systemic and local factors. Cleidocranial dysplasia, Down syndrome, endocrine deficiencies (hypothyroidism and hypopituitarism), febrile diseases, and irradiation, are some of the systemic factors that may influence impaction of permanent teeth. More commonly, local factors include prolonged deciduous tooth retention, malposed tooth germs, arch-length deficiency, supernumerary teeth, odontogenic tumors abnormal eruption path, and cleft lip and palate.<sup>3</sup>

In recent days the change in the dietary pattern with advancement of civilization from hard food to soft food, probably one single factor which is responsible for reduction in jaw size.<sup>4</sup>

The most frequently retained teeth are molars (90%), with higher prevalence in mandible (60%) than maxilla (30%), followed by upper canine teeth (5%), lower bicuspid and supernumerary teeth (5%).<sup>5</sup> Upper and lower 3rd molars are the last teeth to erupt, regardless race and gender, and normally do not erupt at occlusal plane until mandibular growth is complete.<sup>6-8</sup> Where mesio-angulation is the most common in mandible,<sup>7</sup> some authors said vertical angulation is most common.<sup>5,9</sup>

The highest retention incidence of 3rd molar, especially those located in mandible, results in a large number of studies because of their position variations, higher surgical treatment challenges, and their more frequent association to pathologies.<sup>10-13</sup> If retained teeth are left within the alveolar ridge, it is likely that one or more problems occur and the patient may present a higher incidence of local tissue morbidity, such as bone and neighboring tooth loss and potential lesion to the surrounding vital structures.<sup>14</sup> Among the complications, the most important and common are pericoronaritis and the formation of odontogenic cysts and tumors arising from dental follicle. Therefore, it is important to evaluate the state of third molars, to prevent the aforementioned and other complications, such as periodontal disease, dental caries, root resorption, and mandibular fractures.<sup>6, 15, 16</sup>

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## Patients & Methods

The descriptive cross sectional study of 53 impacted mandibular 3rd molar teeth of 33 consecutive patients were done by using Orthopantomogram with following inclusion & exclusion criteria in the Dept of Oral & Maxillofacial Surgery, Dhaka Dental College Hospital.

### Inclusion Criteria :

1. Both genders.
2. Age range from 21 to 70 years.
3. Presence of at least one impacted mandibular 3rd molar.

### Exclusion criteria :

1. Absence of impacted lower 3rd molar in orthopantomograph.
2. Orthopantomograph with absence of name, age & sex marking.

### Evaluation Procedure

In this study 3 types of classification were considered, such as:

#### 1. Winter's classification-

Based on position of long axis of impacted lower 3rd molar in relation to long axis of lower 2nd molar

#### 2. Pell & Gregory's classification-

Based on relationship of the impacted lower 3rd molar to the ramus of the mandible and the lower 2nd molar.

**Class I:** Where there is sufficient amount of space between anterior border of ascending ramus & the distal side of the 2nd molar for the eruption of the 3rd molar.

**Class-II:** The space between distal surface of 2nd molar & anterior border of ascending ramus is less than mesio-distal diameter of the crown of the 3rd molar

**Class-III:** When the 3rd molar is located within the ramus of the mandible.

#### 3. Based on depth of the impacted 3rd molar in

relation to the occlusal surface of the 2nd molar-

**Position A:** When highest point of the 3rd molar is at the level of occlusal line or above it

**Position B:** When the highest point of 3rd molar is below the occlusal level but above the cervical line of 2nd molar.

**Position C:** When the highest point of 3rd molar is below the cervical line of the 2nd molar.

## Result

The age study subjects ranged from 21 to 70 years with mean age 33.39%.

**Table1: Age & sex wise distribution**

Age Group (Years)	Total		Male		Female	
	Patients	Teeth	Patients	Teeth	Patients	Teeth
21-30	16 (48.48%)	25(47.17%)	10(30.30%)	15(28.30%)	6(18.18%)	10(18.87%)
31-40	11(33.33%)	19(35.85%)	7(21.21%)	13(24.53%)	4(12.12%)	6(11.32%)
41-50	2(6.06%)	3(5.66%)	0(0%)	0(0%)	2(6.06%)	3(5.66%)
51-60	3(9.09%)	5(9.43%)	3(9.09%)	5(9.43%)	0(0%)	0(0%)
61-70	1(3.03%)	1(3.03%)	1(3.03%)	1(3.03%)	0(0%)	0(0%)

**Table 2: Age & sex wise distribution**

Sex	Total	Bi-lateral	Unilateral		
			Total	Right	Left
Male	21(63.63%)	13(39.39%)	8(24.24%)	7(21.21%)	1(3.03%)
Female	12(36.36%)	7(21.21%)	5(15.15%)	2(6.06%)	3(9.09%)

**Table 3a: Types of impaction:**

### Angles classification

Types	Total	Right	Left
Mesio-angular	4(7.54%)	3(5.66%)	1(1.87%)
Disto-angular	1(1.87%)	0(0%)	1(1.87%)
Vertical	39(73.59%)	17(32.09%)	22(41.5%)
Horizontal	8(15.09%)	3(5.66%)	5(9.43%)
Transverse	0(0%)	0(0%)	0(0%)
Inverted	0(0%)	0(0%)	0(0%)
Aberrant	1(1.87%)	1(1.87%)	0(0%)

### Pell & Gregory's Classification



**Table 3b: Pell and Gregory's Classification**

Types	Total	Right	Left
Class-I	2(3.78%)	1(1.89%)	1(1.89%)
Class-II	43(81.12%)	20(37.73%)	23(43.40%)
Class-III	8(15.09%)	3(5.66%)	5(9.43%)

**Table 3c: Based on depth of the impacted 3rd molar in relation to the occlusal surface of the 2nd molar**

Types	Total	Right	Left
Position A	29(54.71%)	14(26.41%)	15(28.30%)
Position B	18(33.97%)	6(11.32%)	12(22.64%)
Position C	6(11.32%)	4(7.54%)	2(3.78%)

### Discussion

A total 33 patients with 53 impacted lower 3rd molar were evaluated radiographically with age ranged from 21 to 70 years. The maximum number of patients were in 21-30 years group (25 teeth, i.e 47.17%).

