The effect of off-axis seating on the marginal adaptation of full coverage all ceramic crowns

SADJ July 2020, Vol. 75 No. 6 p303 - p310

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ABSTRACT

Introduction

No studies on the marginal gap or internal fit of crowns have reported the effect of non-axial seating which may often occur inadvertently clinically.

Aim

Therefore this *in vitro* study sought to investigate the offaxis seating of CAD/CAM crowns and its effect on the marginal gap and internal fit.

Method

A standardised crown preparation on a typodont tooth was used to design and mill 30 crowns with a flat occlusal surface. Ten Zirconia (Dentsply Sirona, Germany), 10 Enamic (Vita, Austria), and 10 Brilliant Crios (Coltene, Switzerland) crowns were milled, five of each milled with a luting space of 100 μ m, and five of 200 μ m. The marginal gap was measured in two and three dimensions after luting with silicone on a 3D-printed metal replica. Seating occurred axially, at 5° buccally and 5° lingually. The silicone was used to calculate the internal fit.

Results

Axial seating with a 100 μ m luting space obtained the smallest marginal gap, irrespective of material or luting space. 3D measurements were larger than 2D measurements, but not significantly. The maximum off-axis gap was 117 μ m, on the opposite side to which pressure was applied.

Conclusions

Care must be taken clinically to ensure that luting takes place in an axial direction only.

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Keywords

Marginal gap, Internal fit, Luting Space, full crown.

INTRODUCTION

The introduction of Computer-Aided Design and Computer-Aided Manufacturing (CAD/CAM) technology has allowed for improved aesthetics compared with ceramometal crowns.¹

The luting space within a prosthesis is created to allow the formation of a film of luting agent between the tooth and prosthesis. With CAD/CAM this space is created by selecting the milling parameters within the software to produce a pre-defined cement space when the restoration is milled. However, different manufacturers (of which there are now more than 70)² have recommended different luting spaces, and several studies have linked luting space to marginal gap measurements.³⁻⁷

Recommendations have ranged from 10 μm to 100 μm , with the larger spaces generally producing the smaller marginal gaps both before and after actual or replicated cementation. $^{4-6,8-12}$

The milling process to achieve the luting space is limited by the size of the burs used, and the movements of the axes of the milling machine. This in turn influences the preparation form, and in case that form is not ideal, manufacturers have recommended a luting space of up to $100\,\mu$ m. The smallest diameter bur is generally 1 mm and so any sharp edges in a preparation would not be reproduced, hence the $100\,\mu$ m recommended space.

The marginal gap can be defined as the vertical and horizontal dimension from the finish line of the preparation to the margin of the restoration. The internal fit can be described as the area between the crown and the tooth that will be occupied by cement.¹³

Failure of restorations to seat completely can result in a sizeable marginal gap and occlusal prematurities resulting in sensitivity, and may cause the prosthesis to loosen prematurely.¹⁴

Discrepancies in the marginal gap can lead to microleakage;¹⁵ plaque retention at the margin;¹⁶ secondary caries and pulpal involvement;^{17,18} and changes in the microflora causing the development of periodontal disease,¹⁹⁻²¹ any and all of which could ultimately result in failure of the crown.

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Many studies have measured the marginal gap and internal fit of full coverage restorations using different methods, with varying results. It is generally accepted that marginal gaps below 120 µm are clinically acceptable.²²⁻²⁶ With regards to the internal fit, it is clinically relevant to ensure that adequate space is created to allow an even thickness of dental cement.

The marginal gap was was originally measured at a few points around the circumference but it has been found that to determine an accurate marginal gap it is necessary to measure at least 18 locations around the circumference of the tooth.²⁷

Several methods have been used to evaluate the marginal gap including the use of an optical microscope;²⁸⁻³³ using a profile projector;³ profilometry;³⁴ embedding in epoxy resin and sectioning and measured with a threedimensional microscope;³⁵⁻³⁹ cementation and use of microCT;⁴⁰⁻⁴³ and the use of a silicone luting replica technique.^{3,13,30,35,41,43,44} The silicone replica technique can also be used to measure the overall total fit of the crown and provides a correlation with the marginal gap.

None of the studies have reported on the effect of non-axial seating discrepancies, and these are known to happen in the clinical environment, as finger pressure is used to cement a crown.

