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## **Comparative Evaluation of Aluwax, Polyvinyl Siloxane, Polyether and Bis-Acryl Bite Registration Material at Different Time Interval: An *In-Vitro*-Study**

<sup>1</sup> Shah Saloni, <sup>2</sup> Nadgere Jyoti, <sup>3</sup> Iyer Janani, <sup>4</sup> Parmar Bhoomi, <sup>5</sup> Terni Prachiti, <sup>6</sup> Sampat Saumil

<sup>1</sup> M.D.S, B.D.S, Department of Prosthodontics, Crown & Bridge, MGM Dental College and Hospital, Navi Mumbai, India

<sup>2</sup> M.D.S, B.D.S, Head of the Department, Department of Prosthodontics, Crown & Bridge, MGM Dental College and Hospital, Navi Mumbai, India

<sup>3</sup> M.D.S, B.D.S, Professor, Department of Prosthodontics, Crown & Bridge, MGM Dental College and Hospital, Navi Mumbai, India

<sup>4,5,6</sup> M.D.S, B.D.S, Reader, Department of Prosthodontics, Crown & Bridge, MGM Dental College and Hospital, Navi Mumbai, India

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Corresponding Author: **Shah Saloni**

### **Abstract**

**Aim:** The aim of this study was to evaluate the accuracy and dimensional stability of Aluwax, polyvinyl siloxane, polyether and bis-acrylic bite registration materials at 8hours, 24hours and 48hours.

**Materials and Methods:** Four commonly used bite registration materials were used in this study which includes Aluwax, Polyvinyl Siloxane (Orange bite), Polyether (Ramitec) and Bis-Acrylic bite registration material (Luxabite). The sample size was 76 (19 in each group). The samples fabricated were then grouped into Group 1- Aluwax, Group 2 – Polyvinyl siloxane bite registration material, Group 3 – Polyether bite registration material and Group 4 – Bisacrylic bite registration material.

**Result:** Using Student's paired and unpaired T-test, statistical analysis was performed to determine the

material's accuracy. The results of which were taken at 5% ( $p>0.05$ ) and 1% ( $p>0.01$ ) level of significance. While, Kruskal-Wallis and ANOVA were the tests used to determine dimensional stability over time intervals at 5% ( $p>0.05$ ) and 1% ( $p>0.01$ ) level of significance. The result was statistically significant for both intra-group at different time intervals and between the test groups for accuracy and dimensional stability.

**Conclusion:** Polyvinyl siloxane bite registration material exhibited better accuracy and dimensional stability at time interval, followed by Polyether, Bis-Acrylic and Aluwax. Additionally, this research paper found that the dimensional stability was influenced by the time interval, as time increased the dimensional stability of the material decreased.

**Keywords:** Bite Registration Materials, Accuracy, Dimensional Stability, Polyvinyl Siloxane, Interocclusal Records

### **Introduction**

Oral rehabilitation involves a series of precise clinical steps, each contributing to the success of diagnosis, treatment and long-term prognosis. Right from the initial step of diagnosis till restorative and prosthetic procedure, every step requires precision. An interocclusal record is the registration of the positional relationship of opposing teeth or the jaws to each other [1]. Capturing the tooth-to-tooth and arch-to-arch relationship accurately and transferring it to the articulator is of utmost importance [2]. In order to achieve functional occlusion at the end of prosthetic rehabilitation, it is mandatory that the occlusal relationship between upper and lower teeth is registered properly [3].

An error during the bite registration process can be caused by biological characteristics of stomatognathic system, to faulty techniques of jaw manipulation or to mishandling of the interocclusal recording medium by clinicians [4]. Iatrogenic errors caused by bite registration materials can be controlled by skilled clinicians who have a deep understanding of the procedures and appropriate materials [2]. In order to select the most appropriate material for bite registration, the material should be dimensionally stable, accurate, easy to manipulate, biocompatible, limited initial resistance to closure. However, there is no single material that has all the requirements [5].

The first interocclusal records were made by Phillip Pfaff in 1756 using natural waxes followed by which many other materials have been introduced which includes plaster, modelling compound, waxes, acrylic resin of which modelling wax is the most commonly used [6]. Due to poor dimensional stability and thermoplastic nature of modelling wax, aluminum particles infused in the modelling wax which is known as Aluwax was introduced [7]. Aluwax helped in distributing heat evenly and prevents excessive cooling contraction which overcomes the disadvantages of using modeling wax [8].