Out of 33 patients (53 teeth) of present study, 21 (63.64%) were males and 12 (36.36%) were females. For gender distribution this study is in accordance with study of Hazza'a et al<sup>17</sup>. However, studies of Linden et al., Hattab et al., Yamaoka et al., Sandhu and Kapila, and Odusanya and Abayomi showed female predominance<sup>18-22</sup>.

Highest number of mandibular third molars were in vertically position (39 teeth, i.e 73.59%), followed by horizontal, mesio-angular, and distoangular position. Results of present study are in accordance with the study of Hazza'a et al<sup>17</sup>. as they also found highest number of vertically placed third molars. Rajasuo et al<sup>23</sup>. also found highest number of vertically placed third molars in their study. Number of mesioangular third molars in present study are in accordance with the study carried by Valmaseda-Castellon et al<sup>24</sup>., as they found 358 mesioangular mandibular third molars in a total of 1000 teeth they evaluated, but result was not in agreement for vertically placed, distoangular, and horizontally placed third molars. Linden et al., Hattab et al., Knutsson et al. and Sedaghatfar et al. in their study found maximum number of third molars to be mesioangular<sup>19,20,25,26</sup>. In study of Richardson<sup>27</sup>, he

found maximum number of third molars in horizontal position. In another study by Chu et al<sup>28</sup>., they found that maximum number of third molars (80% of 3178 mandibular third molars) were horizontal or mesioangular. These variations in angular position of mandibular third molars may be because of the fact that the studied population in each study was quite different from each other.

Present study shows maximum number of third molars at level A (29 teeth, i.e., 54.71%), followed by level B (18 teeth, i.e., 33.97%) and level C (06 teeth, i.e., 11.32%). Level of eruption in the present study is in agreement with that of Jerjes et al<sup>29</sup>. and also with study of Hattab et al<sup>20</sup>. Study of Sandhu and Kaur, Susarla and Dodson found maximum third molars at level B followed by level A and level C<sup>7,30</sup>.

It is found that 43 out of 53 teeth (81.12%) mandibular third molars are in class II relation, followed by 8 teeth (15.09%) in class I and 2 teeth (3.78%) in class III. Results of present study are in accordance with that of Susarla and Dodson<sup>30</sup> as they also found maximum third molars in class II relations followed by class I and class III relations. Results were not in agreement with that of Jerjes et al<sup>28</sup>. as they found maximum number of mandibular third molars in class I relation followed by class II and class III.

### Conclusion

The pattern of Impaction of mandibular 3rd molar is characterized by high prevalence in male. The most common angulation is vertical, the most common position is position A and the most common class is Class-II. The vertically impacted teeth were mostly in Position B and the horizontally impacted teeth were mostly in Class-III position. Most of the cases site of impaction is bi-lateral. Future studies with larger sample size with multi-centre involvement are required to evaluate the pattern of third molar impaction in mandible in Bangladeshi patients.



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# The future of dental treatment and prosthesis using CAD/CAM systems

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## Abstract

*This review article explores the efficient use of three-dimensional CAD/CAM system for precision prosthesis. The authors describe the gradual improvement and progress in casting technology through years of experimentation and invention of novel technologies for practical purposes in dental industry.*

**Key words :** Dental Prosthesis, CAD /CAM

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## Introduction

Casting techniques have played an important role in prosthodontic treatment and dental prosthesis for a long time. Casting is a technique used to make molded products by pouring molten metal into a mold. We can reconstruct the shape of teeth using materials such as wax, for easy processing and molding. The metal is then cast in the mold and the wax is eliminated so that just the cast metal remains. The wax pattern is invested into a mold, then the wax is eliminated by means of firing and replaced by cast metal. Including those used in the dentures, approximately five main metals - gold alloy (including gold-platinum alloy), gold-silver-palladium alloy, silver alloy, cobalt-chromium alloy, and titanium alloy - and two types of mold materials - phosphate-based and cristobalite-based (plaster-based) - are adequate.

Recently, although they are more expensive, the performance of automatic casting machines has improved, making fail-safe, stable production possible. The fitting accuracy and clinical prognosis of metal crowns made using dental precision casting is good, with accuracy within 100  $\mu$ m. In the case of short-span bridges, crown prosthetics can be fabricated easily and fitted within a margin of 100  $\mu$ m accuracy. Sato et al<sup>1</sup> described in detail the suitable accuracy of the margin of the crown required for clinical purposes. In addition,

Shiozawa et al described the fitting accuracy of crowns fabricated using the conventional method.<sup>2</sup> As a guideline, these are within approximately 100  $\mu$ m. The techniques and materials used, such as the scale of the equipment for casting, and instruments, are similar to those required by and used in the jewelry industry.

As such, precision dental casting technology has been carried out based on years of experience, but there are still unavoidable problems due to the metal. One such problem is metal allergy: a small percentage of the total number of outpatients at Hokkaido University Hospital complain of metal allergies. However, when we conducted patch tests on healthy human volunteers (such as students and medical staff), almost all of them showed some kind of reaction regardless of the amount of metal. Therefore, we believe that everyone has a risk of metal allergy and this may be due to the metal crown that is attached to the abutment tooth. Currently in Japan, if you are found to have the symptoms of metal allergy, it is almost impossible to find an alternative material that can be used under the health insurance system. For this reason, sometimes the patient is reluctant to receive adequate treatment due to the soaring costs. Therefore, metal-free materials are desired.<sup>3,4</sup>

Another problem, of course, is the matter of esthetics. Even if we produce a pale golden color with a gold alloy, for example, the color of the metal differs greatly to that of the natural teeth. Many years ago in Japan, if someone had a gold tooth, people might have felt it was a status of wealth, but not anymore. Nowadays a skilled dental technician can make a crown with a very natural texture, indistinguishable from the natural teeth of the patient. Almost all patients tend to prefer teeth that look slightly whiter than their natural teeth, and nobody wants cast gold tooth crowns. For the premolar region, health insurance does not cover the application of facing crowns using composite resin.

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This leaves us with a choice of an Au-Ag-Pd alloy crown (so-called silver under colored tooth) and a resin jacket crown that are allowed the health insurance, or an expensive all-ceramic crown that is not covered by the health insurance, leaving us with the dilemma of which to recommend to the patient.