Therefore this *in vitro* study sought to investigate the off-axis seating of CAD/CAM crowns and its effect on the marginal gap and internal fit, using three different materials, a zirconia (Dentsply Sirona, Germany), a polymer infiltrated ceramic network (PICN) (Enamic, Vita, Austria), and a composite (Brilliant Crios, Coltene, Switzerland).

METHOD

A resin typodont molar tooth was prepared to produce a standardised crown preparation with a total convergence angle of 12 degrees as measured digitally from the scanned image using Finite Element Analysis (FEA) Software (Solidworks, SolidWorks Corp, United States), internally rounded shoulder margins of 1.5 mm circumferentially, and an occlusal reduction of 1.5 mm. All line angles were rounded. The surface area of the preparation was calculated from the scan using the FEA software, to aid in the internal fit calculations.

The typodont tooth was scanned with the CEREC Omnicam intra-oral scanner (Sirona Dental Systems. Germany), and 30 crowns were milled with a flat occlusal surface. The flat occlusal surface of the crowns aided in seating the crown off-axis and axially. Ten Zirconia, 10 Enamic, and 10 Brilliant Crios crowns were milled using a CEREC MC X milling machine (Sirona Dental Systems, Germany). In each group, five crowns were milled with a luting space of 100 µm and the other five crowns with a luting space of 200 µm.

Each crown was then seated on the metal replicated tooth set in a typodont model with adjacent teeth to provide contact points. The typodont model was set on a custom-made tilting device (adapted from the model-



Figure 1. A milled crown with a flat occlusal surface. Figure 2. Images showing the replicated typodont tooth into metal.



Figure 3. Two-dimensional measurement only records the vertical height (V) but does not consider any overlap (either positive or negative), hence the 3-dimensional measurement taking into account both the horizontal (H) and vertical gap in three dimensions (3-D) is a more realistic representation of the marginal gap.

holding device of a model surveyor) that allowed the model to be tilted 5 degrees to either side, and a standard 3 kg weight was lowered parallel to the ground simulating cementation pressure.

Each crown was filled with light-body polyvinyl siloxane material (Express XT light-body quick, 3MESPE, Germany) to represent the luting agent and seated onto the tooth. A constant load was placed on the crown with the 3 kg weight for 10 minutes with the model either straight, til-ted 5 degrees buccally or 5 degrees lingually.

Excess impression material was removed using a scalpel. Thereafter the marginal gap was measured at 12 points according to marking points on the metal tooth at 6 points buccally and 6 points lingually.

The marginal gaps were measured at these points using a Reflex Microscope (Reflex Measurement Ltd., Cambridge, UK) which is a microscope and an optical plotter that uses a virtual point of light to measure objects in

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two and three dimensions (Fig. 3), with an accuracy of $4\,\mu$ m.⁴⁵ There is some difficulty in locating the virtual point of light, especially on the z-axis, and so the entire experiment was repeated on three separate occasions to assess measurement consistency.

The crown was then removed, and the silicone impression material removed and weighed to calculate the overall internal fit according to the formula:³

thickness (*internal gap*) = $\frac{\text{weight}}{\text{surface area x density}}$

Sample size and statistical analysis

The literature review has shown that marginal gaps of greater than 120 μm were considered the limit of clinical acceptability.

Given an expected mean marginal gap of 110μ m for any group, and aiming to detect a difference of more than 20% from this, given a within-group relative standard deviation of 22% (which corresponds to an effect size of d=0.83), 80% power and the 5% significance level, a total sample size of 24, i.e. 4 per group, would be required.⁴⁶ It was decided, however, to use 5 per group as the expected mean gap may differ from the above.

Reliability was tested by the Intra-class Correlation Coefficient (ICC). Test-retest reliability for whether or not the marginal gap exceeded $120\,\mu m$ was determined by Cohen's kappa.

Post-hoc tests were carried out using the Tukey-Kramer adjustment for unequal group sizes (to allow for the deletion of outliers). From the *post-hoc* tests, the materialluting space combinations which had the smallest values for the outcomes were determined.

All measurements were below the limit of 0.120 mm, so it was not necessary to measure comparisons between the experimental groups. Comparison of the marginal gap between matching 3D measurements was carried out using the paired samples t-test.