Newer materials include polyvinyl siloxane and polyether. Chemically they are similar to rubber-based impression materials, which have been modified by adding plasticizers and catalyst to provide better handling characteristics of the materials [9]. A study conducted by Tripodakis A. P., concluded that elastomeric materials have been proven to be more stable, dimensionally more accurate and do not require trays or special equipment [10]. Apart from waxes and elastomeric materials, bis-acrylic based materials are also recently being used to record inter-occlusal bite records. Bis-acrylic is a composite based material with elastomeric additives, which gives it an advantage of elastomeric material of being stable and easy to handle and due to presence of glass filler, it reduces the rubbery consistency of the material [8].

Dimensional stability is an important parameter to be considered while selecting an appropriate bite registration material as transferring the registered interocclusal record from the clinics to dental laboratory followed by the articulation of patient's casts is a long procedure which can be delayed due to various reasons. It is therefore crucial to use dimensionally stable material which can be reliable for longer period of time [5]. Another important parameter to be considered is the accuracy. Accuracy is defined as the condition or quality of being true, correct or exact. It has a significant impact on the representation of maxillary-mandibular relationship as it directly impacts the proper alignment and fit of the restorations ensuring the final prosthesis matches the patient's natural bite [2].

Accuracy and dimensional stability of the bite registration materials with the passage of time are an important factor that play crucial role and providing clear idea to the clinicians about the different materials with regards to its usage in routine practice. Considering the mentioned factors, this study aimed to evaluate the accuracy and dimensional stability of Aluwax, polyvinyl siloxane, polyether and bis-acryl bite registration materials at different time intervals.

### Materials and Method

Four bite registration materials were included in this study. These materials were divided into 4 different groups. Group 1: Aluwax bite registration material (Aluwax, Aluwax Dental Products, USA), Group 2: Polyvinyl siloxane bite registration material (OrangeBite, Medicept Dental, India), Group 3: Polyether bite registration material (Ramitec, 3M ESPE, USA), Group 4: Bis-acrylic bite registration material (LuxaBite, DMG-USA).

#### Preparation of stainless-steel die

A study mold was prepared according to revised American Dental Association specification no. 19. It consisted of a ruled block, test material mold and a riser. The ruled block had three horizontal lines A, B and C and two vertical lines

P and Q. The point of intersection of line P and Q with A and C are 1, 2, 3, 4. The line B was be 1 mm in depth, which was standardized for evaluating the accuracy. The vertical lines were separated from each other by 25 mm; which was the standardized distance for evaluating the dimensional stability. The test mold was a cylinder of inner diameter of 30 mm, outer diameter of 38 mm and depth of 6 mm where the bite registration material was placed.

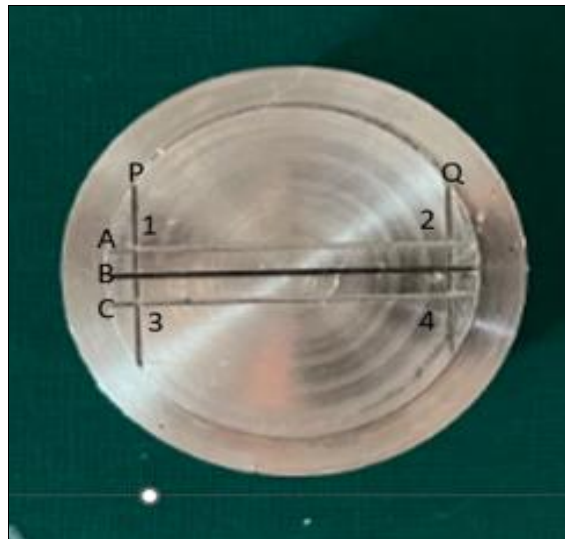


Fig 1: Two vertical lines P and Q, Three horizontal lines A, B and C

#### Sample manipulation and fabrication

For fabrication of samples in group 1, since Aluwax was supplied in the form of sheet it was first softened uniformly and placed inside 20 mm syringe and then injected. For fabrication of samples in group 2 and 4, polyvinyl siloxane and bis-acrylic were supplied in the form of cartridge. Using auto-mixing dispensing gun, the material was spread over the surface of the die. For fabrication of samples in group 3, polyether was supplied in form of 2 pastes which was mixed homogeneously using spatula and the material was loaded on the surface of the die. Once the material was manipulated and loaded on the stainless-steel die, the material was covered using a glass plate on which a weight weighing 500 gms was placed. This created a total force of 5.56 N which simulated the intra-oral pressure that was required to compensate the initial resistance of bite registration material.