### Introduction of industrial-use three-dimensional CAD/CAM systems in dentistry

It is said that fully-fledged three-dimensional computerized design was first used for aircraft design by Boeing, and on the body design of the Mercedes-Benz. In order to meet those requirements, mathematical theories such as Rational bézier surface and NURBS (Non-uniform rational B-spline surface) (Fig. 1), which are now in standard use on computer 3D CAD systems, were researched and developed. These 3D CAD systems with such mathematical theories can be used on our own personal computers now. However, in the 1980s, the capacity of the computer was insufficient, and a super mini-computer the size of several lockers, costing hundreds of millions of yen, had the same capacity as the game consoles used by children today (Fig. 2). Some three-dimensional CAD software was already available, such as CATIA developed by IBM, which continues to be used now, but because it was so expensive and the capacity of the computer was insufficient, its main use was to design molds for products for mass production in factories, not individual custom-made products, and three-dimensional CAD was never applied. Nevertheless, because of the scale of production in the automotive industry and the like, even if cost reductions were only slight, the impact on the entire production system was significant, leading to the rapid introduction of CAD/CAM systems in those industries.

Under such circumstances, in the field of dentistry, the CEREC system that attempted to produce ceramic inlay restoration by means of a CAD/CAM system was first developed jointly by Siemens AG in Germany and Prof. Mörmann at the University of Zurich, Switzerland (Fig. 3). At that time, biocompatibility and esthetically superior treatment was required as an alternative to amalgam fillings previously used in Europe and the United States, and the CEREC system was developed in response, with the potential for enabling immediate, chairside ceramic restoration.<sup>5</sup>

The first generation CEREC system was completed and introduced in Japan between the late 1980s and early '90s. By that time, the mathematical theory of handling three-dimensional curved surfaces had been introduced

to the Japan Prosthodontic Society, and the development of CAD/CAM systems for the production of dental crowns and bridges began in unison from there. Also, around the same time, the theory of stereo laser lithography was proposed almost simultaneously in Japan and the United States, laying down the foundations for the three-dimensional printer.<sup>6,7</sup>

### Completion of the dental CAD/CAM system and advent of zirconia ceramics

Development of dental CAD/CAM systems in Japan was carried out by several university-based groups. The most typical system, GN-I was released by GC Corp. (Fig. 4). Dr. Yoichi Uchiyama, who was a professor of Hokkaido University (currently professor emeritus of Hokkaido University), became chairman of this system's development committee. GN-I was developed as a national project under the New Energy and Industrial Technology Development Organization (NEDO) in Japan. The final evaluation report is available on the website of Japan's Ministry of Economy, Trade and Industry.

However, almost all medical care in Japan is carried out under the health insurance system. Since expenditure for dental prosthetic treatment is very low, we did not expect to obtain sufficient revenue to use the CAD/CAM system within this framework, so therefore the popularity of the device at the time did not spread. In addition, staff of the university had hoped to use ceramic materials, but problems regarding development capabilities and areas of expertise of the manufacturer meant that the use of ceramic-based materials did not spread easily.

As a result of such circumstances, development of these systems ended in Japan and dental CAD/CAM-related topics began to gradually fade out in the early 2000s. Meanwhile, application of zirconia ceramics in dental treatment progressed in Europe (especially in Germany) during this period, and dental CAD/CAM systems have been attracting increasing attention. The main reason for this is that zirconia ceramics cannot be processed by the traditional manual techniques of dental technicians. The firing temperature of zirconia ceramics is around 1,500°C, approximately 500°C higher than the conventional dental porcelain firing temperature. Although the strength and properties of zirconia ceramics after firing are superior to those of conventional dental porcelain, it becomes extremely difficult to process after firing. Therefore, it is necessary to process the ceramic before firing, but as we know that its volume shrinks by approximately 25 to 30% during firing, it is difficult to compensate for this, manually.



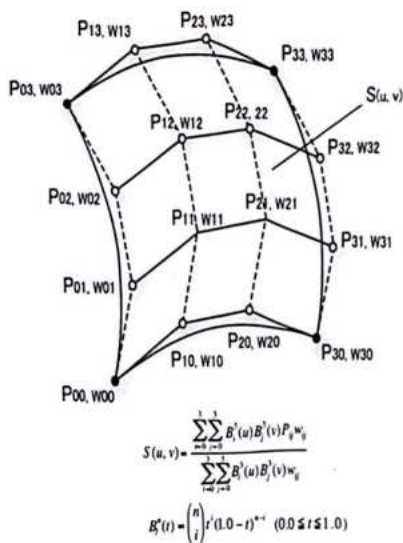


Fig.1 Bicubic rational Bézier curved surface  
(S : surface, B : parameter, P : control point, w : weight parameter)

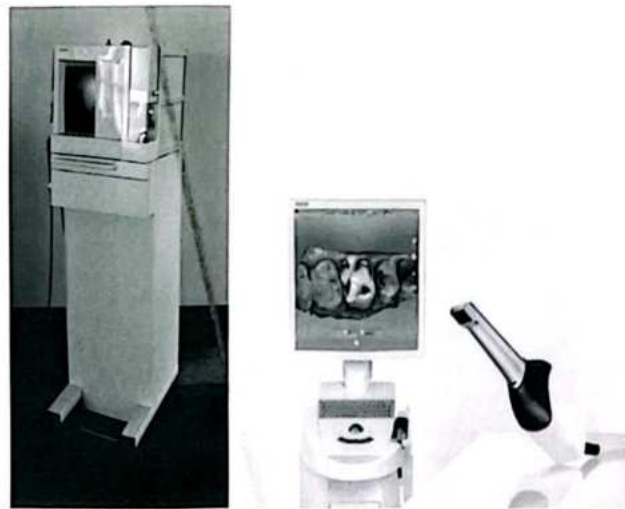


Fig.3 CEREC system (1st generation) (Left) and CEREC Omnicam system (right) (Sirona).  
CETEC system was a dental CAD/CAM system, which was released at the earliest in the world.  
URL : <http://www.sirona.com/en/news-events/campaigns/the-new-cerec-omnicam/>



Downsizing  
and  
High performance



Fig.2 Old Super mini-computer and new gaming machine

A : Vax 11/780 Super mini-computer (Digital Equipment Corporation /DEC).  
This computer was released in 1977, and its speed is only 1 MIPS.  
URL : <http://en.wikipedia.org/wiki/VAX-11>

B : Onyx visualization system (Silicon Graphics, Inc./SGI). This computer was released in 1993, and as for at the time, this computer was equipped with an excellent three-dimensional graphics system.  
URL : [http://en.wikipedia.org/wiki/SGI\\_Onyx](http://en.wikipedia.org/wiki/SGI_Onyx)

C : Playstation (SONY). This gaming machine was released in 1994, and in part, its performance was as good as the Onyx.  
URL : <http://en.wikipedia.org/wiki/Playstation>





Fig.4 GN-I dental CAD/CAM system (GC). This device was being developed by three principal companies that were GC Corporation, Nikon Corporation and Hitachi-seiko Corporation. Professor Uchiyama Y. of Hokkaido University had served as Chairman of the Development Committee. I was involved in the development of CAD software.