Within each material, across both luting spaces, the difference between buccal and lingual readings for each seating direction was compared using the paired samples t-test. The effect of luting space on the 2D and 3D outcomes for each direction of seating, was determined by a repeated measures ANOVA with the outcome as the dependent variable, luting space as the independent variable, and experiment as the repeated measure. The effect of material was determined similarly. Data analysis was carried out using Statistical Analysis Software (SAS) version 9.4 for Windows. The 5% significance level was used.

RESULTS

The Intraclass Correlation Coefficients (ICC) for the marginal gap measurements ranged between 0.78 and 0.99, representing excellent agreement and so the average of all three sets of measurements was used for further analysis.

Marginal gap measurements

Table 1 shows the minimum, maximum, and mean mar-ginal gap measurements in all scenarios.

In all circumstances, the marginal gap did not exceed 120 μ m. For all materials and luting spaces, the maximum value (117 μ m) occurred on the buccal marginal gap when seating was applied at an angle to the lingual, and on the lingual marginal gap (112 μ m) it occurred when seating was applied at an angle to the buccal.

For all 2D and 3D measurements and their differences between buccal and lingual, the ANOVA showed that the three-factor interaction was significant for each measurement set (Table 2).

The differences between the buccal and lingual marginal gap measurements for each seating direction were calculated and then compared, combining the 100 and 200 μ m measurements.

In all cases, the buccal and lingual measurements differed significantly for buccal and lingual seating angles except for two 3D measurements for Enamic and Zirconia which were not significantly different for axial seating, but this direction yielded the smallest difference ranging from $3.2 \,\mu\text{m} \cdot 20.1 \,\mu\text{m}$.

When comparing materials, there were no significant differences between materials for any seating angle. The smallest differences were again found for the axial seating. For 2D measurements, this ranged from 10.4 μ m – 20.1 μ m, and for 3D measurements, this ranged from 3.5 μ m - 9.4 μ m.

For all materials, the differences between the buccal and lingual marginal gaps were grouped into the buccal, axial and lingual seating directions, to compare the luting spaces (Table 3).

The only statistically significant differences between the 100 μ m and 200 μ m spaces, for both the 2D and 3D measurements, were for the axial direction of seating. The actual gaps averaging all buccal and lingual measurements for the axial seating only are shown in Table 4.

All 2D and 3D measurements, irrespective of material, pressure and luting space were then compared. The 3D measurements for the buccal marginal gap were an average of 13.5 μ m higher than the corresponding 2D measurements (95% confidence interval: 12.0-15.0 μ m; p<0.0001).

The 3D measurements for the lingual marginal gap were an average of 13.4 μm higher than the corresponding 2D measurements (95% confidence interval: 10.9-15.8 $\mu m; \, p < 0.0001).$

When the buccal and lingual gaps were combined, the 3D measurements were an average of $13.4 \,\mu\text{m}$ higher than the corresponding 2D measurements (95% confidence interval: $11.7-15.1 \,\mu\text{m}$; p < 0.0001).