Fig 2: Armamentarium for fabrication of samples for Group 1,2,3 and 4

The whole assembly was then submerged in the water bath of temperature  $36 \pm 1^{\circ}$  centigrade before initial set. The material was kept in the water bath for an additional 2-3

minutes after the setting time mentioned by the manufacturers to ensure complete setting/polymerization of the material. The water bath was used to simulate the oral temperature while recording the bite.

After removing it from the water bath the material was separated from the die and the excess flash was trimmed using Bard Parker Knife and No. 15 blade. Thus, the samples of 30 mm in diameter and 3mm in thickness with three horizontal and two vertical lines on it were fabricated.



Fig 3: Samples Fabricated

**Testing of Samples**

**Testing for Accuracy**

The samples were tested for accuracy immediately after fabrication. Testing for accuracy was done by using travelling microscope to measure the depth of line B obtained on the fabricated samples whose depth was standardized in the stainless-steel die which is 1 mm. The values of the line B obtained from the samples were noted and tabulated.

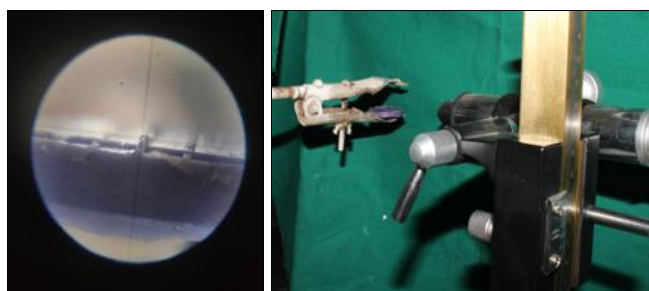


Fig 4: Testing the samples for accuracy

**Evaluating change in accuracy**

- The values for the depth of line B in samples were obtained from the travelling microscope by recording the **mean scale reading (MSR)** on travelling microscope and its **co-incident division (CD)**.
- **Total reading** was obtained by the formula MSR + (CD\*0.0001).
- Similarly, the values were obtained for all samples in each group.
- Change in the accuracy will be calculated according to the formula:  $\frac{D-E}{D} \times 100$

Where D is the standard depth of line B and E is the observed depth of the line B in samples fabricated.

**Table 1: Total readings of all samples in 4 groups**

Sample Number	Total readings for the depth of the line B on the samples (E) (mm)			
	GROUP 1 Aluwax	GROUP 2 Polyvinyl siloxane	GROUP 3 Polyether	GROUP 4 Bis-acrylic
1	0.4	0.45	0.41	0.45
2	0.25	0.54	0.92	0.42
3	0.08	0.59	0.86	0.35
4	0.05	0.98	0.10	0.31
5	0.09	0.90	0.91	0.36
6	0.6	0.79	0.48	0.47
7	0.35	0.58	0.96	0.46
8	0.04	0.53	0.89	0.39
9	0.09	0.94	0.12	0.33
10	0.09	0.60	0.55	0.33
11	0.08	0.78	0.92	0.45
12	0.05	0.54	0.86	0.42
13	0.09	0.59	0.81	0.35
14	0.6	0.98	0.91	0.31
15	0.35	0.70	0.48	0.36
16	0.04	0.79	0.96	0.47
17	0.09	0.58	0.89	0.46
18	0.889	0.53	0.12	0.39
19	0.25	0.94	0.55	0.33
Mean	0.23	0.71	0.66	0.39

**Testing for dimensional stability**

The distance between two vertical lines P and Q were measured at the point of intersection of lines P and Q with lines A, B and C followed by measuring the mean of readings obtained by using a travelling microscope. The mean readings obtained were then compared with the distance between the lines P and Q which is standardized at 25 mm. The readings were noted at 0hours, 8hours, 24 hours and 48hours respectively for each sample and tabulated.