URL : <http://www.gcidental.co.jp/cadcam/system/gni.html>



Fig5 3 dimensional scanner D810 (3Shape). This device has become the de facto standard for virtually.

URL : <http://www.3shapedental.com/restoration.aspx>

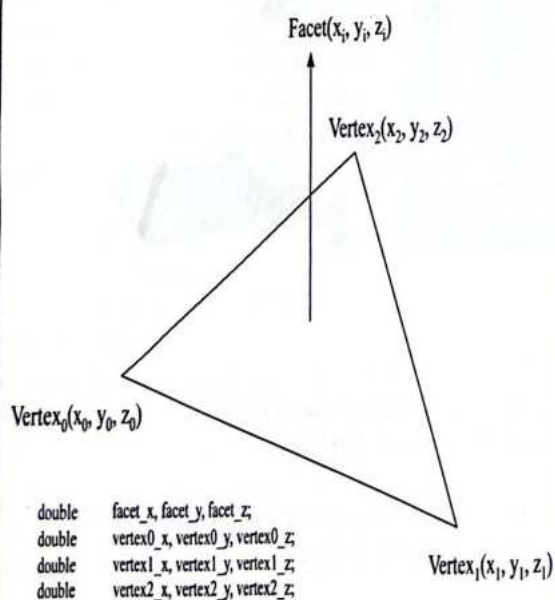


Fig.6 STL (Stereo lithography) format.

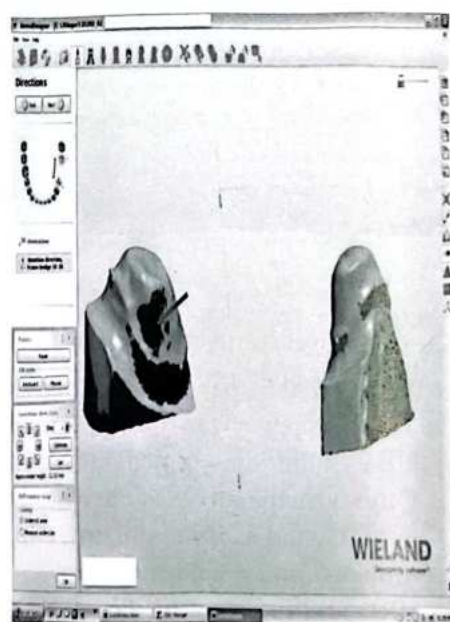


Fig.7 CAD screen of a bridge abutment teeth were pointed out to undercut (red arrow).



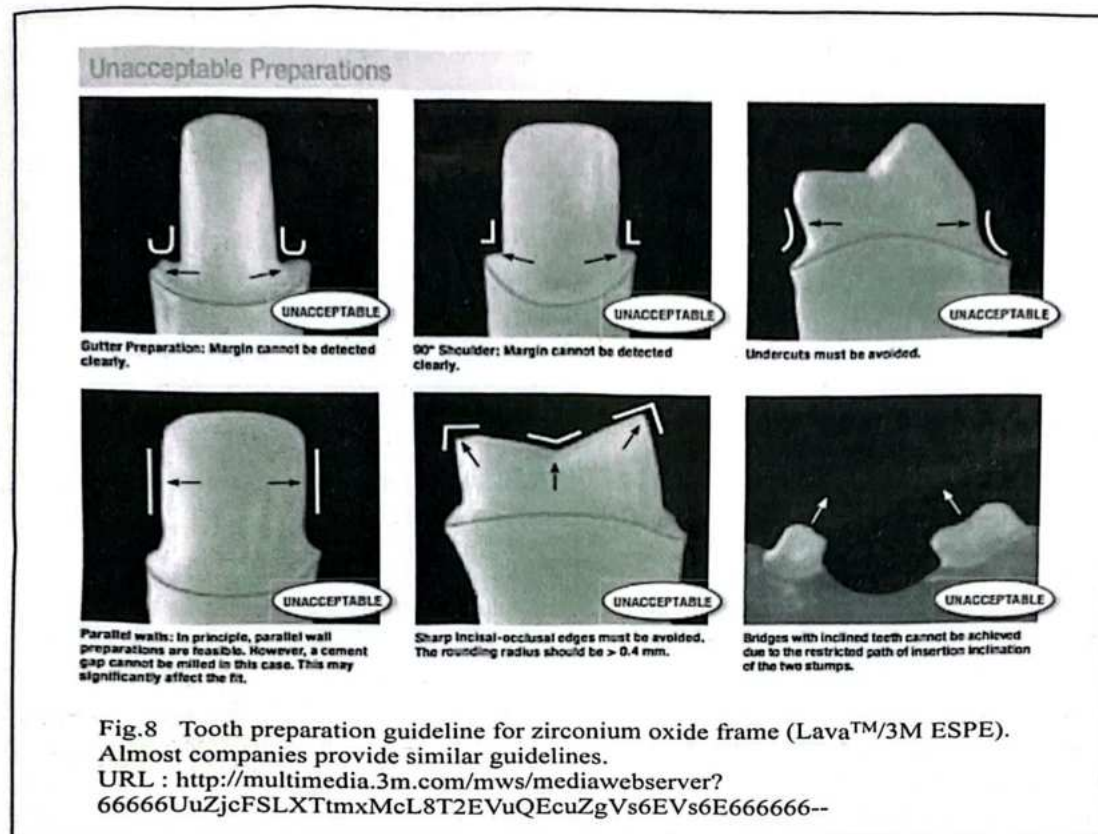


Table 1 Dental CAD/CAM systems main running in Japan

3M ESPE Lava  
 3Shape Dental System  
 DeguDent Cereon  
 Delcam iMetric  
 Dental Wings  
 DENTSPLY Compatis  
 GC Aadvia  
 KaVo ARCTICA & Everest  
 Kuraray Noritake Dental Inc. Katana system  
 Nobel Procera  
 Panasonic C-Pro System  
 Sirona CEREC  
 Straumann CARES  
 WIELAND Dental ZENOTEC  
 DMG ULTRASONIC 10/20 (Milling machine)  
 Zirkonzahn 5-TEC (Milling machine)  
 3D Systems Projet DP-3000 (Rapid prototyping machine)  
 envisionTEC Perfector (Rapid prototyping machine)  
 EOS EOSINT M280 (Rapid prototyping machine)