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Table 1. Summary of buccal and lingual marginal gap readings for all three materials and both luting spaces. Shading indicates the largest gaps in each measurement group of material and luting space combination.							
Material	Luting space	2D/3D	Seating direction	Reading	Min µm	Max µm	Mean µm
				Buccal MG	35.7	44.3	40.1
			Buccal	Lingual MG	53	91.3	70.9
				Buccal MG	37.3	57	44.4
		2D	Axial	Lingual MG	43.7	55.3	49.3
				Buggal MC	40.7	97.2	72.0
			Lingual	Buccar MG	00.7	07.3	73.9
Zirconia				Lingual MG	35.3	44.3	40.9
			Buccal	Buccal MG	47.3	59	55.4
				Lingual MG	70.3	101	88.3
		3D	Axial	Buccal MG	48.7	65.7	58
				Lingual MG	45.7	59.7	53.1
			Lingual	Buccal MG	78	101	86.8
			Engua	Lingual MG	43	59	50.2
			Ruccal	Buccal MG	40.7	47.3	44.6
			Duccai	Lingual MG	51	63	58.6
		00	A	Buccal MG	45.3	54.3	48.8
		20	Axiai	Lingual MG	51.7	60.3	56.3
				Buccal MG	63	65.7	64.3
			Lingual	Lingual MG	33.7	45.7	37.9
Enamic	100µm			Buccal MG	53.7	62.3	57.1
			Buccal	Lingual MG	70.7	91 7	81.3
				Buccal MG	55.3	61.7	57.5
		3D	Axial	Lingual MG	57.7	62.2	60.6
				Bussel MC	60.2	75	70.0
			Lingual	Buccar MG	09.3	75	72.0
				Lingual MG	40.3	58	50.1
		2D	Buccal	Buccal MG	41.3	73	51.5
				Lingual MG	54.7	92.7	65.2
			Axial	Buccal MG	41	82.7	51.4
				Lingual MG	44.3	92.3	56.9
			Lingual Buccal	Buccal MG	58.7	102	70.7
Brilliant				Lingual MG	34.7	39.3	36.2
Crios				Buccal MG	50.7	81.7	61.9
				Lingual MG	68	109	85.1
		00	A	Buccal MG	46.7	86	56.3
		30	Axial	Lingual MG	45	95.3	59.7
			Lingual	Buccal MG	67.3	108	78.4
				Lingual MG	35	45.7	40.3
				Buccal MG	57.3	62.7	60.5
		2D 3D	Buccal	Lingual MG	58.7	89	69.9
				Buccal MG	30.7	56	46.8
			Axial	Lingual MG	66.3	87.7	82.1
				Buccal MG	51.3	72.3	62.5
			Lingual	Lingual MG	32	48	40.1
Zirconia				Buccal MG	75	80	90.0
			Buccal		15	100	02.0
				Russel MC	60.0 E1	70	90.9
			Axial	Buccarivig	51	70	00.7
				Lingual MG	66.7	98.7	82.6
			Lingual	Buccal MG	69	81.7	76
				Lingual MG	47	59.3	51.6
			Buccal	Buccal MG	43.3	50.3	47.4
				Lingual MG	66	77.3	71.8
		20	Axial	Buccal MG	40.3	54.3	47.1
		20	7 0104	Lingual MG	49	68.3	60.7
			Linguel	Buccal MG	48.3	94.3	75
Energia	000		Lingual	Lingual MG	32.3	38	35.3
Enamic	200µm	3D		Buccal MG	65	75.7	73.1
			Buccal	Lingual MG	97.3	113	106.9
			Axial	Buccal MG	56.3	65	60.5
				Lingual MG	50	72.7	63.9
				Buccal MG	71.3	103	88.5
			Lingual	Lingual MG	40.7	48.7	44.1

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Material	Luting space	2D/3D	Seating direction	Reading	Min µm	Max µm	Mean µm
		2D	Duesel	Buccal MG	47	54.3	50.9
			Duccai	Lingual MG	69	75.7	72.7
			Axial	Buccal MG	44.3	53	48
Brilliant Crios				Lingual MG	52.3	69.3	63.1
			Lingual	Buccal MG	61.7	105	80.7
				Lingual MG	31	45	39.5
		3D	Puppel	Buccal MG	60	69.3	65.6
			Buccar	Lingual MG	103	112	108.5
			Avial	Buccal MG	50	65	59.5
			Axiai	Lingual MG	62.7	84	74.9
			Lingual	Buccal MG	71.3	117	92.6
			Lingual	Lingual MG	40	58	50.9

Table 2. ANOVA results for the three-factor interaction of material,seating direction, and luting space.					
Measurement	p-value				
2D buccal	0.0039				
2D lingual	0.0095				
2D overall	0.0029				
2D difference buccal / lingual	0.0028				
3D buccal	0.0005				
3D lingual	0.015				
3D overall	0.0006				
3D difference buccal/lingual	0.0003				

Internal fit measurements

The effects of material, seating direction, and luting space, and their interaction, on each outcome were compared and the ANOVA source table is shown as Table 5.

The signifant interactions were between the material and the seating direction, and the material and luting space.