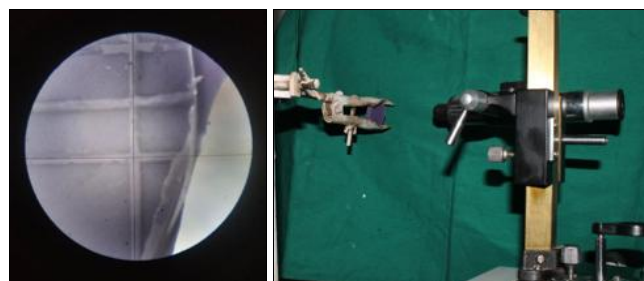


Fig 5: Testing the samples for dimensional stability

**Evaluating change in dimensional stability**

- The values for the dimensional stability were obtained by calculating the distance between points (1) and (2) and (3) and (4) in samples by recording the **mean scale reading (MSR)** on travelling microscope and its **co-incident division (CD)**.
- **Total readings** were obtained by the similar formula MSR + (CD\*0.0001) (Table 2).
- Similarly, the values were obtained for all samples in each group.
- **Mean** of which was calculated and the **change in the dimensional stability** was then calculated according to the formula.
- Change in the dimensional stability will be calculated according to the formula:  $\frac{X-Y}{X} \times 100$

Where X is the standard measurement (µm) between lines P and Q and Y is the observed measurement (µm) between lines P and Q in the sample.

**Table 2:** Testing the samples for dimensional stability

Sample Number	Total readings for distance between two vertical lines in the samples (Y) in (mm)															
	GROUP 1 Aluwax				GROUP 2 Polyvinyl siloxane				GROUP 3 Polyether				GROUP 4 Bis-acrylic			
	0hrs	8hrs	24hrs	48hrs	0hrs	8hrs	24hrs	48hrs	0hrs	8hrs	24hrs	48hrs	0hrs	8hrs	24hrs	48hrs
1	25.15	25.17	25.18	24.15	25.17	24.6	24.65	23.78	25.49	24.67	25.15	24.37	25.49	25.49	24.70	25.65
2	25.15	25.25	20.79	24.15	24.75	25.22	25.41	25.06	25.94	24.96	25.15	24.42	25.24	25.04	25.23	25.03
3	24.99	25.55	25.25	24.74	25.08	25.17	25.10	24.98	25.8	24.47	24.74	24.95	25.8	25.8	24.75	25.69
4	25.07	24.67	24.99	23.97	25.13	25.77	12.6	23.94	25.6	24.4	25.07	24.93	25.6	25.6	25.08	24.58
5	25.66	25.6	25.68	24.62	25.65	25.17	24.92	24.98	20.48	24.06	25.62	25.07	22.48	20.48	25.13	25.49
6	25.74	24.67	24.6	24.67	25.03	25.49	25.17	25.83	24.70	25.49	24.67	24.6	24.7	24.70	23.78	26.50
7	25.47	24.96	25.22	23.96	25.69	25.94	25.18	23.65	25.49	25.94	24.26	25.22	25.49	25.49	24.65	24.74
8	24.76	24.47	25.17	24.47	24.58	25.8	25.15	24.84	24.47	25.8	24.47	25.17	24.47	24.47	25.17	24.47
9	24.96	24.4	25.77	24.4	25.94	25.6	24.6	24.43	25.8	25.6	24.14	25.77	25.8	25.8	25.15	29.84
10	24.97	24.06	25.17	24.06	25.8	20.48	24.65	24.37	24.6	20.48	24.06	25.17	24.6	24.6	23.78	24.37
11	24.79	25.49	24.65	24.74	25.6	24.70	23.78	24.50	25.65	24.70	24.74	23.78	25.65	25.65	24.70	25.18
12	24.88	25.94	25.41	24.71	20.48	25.23	24.67	25.49	25.55	25.23	24.11	25.06	25.55	25.55	25.25	24.74
13	25.29	25.8	25.10	20.29	24.70	24.75	24.74	24.39	25.77	24.75	20.29	24.98	25.77	25.77	12.6	23.94
14	25.10	25.6	12.6	24.1	24.6	25.08	24.79	23.78	25.77	25.08	25.1	23.94	25.77	25.77	23.94	24.93
15	24.95	20.48	24.92	23.85	25.22	25.13	25.94	25.17	25.49	25.13	24.35	25.41	25.49	25.49	24.99	25.21
16	24.98	24.6	23.78	24.37	25.17	25.65	24.70	25.18	25.22	25.03	24.37	25.10	25.22	25.22	25.06	24.42
17	25.72	25.22	25.06	24.42	25.77	25.03	25.65	25.15	25.03	25.69	24.42	12.6	25.03	25.03	25.65	25.15
18	25.45	25.17	24.98	23.95	25.17	25.69	24.78	24.6	25.09	24.58	24.45	24.92	25.69	25.69	20.79	12.6
19	24.83	25.77	23.94	23.93	25.49	24.58	25.59	24.65	24.75	25.49	24.23	25.21	24.75	24.75	24.74	24.39
Mean	<b>25.15</b>	<b>24.88</b>	<b>24.11</b>	<b>24.08</b>	<b>25.00</b>	<b>25.004</b>	<b>24.31</b>	<b>24.67</b>	<b>25.08</b>	<b>24.81</b>	<b>24.40</b>	<b>24.245</b>	<b>25.18</b>	<b>25.07</b>	<b>23.95</b>	<b>24.57</b>