Table 2 The world's major Intra-oral optical scanners

Sirona CEREC Omnicam  
 D4D Technologies E4D Dentist  
 3M ESPE Lava Chairside Oral Scanner C.O.S  
 CADENT iTero  
 3Shape TRIOS



We have to compensate for the volume shrinkage in advance using computer simulation. By firing restorations that are designed and milled to a slightly larger size, it is finally possible to utilize zirconia ceramics in dentistry. In order to produce such zirconia restorations, the use of CAD/CAM systems has become essential. In addition, the price of personal computers has decreased and their capabilities now surpass those of the super-mini computer. The function and usability of three-dimensional scanners used in the initial part of work using CAD/CAM systems have improved significantly, creating the conditions for the rapid spread of CAD/CAM systems in dentistry around the world from the late 2000s through the 2010s.<sup>8-17</sup>

### **Selection of three-dimensional scanners and CAD software has already begun**

Of the dental CAD/CAM systems revealed around the world, the main ones introduced in Japan as of the period around 2010 are shown in Table 1. Initially, many of these systems were equipped with the company's original three-dimensional scanners, and many companies also had their own original design software, too. However, in the past couple of years, manufacturers have withdrawn from the development of scanners, and many systems have adopted the 3Shape company's 3D scanners (Fig. 5). If you compare the principles of measurement, 3Shape, Dental Wings, KaVo, and Hint-Els<sup>7-10</sup> use similar techniques, characterized by high-speed scanning by means of an encoded pattern projection method, to scan plaster working models. Of these, we believe the color-coded pattern method is the most technically advanced as it is fast and can acquire an enormous amount of information. However, the cost of 3Shape's machines decreased and they are now becoming the de facto standard, as many manufacturers began to adopt 3Shape's scanner as an OEM machine. We believe that within the next few years, these scanners will progress by means of natural selection. In this case, if one company monopolizes the market, there will be no choice, and therefore the cost will not go down, which is undesirable. If several companies remain and the de facto standard is narrowed down, easier data compatibility can be anticipated.

On the other hand, with regard to dental CAD software, 3Shape, SensAble Technologies Inc., Delcam, Dental Wings Inc, etc. continue to occupy a major position. Each company maintains its own independent internal format for CAD data. Currently, each company is reluctant to support the compatibility of data output in a

general-purpose CAD format. However, most of these support CAM data output in STL (Stereolithography) format (Fig. 6). Therefore, once the design is finished, the data output in STL format can be processed by any other CAM system. STL format is a very simple data structure, and the processing accuracy required for prosthodontic fitting is less than 100  $\mu$ m. For small factories that produce metal dies, this is an undemanding level of request that is extremely simple. As a result, future zirconia frame processing may be carried out as a side business of such small factories, enabling mass production in different locations from the dental laboratory.

### **Careful review of abutment tooth formation is important**

With the introduction of dental CAD/CAM systems, many plaster working models were measured three-dimensionally, and it became clear that many abutment teeth were not formed properly. This problem had not surfaced before because it had been covered by the skill of the dental technician's work. Abutment teeth of bridges we thought were parallel were surprisingly undercut (Fig. 7). However, when it became clear what needed to be done, the only thing that could deal with it flexibly was human skills. If we want to produce zirconia frames using non-contact optical measurement and milling technology, the form of the abutment teeth must be a little different from conventional ones (Fig. 8). In addition, if dentists can actively utilize dental CAD/CAM systems, they will be able to fix abutment tooth formation problems themselves and provide more accurate treatment.

### **Do intra-oral scanners for optical impressions have a future?**

Currently, there are approximately five companies in the world that sell intra-oral scanners for optical impressions (Table 2). From the point of view of those of us who are accustomed to dental handpieces, such as turbines and engines, the probe of the intra-oral scanner is quite large and looks difficult to operate. But when actually tested, the scanning and processing speed of the intra-oral scanner seemed very fast, and it is possible to capture the three-dimensional shape of the teeth in quick succession. This technique promises to provide an alternative way of taking impressions for and fabricating study models, and would be ideal for recording the three-dimensional shape and occlusion of the teeth efficiently. It is believed that the time and cost of the work can be significantly reduced. On the other hand, because this is



an optical measuring method, it is impossible to measure the shape of the parts that are not visible. So if the margin line of the abutment tooth is hidden beneath the gingival margin, unless gingival retraction is carried out and the margin line can be seen directly, it is difficult to scan the shape. It is hoped that there will be a technological breakthrough to enable the subgingival margin to be measured even if it isn't directly visible, but this may be difficult. In this regard, although we cannot completely avoid the risk of radiation exposure, the authors are pinning their hopes on three-dimensional reconstruction image data of dental cone beam CT. If the resolution of cone beam CT were 100 times better than now, and if the dose can be reduced to a fraction of what it is, there is a possibility that the data obtained can be used for crown and bridge fabrication. The problem is, the people who have developed dental CT do not believe there is such a market or demand. As a result, devices remain expensive, shipments do not increase and the cost does not go down. However, when researchers specializing in radiation were questioned about this, they predicted that it will be technically possible to improve the resolution to 100 times that of now, within a decade. Regardless of whichever technology becomes more advanced first, there is no doubt that the impressions and bite-record methods used now will no longer be used in the very near future.