Table 3. Table comparing the buccal and lingual marginal gaps for different milled internal gaps; * denotes statistical significance.								
Material	Luting space	Seating direction	n	Metric	Mean difference	95% CI f	or Mean	p-value
All motoriala	100 µm	Russel	15	2D Buccal	-19.6	-25.9	-13.3	0.90
All materials	200 µm	Buccai	15	vs. Lingual	-18.6	-24.9	-12.4	0.02
All motoriala	100 µm	Lingual	15	2D Buccal	31.4	23.7	39.0	0.59
All materials	200 µm	Linguai	15	vs. Lingual	34.3	26.7	41.9	0.56
	100 µm	Avial	15	2D Buccal	-6.1	-11.9	-0.3	0.0007*
All materials 2	200 µm	Aniai	15	vs. Lingual	-21.4	-27.2	-15.6	0.0007
All motoriala	100 µm	Russel	15	3D Buccal	-26.9t	-33.4	-20.4	0.49
All materials	200 µm	Duccar	15	vs. Lingual	-30.2	-36.7	-23.6	0.40
	100 µm	Lingual	15	3D Buccal	32.4	24.2	40.7	0.44
All materials	200 µm	Linguai	15	vs. Lingual	36.9	28.6	45.2	0.44
All motoriala	100 µm	Avial	15	3D Buccal	-0.7	-6.2	4.7	0.010*
All materials	200 µm	Axial	15	vs. Lingual	-10.9	-16.3	-5.4	0.012"

Table 4. Mean of buccal and lingual measurements for axial seating direction.						
Material	Luting space	Measurement	n	Mean buccal and lingual		
	100 um	2D	15	46.9		
Ziroonia	του μπ	3D	15	55.6		
Zircoma	200 um	2D	15	64.5		
	200 µm	3D	15	75.7		
	100 um	2D	15	52.6		
Enomio	του μπ	3D	15	59.1		
Enamic	200 um	2D	15	53.9		
	200 µm	3D	15	62.2		
Crios	100 um	2D	15	54.2		
	του μπ	3D	15	58.0		
	200 um	2D	15	55.6		
	200 µm	3D	15	67.2		

Post-hoc tests revealed the following significant differences:

• The mean internal fit was significantly higher for all Zirconia seating angles (p<0.0001) compared with Enamic and Crios, but not within Zirconia.

Table 5. The effects of material, seating direction, and luting space,and their interaction on the internal fit; *significant differences.							
Effect	Num DF	Den DF	F Value	p-value			
Material	2	22	90.35	<0.0001*			
Seating direction	2	21	7.26	0.004*			
Luting space	1	22	2696.22	<0.0001*			
Material* Seating direction	4	24.4	3.12	0.033*			
Material* Luting space	2	22	36.52	<0.0001*			
Pressure* Luting space	2	21	2.81	0.083			
Material* Seating direction* Luting space	4	24.4	1.56	0.22			

- Within Enamic, the mean internal fit for lingual seating was greater than buccal (p=0.0088) and axial (p=0.0052).
- Within Crios, the mean internal fit for buccal seating was greater than for the occlusal (p=0.014).

When comparing the luting spaces, Post-hoc tests revealed the following significant differences:

- The mean internal fit was significantly higher for all 200 µm experiments compared with all 100 µm experiments (p<0.0001).
- Within the 100 μm experiments, the mean internal fit decreased in the order Zirconia > Enamic (p<0.0001) > Crios (p<0.0078).
- Within the 200 μm experiments, the mean internal fit decreased in the order Zirconia > Enamic (p < 0.0055) > Crios (p < 0.0034).

DISCUSSION

This is the first study to be carried out to measure and compare the effect of off-axis seating on the adaptation of full coverage crowns using the marginal gap and internal fit as excellent proxies for the clinical quality and success of a restoration.

Discrepancies in the marginal gap can lead to a variety of problems which could ultimately result in failure of the crown.¹⁵⁻²¹ It is generally accepted that marginal gaps below 120 µm are clinically acceptable.^{22-26,47,48}

With regards to the internal fit, it is clinically relevant to ensure that adequate space is created to allow an even thickness of dental cement. Theoretically, the space required for the cement to lute is $20-40\,\mu$ m, as cement thickness ranges from $25-50\,\mu$ m, and an acceptable practical guide was set between $50\,\mu$ m and $100\,\mu$ m.⁴⁹

In CAD/CAM restorations, a luting space is used to allow for this, and several studies have linked luting space to marginal gap measurements.³⁻⁷ In the literature, luting space recommendations have ranged from 10 μ m to 100 μ m. The larger spaces have produced the smaller marginal gaps both before and after actual or replicated cementation.^{4-6,8-12}

In this study, it was decided to use luting spaces of $100\,\mu\text{m}$ and $200\,\mu\text{m}$. In a pilot study it had been observed that, as finger pressure is used to cement a crown, it is possible that it may not seat evenly if seated at an angle to the occlusal. No studies have reported on the effect of non-axial seating discrepancies.