**Results**

- Statistical analysis was done by descriptive statistics as mean, SD, percentage and proportions etc.
- The comparison of mean values all parameters in group 1, 2, 3 and 4 and normality was assessed by Student's Paired and Unpaired 't' test at 5% (p,0.05) and 1% (p,0.01) level of significance.
- Kruskal-Wallis and ANOVA for the overall comparison was used at at 5% (p,0.05) and 1% (p,0.01) level of significance.
- The statistical analysis software namely SYSTAT Version -12 (made by Crane's Software's, Bangalore) – a licensed copy was used to analysis of data under study.
- By applying Student's Unpaired t test there is a significant difference between mean values of measuring the depth of the line B on the samples tested for accuracy when Group 1 compared with Group 2, Group 1 compared with Group 3, Group 1 compared with and in Group 4, Group 2 compared with Group 4 and Group 3 compared with Group 4. By applying Student's Unpaired t test there is no significant difference seen in between mean values of measuring the depth of the line B on the samples tested for accuracy when Group 2 compared with Group 3.
- By applying Kruskal-Wallis and ANOVA for the overall comparison of measuring the depth of the line B on the samples (mm) tested for accuracy there is a significant difference were found between values of measuring the depth of the line B on the samples (mm) tested for accuracy in Group 1, Group 2, Group 3 and in Group 4 compared together. By applying Student's Paired t test there is a significant difference between mean values of measuring the distance between 2 vertical lines P and Q at different time intervals (y) (mm) from time interval 0 hrs. to 48 hrs. Group 1, Group 2, Group 3 and in Group 4.
- One-way ANOVA with posttest: Kruskal-Wallis and ANOVA for the overall comparison By applying

Kruskal-Wallis and ANOVA for the overall comparison of measuring the distance between 2 vertical lines P and Q at different time intervals (y) (mm) there is a significant difference were found between values of measuring the distance between 2 vertical lines P and Q at different time intervals (y) (mm) in Group 1, Group 2, Group 3 and in Group 4 compared together.

**Table 3:** Mean, SD and value of significance for calculating change in accuracy

n=19	Group 1	Group 2	Group 3	Group 4
Depth of line B on the samples tested for accuracy	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
	0.23±0.24	0.71±0.16	0.66±0.31	0.39±0.05

Group 1 V/s Group 2	Value of 't' = 7.173, p=0.0001, Significant
Group 1 V/s Group 3	Value of 't' = 4.833, p=0.0001, Significant
Group 1 V/s Group 4	Value of 't' = 2.712, p=0.0102, Significant
Group 2 V/s Group 3	Value of 't' = 0.6297, p=0.5328, Not significant
Group 2 V/s Group 4	Value of 't' = 8.083, p=0.0001, Significant
Group 3 V/s Group 4	Value of 't' = 3.886, p=0.0004, Significant