#### **Expectations for all-ceramic restoration using zirconia frames**

It is said that the flexural strength of zirconia ceramics is approximately 1,200 MP. If there are no mistakes in the design of the frame, it can withstand nearly twice the maximum bite force of the posterior region. Results of aging tests published by some manufacturers show strengths to be approximately half of the initial value after five years. Therefore, it is expected that long-term strength can be secured to some extent. It is almost always the aesthetic advantages of all-ceramic restoration that are mentioned, but it is thought that one of the main features is that, compared with resin- and metal-based materials, it becomes stained much less easily. Therefore, if the cervical margin is set properly, we can minimize the deposition of plaque on the

lingual cervical area, where brushing is the most difficult. In this way it is considered possible to reduce the risk of retreatment of secondary caries. Unfortunately, sufficient data from the course of prognosis has not yet been obtained, but once it becomes apparent, it will surely become a major force in the promotion of all-ceramic restoration.

Since it is difficult to process zirconia ceramics, the cost of production has not decreased sufficiently. However, the raw materials are available at a very low price. Most of the world's powdered raw material of zirconia ceramic is supplied by Tosoh Corporation and DAIICHI KIGENSO KAGAKU KOGYO CO., LTD. in Japan. Meanwhile, three-dimensional printing technology representative of the additive manufacturing technology has been making steady progress. If the ceramic materials are available to this technology, a significant cost reduction in all-ceramic restoration will be possible and a significant global-scale spread can be expected.

#### **Future of dental technicians in Japan**

According to the statistical data of Japan's Ministry of Health, Labour and Welfare, the number of dental technicians over the age of 50 in Japan reached 39% at the end of 2010. Because of the balance of the pensionable age in Japan, the government is trying to promote a mandatory retirement age of 65, which means that nearly 40% of dental technicians currently working will quit their jobs within the next 15 years. Although caries prevalence is declining year by year in Japan, aging of the population is progressing rapidly and it is unlikely that demand for prosthetic dental treatment will decrease rapidly. As an efficient way of continuing to substitute the work of skilled dental technicians, the spread of dental CAD/CAM systems is desired.

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## A case of malunited mandibular fracture treated by intentional osteotomy

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### Abstract

Fracture in facial region for various reasons is common problem in our country. Commonly this fracture occurs in mandible. To restore the functional and masticatory load, intentional osteotomy can be chosen. Here we present a case of malunited mandibular fracture in 38 years old man where intentional osteotomy was performed successfully.

**Key Words:** Mandibular fracture, Intentional osteotomy

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### Introduction

Fracture may be defined as loss of continuity of bone with or without loss of bone contour due to trauma or pathology.<sup>1</sup> So mandibular fracture also defined as breach of normal anatomical contour of mandible. This fracture can be classified according to type, site, direction, cause and treatment option.<sup>2</sup>

The treatment option for mandibular fracture is open or close reduction, immobilization & fixation.

### Presentation of case

A 38 years old man reported to Sapporo Dental College & Hospital in 28th July, 2012 with the history of road traffic accident 2 months back which caused fracture in lower jaw and right lower limb. Then he reported to a renowned college and hospital for treatment. His right lower extremity fracture was treated successfully & he was advised to take rest for several weeks for jaw fracture. However he was unable to chew from his lower right jaw and feel pain and discomfort during chewing.

On extra oral examination facial asymmetry & step deformity in lower border of the mandible was found. Intra oral examination revealed occlusal disharmony in the left side of lower jaw and missing all anterior incisor teeth. His vital signs were within normal range & there was no significant medical problem in any organ.

The Orthopantomogram (OPG-X-Ray) revealed a radiolucent line extending distally & laterally from the mesial aspect of lower left canine to the lower border of the mandible in the region of lower right first molar. His

routine hematological picture, blood sugar level, ECG & chest-X-Ray were within normal limit.

The case was diagnosed as compound horizontal unfavorable malunited fracture of the right side of the mandible. Since 2 months had elapsed after the trauma an union of the fracture fragments had occurred in malposition. As the occlusion of right side was normal & all incisors were missing, decision was taken for osteotomy in the symphyseal region through an intra-oral approach to bring back the left side into normal occlusion.

Open reduction & immobilization was done on 1st August, 2012 under General Anesthesia. Arch bar was fixed on upper & lower jaw. A semilunar incision was given from left lower first premolar to right lower second premolar and mucoperiosteal flap was raised with the help of periosteal elevator. Osteotomy was performed with micromotor & chisel mallet at the symphyseal region of the mandible. Then the left side of the lower jaw was brought into its normal position & immobilization was done with Trans-osseous wiring & bone plate. Finally the wound was closed in layers & the postoperative phase was uneventful.

Two months postoperatively, the occlusion was normal & mouth opening was satisfactory.

### Pre-operative pictures

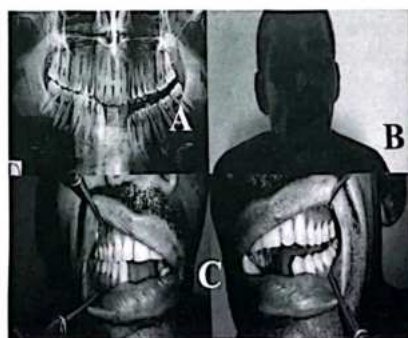


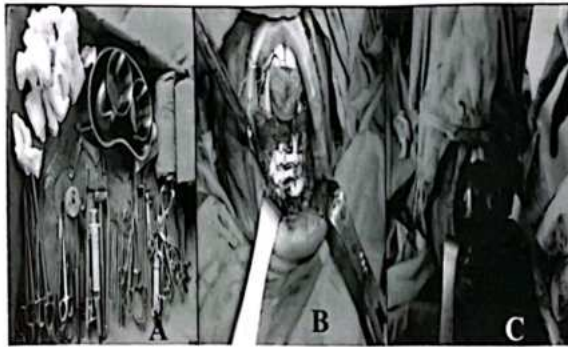
Fig.1 A: Radiographic, B: extraoral and C: intraoral disharmony

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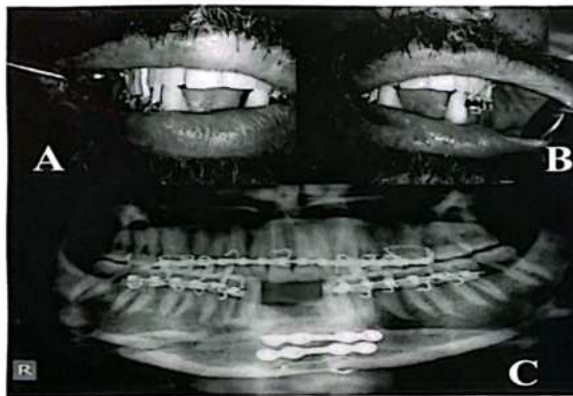
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*Per-operative pictures*