It was evident that seating the crown off-axis at just 5° did affect the marginal gap: there was a significant difference between the buccal and lingual marginal gap measurements in all cases when the crowns were seated off axis, but it was interesting to note that none of the marginal gaps measured exceeded $120\,\mu$ m. However, the greatest discrepancies were observed in off-axis seating with a luting space of $200\,\mu$ m for all materials, indicating that that luting space is proably too large and may produce more off-axis seating clinically.

There were statistically significant differences between the $100\,\mu\text{m}$ and $200\,\mu\text{m}$ spaces, for both the 2D and 3D measuremets, for the axial direction of seating, indicating that the luting space did affect the marginal gap.

The smallest gaps were from the axial seating using the 100 μm luting space. Overall, for all materials these differences for the 2D measurements ranged from 10.4 μm – 20.1 μm , and for 3D measurements, from 3.5 μm – 9.4 μm .

Overall the 3D measurements were 13.4 μ m greater, but not significantly different from the corresponding 2D measurements (p=0.92). These measurements are to be expected, as the 3D gap is likely always to be higher

than the 2D measurement, but they are nevertheless all extremely low, which is a testament to the accuracy of the milling of these crowns. As with the marginal gaps, within each material, axial seating yielded the smallest internal fit when compared with off-axis seating.

The internal fit of a crown is just as important as the marginal gap, as it enables the seating of the crown and expression of cement, while also aiding in retention and resistance.³¹ The mean internal fit for all $200 \,\mu\text{m}$ crowns was significantly higher than the $100 \,\mu\text{m}$ crowns, which was expected.

This also shows that the CAD/CAM process is highly accurate, generating an internal fit for each crown which closely resembles the luting space chosen. Clinically the results obtained in this study have implications.

Irrespective of material used when seating a crown, a minor tilt of even 5 degrees can result in a larger marginal gap specifically on the opposite side to the pressure being applied.

Although this study did not find these measurements to be above $120\,\mu\text{m}$, some marginal gaps were still large, with one reaching $117\mu\text{m}$.

Previous studies which measured the marginal gap of crowns found that they ranged from $<70 \mu m^{50}$, $52 \mu m$ to $74 \mu m^3$, a median of 130.2 and 132.2 μm , 51 below $90 \mu m.^{52}$ The marginal gaps measured in this study which more closely resemble those of other studies are the values measured for axial seating.

Should other studies have taken into consideration the tilt that may be found when seating off axis, they may have measured larger results. In this study the marginal gaps ranged from $36 \,\mu\text{m} - 117 \,\mu\text{m}$ with off-axis seating and $31 \,\mu\text{m} - 99 \,\mu\text{m}$ with axial seating.

The other factor not taken into consideration in other studies is measuring the marginal gap buccally and lingually separately. Gassino et al. (2004)²⁷ found that to obtain an accurate overall marginal gap measurement requires at least¹⁸ points around the circumference of the tooth to be measured.

However, this again did not take into account the tilt and used an average of all measurements to arrive at a marginal gap. Considering that a larger marginal gap will be found on the opposite side to the pressure being applied it is necessary to measure the buccal and lingual sides separately, to yield an accurate result that resembles the correct fit of the crown.

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CONCLUSION

Within the limitations of this study, it was found that, irrespective of the material, seating off-axis at 5 degrees buccally or lingually resulted in a marginal gap which was larger on the opposite side to which pressure was applied. The smallest marginal gaps and internal fit were obtained when seating axially, with a luting space of $100 \,\mu\text{m}$.

All measurements made in three dimensions were larger than those derived for two-dimensional measurement, but the difference, average of $13.4 \,\mu\text{m}$, was not significant. None of the measurements, whether cemented axially or off-axis were larger than $120 \,\mu\text{m}$. However, when seating off axis, the largest gap was $117 \,\mu\text{m}$ as opposed to seating axially which yielded a mean maximum marginal gap measurement of $76 \,\mu\text{m}$.

It is recommended that future studies should measure the marginal gap both buccally and lingually separately and not just use an average to obtain an accurate measurement, and that a method needs to be devised to cement crowns axially in the clinical environment to provide the best fit possible and minimise complications.

Acknowledgements

We are grateful to Dr P Gaylard for statistical advice and analysis.

Declaration

The authors declare no conflict of interest.

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