**Table 4:** Mean, SD and value of significance for calculating change in dimensional stability

Time interval	Group 1	Group 2	Group 3	Group 4
n=19	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
0 hours	24.77±0.16	24.65±0.16	24.69±0.16	24.71±0.17
8 hours	24.36±1.00	24.30±0.22	24.58±0.23	24.54±0.21
24 hours	23.91±0.42	24.27±0.29	24.15±0.29	23.91±0.24
48 hours	23.24±0.38	24.11±0.32	23.89±0.24	23.83±0.28

From 0 hours to 48 hours	Value of t= 18.766, p=0.0001, significant	Value of t= 6.772, p=0.0001, significant	Value of t= 10.394, p=0.0001, significant	Value of t= 10.977, p=0.0001, significant
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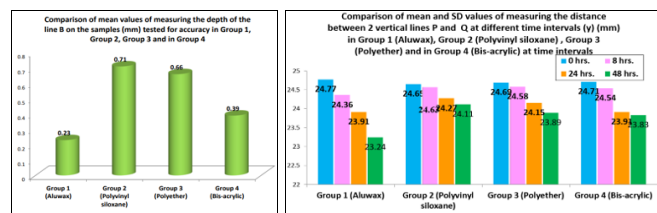


Fig 6: Comparing the mean values for accuracy and dimensional stability

**Discussion**

Interocclusal bite records should be an accurate and dimensionally stable representation of the interocclusal space that is subsequently transferred to an articulator [11]. Accuracy of the material is essential for precise transferring of the maxillomandibular relationship to the articulator, while dimensional stability ensures that these records maintain their shape over a period of time to avoid errors while aligning the casts and hence minimizing the need for adjustments during prosthesis placement [12].

The most commonly used materials like bite registration wax, polyvinylsiloxane and polyether along with a recent material which is bis-acrylic have been tested in this study. These materials either undergo setting reaction or polymerization. As a result of which dimensional changes of these materials even after initial setting are continuous. Hence, in this study various time intervals are taken and the materials for dimensional stability have been checked at 8hrs, 24 hrs and 48hrs.

In this study the accuracy for polyvinyl siloxane had minimal variation in depth while Aluwax showed significant spikes in depth measurements and hence this study concluded that polyvinyl siloxane provided with the best accuracy amongst the 4 materials compared and the choice of material influenced the accuracy of the bite to be registered. The results of this study are in agreement with the study by Vassilis K. *et al* where they are comparing the same materials for vertical and horizontal accuracy [13]. The results of that study showed that lowest discrepancy was displayed by polyvinyl siloxane followed by polyether and the greatest discrepancy was displayed by the wax. This study also concluded that not only the choice of material but also the technique of recording the interocclusal bite influenced the accuracy.

Similarly, in a study by Anup G. *et al*, were they compared polyvinyl siloxane to zincoxide eugenol and Aluwax, they concluded that accuracy and dimensional stability of polyvinyl siloxane is better than both zinc-oxide eugenol and Aluwax [6]. Also, they concluded that not only dimensional stability but accuracy is also influenced by time factor. The findings of the present study also show an increasing influence of time on the dimensional stability of all the materials but the limitation of this study is that influence of time on accuracy was not considered in this study. Another study favoring the use of polyvinyl siloxane over ZoE and bite registration wax is by Kumar P. *et al* where the time intervals used were 1hr, 8hrs, 24hrs, 48 hrs and polyvinyl siloxane confirmed higher dimensional stability and surface hardness at all the given times [14]. They

also concluded that correctness of polyvinyl siloxane samples was statistically significant. The findings in the present study showed substantial and significant change in the dimensional stability of different materials at increasing time interval. Of all the materials polyvinyl siloxane showed least changes in dimensional stability while Aluwax showed the highest changes. Samples of group 3 (polyether) exhibited slightly greater dimensional changes than group 2 (polyvinyl siloxane) despite both being elastomers. One of the causes for this discrepancy could be that polyether is hydrophilic so it may react to the environmental moisture than polyvinyl siloxane [11]. Although the differences in the value were significant at 8 hours and 24 hours for polyvinyl siloxane and polyether. Post 24 hours the values were almost similar and were not significant.