**Fig. 2** A: Instruments, B: After osteotomy immobilization of 2 fragmented part done by bone plate and trans-osseous wiring; C: photograph after suturing

*Post-operative & follow up pictures*

**Fig. 3** A & B: Showing occlusal harmony after the operation; C: OPG-X-Ray showing the fracture line, arch bar wiring, mini bone plate, trans-osseous wiring

**Discussion**

Intentional osteotomy is usually performed to correct skeletal malocclusions, that is discrepancies in tooth position that can not be corrected by simple orthodontic movement and realignment of the temporomandibular joints or to correct facial deformities such as mandibular retrognathia.<sup>3-6</sup>

Several studies were available in literature where intentional osteotomy was performed to treat malunion in mandibular fracture. In Hungary Oblique osteotomy of the body of the mandible has been successfully employed to treat seven cases of malunion following fracture with bone loss due to war injury. Another case in which the technique was used to treat a mandibular deformity resulting from an infection in childhood.

Conventionally, extra oral incision is essential for reduction of fracture of body of mandible. Skin scar formation is a natural phenomenon following extra oral incision. In this case symphyseal region was closed for intentional osteotomy to avoid extra oral skin scar development & injury to the neurovascular bundle in mental foramen. Apart from above mentioned advantages, the procedure described in this paper is very simple, easier & less time consuming.

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## Surgical Exposure and Orthodontic Treatment of Bilaterally Impacted Maxillary Central Incisors: A Case Report

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### Abstract

*Maxillary central incisor impactions occur infrequently. Their origins include various local causes, such as odontoma, supernumerary teeth, and space loss. Supernumerary and ectopically impacted teeth are asymptomatic and found during routine clinical or radiological examinations. The surgical exposure and orthodontic traction of impacted right central incisor after removal of odontomas is presented in this report.*

**Key words:** Impacted teeth, maxillary central incisor, supernumerary teeth, odontoma

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### Introduction

Impaction is the total or partial lack of eruption of a tooth well after the normal age of eruption.<sup>1</sup> The most commonly impacted maxillary tooth is the canine. Ericson and Kurol<sup>2</sup> estimated the incidence at 1.17%. The frequency of maxillary central incisor impaction ranges from 0.06% to 0.2%.<sup>3</sup>

The most common causes of impaction seem to be odontoma, supernumerary teeth, and loss of space. Impactions caused by disturbances in the eruption path related to crowding are somewhat less common.<sup>4</sup> Other causes are crown or root malformation of permanent incisors due to trauma transmitted from the primary predecessors and apical follicular cysts that prevent normal eruption.

The anterior maxilla is a highly demanding area from anesthetic point of view, and orthodontic treatment of impacted maxillary incisor requires a well synchronized and interdisciplinary approach to obtain an acceptable esthetic and functional result.<sup>5</sup>

This report presents the surgical and orthodontic treatment of a case with impacted maxillary right central incisor caused by Odontoma located in the eruption path of the impacted tooth.

### Case presentation:

A 12 year old male patient reported to the Department of Orthodontics & Dentofacial Orthopedics at Dhaka Dental College & Hospital, Dhaka with the chief complaint of delayed eruption of both maxillary permanent central incisor. The patient was physically healthy and had no history of medical and dental trauma. His medical history showed no contraindications to orthodontic treatment (Figure 1).

The patient had a balanced facial pattern. Intraoral examination showed late mixed dentition with good oral health, an Angle Class I molar relationship with missing both maxillary permanent central incisor and no apparent arch length discrepancy in maxillary & mandibular arch (Figure 2).

The panoramic radiograph demonstrated both maxillary central incisor were impacted due to the presence of two impacted odontomas located in their eruption path. The impacted maxillary central incisor was positioned vertically. (Figure 3).

### Treatment objectives:

1. Surgical removal of odontomas and exposure of the impacted maxillary permanent central incisors, apply orthodontic traction with light forces, and align the maxillary dental arch.
2. Establish ideal overbite and overjet.
3. Improve facial esthetics.

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**Treatment progress:**

The patient was transferred to an oral surgeon for surgical removal of odontomas and exposure of the impacted teeth, and the closed eruption technique was used to extrude the impacted incisors. Local anaesthesia was administered using Lignocain with 0.006 mg/ml adrenaline. A full thickness mucoperiosteal flap was elevated, approximately two-thirds of the crown was exposed with appropriate bone removal by means of surgical burs. Odontomas were removed and the impacted incisors was isolated with haemostatic agent. A brackets were bonded on the labial surface of the impacted maxillary central incisors (Fig 4). The flap was returned to the same position and sutured, leaving a tied 0.010-in ligature wire protruding through the mucosa and attached to the bracket. After a week, a light force of 60 to 90 g was applied. Orthodontic traction of the impacted incisors was accomplished with a maxillary removable appliance containing a high labial archwire (Figure 5).

When the incisors reached the occlusal plane, removable appliance were removed and leveling and alignment was done with a 0.016-in nickel titanium wire (Figure 6).

After the teeth had erupted into its position in dental arch and satisfactory interdigitation was achieved, the fixed appliances were removed. The patient was given maxillary Hawley retainers with instructions for initial full-time wear and then long-term night time wear.

**Treatment results:**

The overall active treatment time was 18 months. The impacted maxillary central incisors were brought into an acceptable position within the arch. The most significant change was a dramatic improvement in the patient's smile (Figures 7), and the final appearance of the teeth was esthetically pleasing with gingival margins at the same level and similar clinical crown sizes. After completed treatment, the repositioned incisors had an acceptable gingival contour and width of attached gingiva (Figures 8). The post-treatment radiograph showed the newly positioned incisors showed no periodontal bone loss, minimal root resorption, acceptable root parallelism, and root form (Figures 9).

**Discussion:**

Impacted teeth can cause serious dental and aesthetic difficulties as well as psychological problems especially in anterior regions. Although the impacted maxillary incisor occurs less frequently than the

maxillary canine, it is of concern to parents during the early mixed dentition stage because of noneruption of the tooth.<sup>6</sup>

Maxillary central incisor impactions occur infrequently; their origins include various local causes, such as odontoma, supernumerary teeth, space loss, and disturbances in the eruption path, also trauma and apical follicular cysts.<sup>4,7</sup> In the patient treated here, the upper permanent central incisors was disrupted by odontom.

Impaction of maxillary anterior teeth can be a challenging orthodontic problem. Several reports have indicated an impacted tooth can be brought into proper alignment in the dental arch.<sup>8-11</sup> The following factors are used to determine whether successful alignment of an impacted tooth can take place: (1) the position and direction of the impacted tooth, (2) the degree of root completion, (3) the degree of dilacerations, and (4) the presence of space for the impacted tooth.<sup>12</sup> Holland has recommended the movement axis of the impacted tooth must be considered together with these factors.<sup>13</sup>

The treatment approach of impacted maxillary teeth requires the cooperation of dental specialties such as orthodontics, oral surgery, and prosthodontics. Several reports have demonstrated successfully treated impacted maxillary anterior teeth by proper crown exposure surgery and orthodontic traction.<sup>9-14</sup>

Several techniques are commonly used to uncover maxillary labial impactions. One technique, the apically positioned flap, consists of apically repositioning a raised flap that incorporates attached gingiva overlying the impacted tooth. Another technique, the closed-eruption technique, involves raising a flap that incorporates attached gingiva over the impacted tooth, attaching an orthodontic bracket to the tooth, and then fully replacing the tissue over the tooth and bracket.



Each technique offers certain advantages during forced eruption of impacted teeth. The apically positioned flap technique permits ready reattachment of a bracket if unintended debonding occurs. However, the closed-eruption technique is believed to provide the most aesthetically pleasing result<sup>15,16</sup>. In the present case, the closed eruption surgical technique was used. This technique is more reliable when aesthetic and periodontal health is considered. Vermette et al. recommended the usage of the closed eruption technique when the tooth is in the middle of the alveolus or high near the nasal spine.<sup>15</sup> In this case the periodontal status of the exposed incisor after orthodontic treatment revealed an acceptable gingival contour and attached gingiva. No further mucogingival surgery was recommended.

### Conclusion:

Successful management of anterior impacted maxillary teeth can be challenging in a clinical practice. Proper diagnosis concerning the exact localization of the impacted tooth, an appropriate surgical technique, and a light orthodontic force system can be an effective approach to successfully bring the tooth into occlusion. The closed-eruption technique provided an esthetically pleasing result in this patient.



Figure 1. Pre-treatment facial photographs



Figure 2. Pre-treatment Intraoral photographs

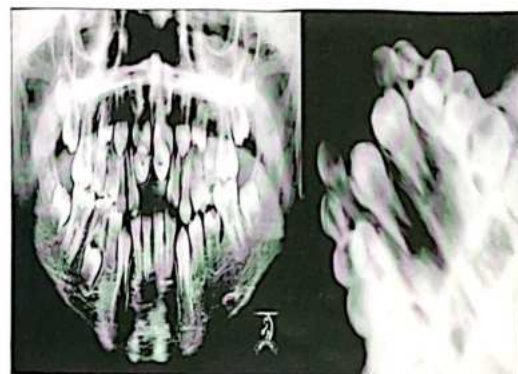


Figure 3. Pre-treatment Panoramic Radiographs

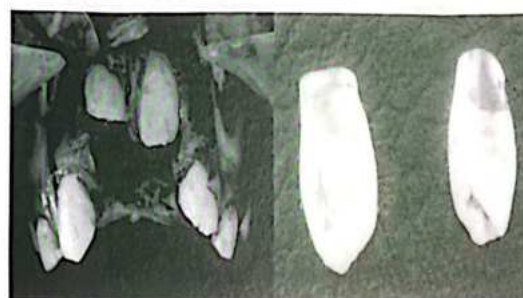


Figure 4. Clinical view at surgical exposure



Figure 5. Orthodontics traction of the impacted incisors



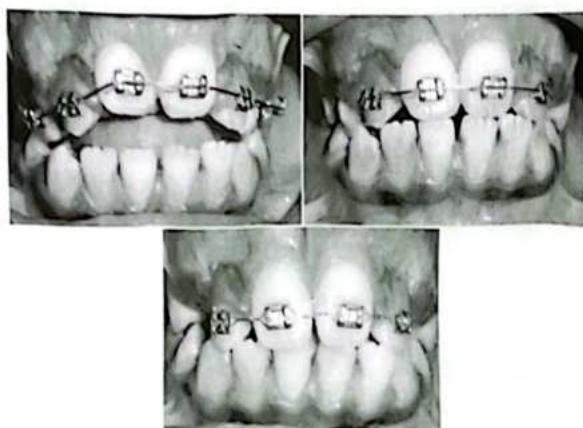


Figure 6. Progress intraoral photographs



Figure 7. Post-treatment facial photographs

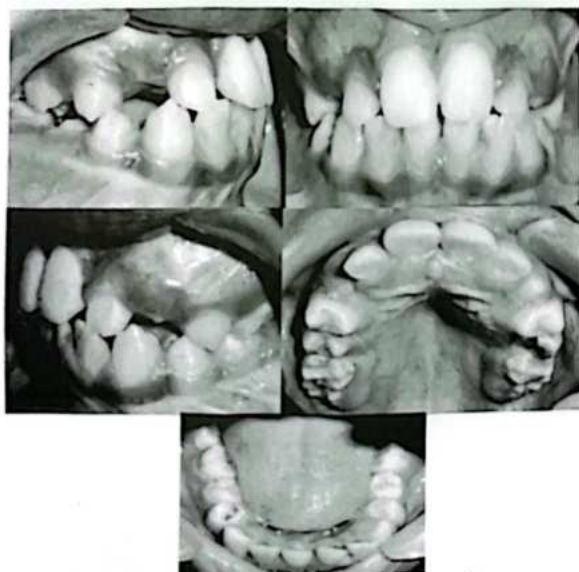


Figure 8. Post-treatment intraoral photographs

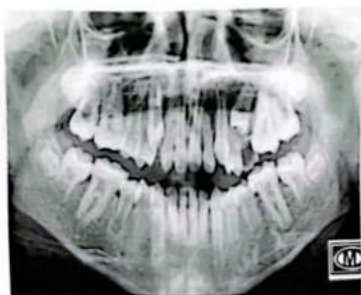


Figure 9. Post-treatment radiographs.

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