As polyvinyl siloxane and polyether are most commonly used elastomeric bite registration materials there are various studies that are in agreement with the results of this study proving polyvinyl siloxane has better stability and accuracy as compared to polyether [1, 6, 11, 15]. Whereas, there are a few studies opposing to the results of this study and they conclude that polyether is dimensionally more stable than polyvinyl siloxane [5, 8, 16]. However according to current study polyether bite registration material should be used for articulation within 24 hours while polyvinyl siloxane can also be used post 24 hours.

Another material used in this study was Bis-acrylic bite registration material as it is a resin-based material. It combines the favorable properties of acrylic resins with improved working characteristics offering high accuracy, low shrinkage and fast setting time. The results in this study showed comparable results of bis-acrylic material with polyether for accuracy and dimensional stability. While, polyvinyl siloxane showed better results as compared to all the other materials for accuracy and dimensional stability. Bite registration wax showed the greatest dimensional changes which was attributed to greater co-efficient of thermal expansion and distortion due to stress released [17]. In various studies conducted with different bite registration materials, one of the studies by Arya S. *et al* where she compared accuracy and dimensional stability of polyvinyl siloxane, bis-acrylic and Aluwax at different time intervals and the results were in agreement with this study that polyvinyl siloxane was found to be the most accurate and the one material that exhibited maximum dimensional stability followed by bis-acrylic and Aluwax respectively [18]. Also, the values were significant till 48 hours for polyvinyl siloxane and bis-acrylic which is also in agreement with this study. Another study in agreement with these results was by El Sayed A. where all tested bite registration materials had comparable results but polyvinylsiloxane showed lower errors than bis-acrylic and polyether bite registration materials [7].

As opposed to the results of this study is a study by Sharma R. where she compared polyvinyl siloxane and bis-acrylic bite registration materials for its accuracy, dimensional stability and reproducibility at different time interval and concluded that dimensional stability was influenced by both time and material with less significant percentage of dimensional change for bis-acrylic material and slightly more significant percentage of dimensional change for polyvinyl siloxane material up to 48hrs [19]. The only disadvantage of using polyvinyl siloxane bite registration

material is its springiness which causes the articulated casts to open in centric closure position<sup>[13]</sup>. There are also studies which conclude that both polyvinyl siloxane as well as bis-acrylate based materials are accurate enough to provide reliable recordings in order to program semi adjustable articulators<sup>[20]</sup>.

According to the recent advancement of digital CAD/CAM technology, there are various scannable and transparent bite registration materials available and a study has been conducted by Firas K. Alqarawi where he has compared 3 scannable and 3 transparent materials over obligatory clinical time intervals and he concluded that all the scannable and transparent bite registration materials showed shrinkage and reduced linear dimensions with Virtual CAD Bite and Maxill Bite having lowest linear disagreement after 1 hour<sup>[21]</sup>.

Hence, according to this study all the materials did show some amount of inaccuracy and instability but the least in both the parameters were seen for polyvinyl siloxane when used for articulation by 48 hours and maximum was seen in bite registration wax even at 8hrs. This information is relevant for the clinicians as in a survey conducted by Maru K. most of the practitioners 54.6% preferred wax as an interocclusal recording material and 62.9% transferred the record to lab in 1-24 hours<sup>[22]</sup>. As a result of which this study will help to develop the practitioner's attitude towards the use of interocclusal bite recording material and selecting them wisely.

### Conclusion

- Within the parameters of this research polyvinyl siloxane was proved to be accurate and dimensionally stable followed by polyether, bis-acrylic and Aluwax at 0hrs, 8hours and 24 hours.
- After 24 hours the change in dimensional stability is similar for polyvinyl siloxane and polyether.
- The choice of material should be based upon the clinical situation. The dimensional stability decreased with increase in time.

### Limitations

- A possible limitation of this study is that it takes only the linear measurement as a parameter for determining dimensional stability as in routine clinical situations, dimensional errors occur in all three dimensions.
- Does not include time intervals for testing accuracy of the materials.
- The study conducted was *in vitro* as a result there can be changes when *in vivo* tests are carried out.

### Acknowledgement

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