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# Assessment of Microbial Adhesion on Provisional Crown Material after Polishing with Different Polishing Agents - An *In-Vitro* Study

Research Article

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## Abstract

**Aim:** To assess themicrobial culture on provisional crown material polished with different polishing agents.
**Materials and Methods:** Discs made of Pro-temp provisional crown material, of a uniform size, were polishes using Rouge, Polishing paste and Pumice, i.e. three different polishing agents. They were then disinfected and immersed in a Streptococcus mutansbacterial broth, and were incubated for 24 hours. The biofilm formed on the discs post incubation were smeared on agar petri plates, to obtain subcultures that can be used for colony counting and determining the extent of biofilm formation on each disc.
**Results:** There is a significant difference in the method of polishing employed and the bacterial adherence and colonisation on the surface of the provisional crown material.
**Conclusion:** Rouge is a better polishing agent, followed by polishing paste, and then pumice, which is inferred from the microbial colonisation on the discs polished with respective polishing agents.
**Clinical significance:** Provisional crowns, also known as interim crowns, are devices placed temporarily, until a permanent replacement is constructed, for protecting the affected tooth, preventing teeth shifting, maintaining aesthetics, and in keeping sensitivity at bay. Also, it is known that microbial colonisation is favoured by rough or irregular surfaces. Thus, with the extensive microbial flora of the oral cavity, it is indispensable that the surface roughness of any material or appliance, that is to be placed inside the oral cavity, must be finished and polished to support least microbial adhesion and growth. The present study aims to assess the effect of various polishing agents on provisional crown material to study the extent of microbial colonisation over it.

**Keywords:** Original Study; Microbial Colonisation; Biofilm; Provisional Crown; Streptococcus Mutans; Rouge; Polishing Paste; Pumice.

## Introduction

With the expanding use of fixed prostheses in current times, considerations into the development of provisional crowns is of growing interest worldwide. As a part of the standard prosthetic therapy, prosthetic crowns play an important role in tooth preparation [1] and in luting the final restoration that provides protection against any form of physical, chemical and thermal traumatic factors on tooth pulp tissues [2]. They help maintain occlusion and space [3], and facilitate speech and masticatory functions in the interim period, before fixing the final crown, thus contributing to strength and aesthetics, which are certain essential aspects of treatment success [4]. Previous studies also establish their role in the maintenance of periodontal health and guided tissue healing [5]. Besides, provisional crowns are guide templates for fabricating the actual crowns [6]. They also prove to be psychological

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management aids in patients undergoing the treatment process [7]. The nuance over here lies in the preparation of a crown, permitting self-cleaning, with a well-polished, stain resistant and plaque resistant finish [8].

The polymer based temporary material used earlier, for fabricating provisional crowns, is polymethyl methacrylate (PMMA) mixed with a methyl methacrylate monomer (MMA) liquid which resulted in an exothermic setting reaction that necessitated the timely removal of the temporary restoration lest it causes pulpal damage [9]. Currently, the most successful and widely used temporary crown material is the bis-acrylate composite, Protemp. With improved mechanical properties, reduced configuration factor, lowered setting temperature, better colour stability, good polishability, and a strength equivalent to that of composites, the composition of Protemp includes organic resins and inorganic fillers [10]. Bisphenol-A-glycidyl methacrylate (bis-GMA) andtriethylene glycol dimethacrylate (TEGDMA) are some Bowen resin derivatives which are used as the organic resin [11] while inorganic fillers including zirconia-silica and fumed silica, account for about half of the composition by weight [12].

A common oral pathogen is the *S. mutans* [13], which is why this particular pathogen was considered for the study. Even though bacterial proliferation is responsible for plaque formation, the initial adhesion of bacteria itself is caused by surface irregularities and roughness [14]. In highly irregular surfaces, due to inadequate salivary flow, bacteria can adhere to the surface of the intra-oral prosthesis, better [15]. Moreover, the extent of bacterial colonisation on any surface is determined by surface characteristics like hydrophobicity and surface charge [16]. On polishing using polishing agent slike rouge, polishing, paste, pumice stone, gypsum, chalk, tripoli, garnet, cuttle, tin oxide etc.,the surface is rendered smooth [17], making this procedure crucial to any prosthesis placed in the oral cavity.There have been previous studies [18] which illustrated various methods for quantifying bacterial adhesion to dental structures. These methods included electron microscopy, radiolabelling, fluorescence testing and direct plate counting.

Thus, with the hypothesis that various polishing methods will have a different impact on the surface roughness of provisional crown materials, the study was conducted to test the efficacy of the polishing agents used, namely, rouge, polishing paste and pumice.

## Materials and Methods

### Fabrication of discs with Protemp

With pro-temp being used commonly nowadays, for the fabrication of provisional crowns, the present study used the same material for fabricating the sample discs. 20 discs of uniform size were fabricated using putty moulds. Gross surface irregularities were removed using burs for shaping, and fine sand paper, for polishing.

### Polishing of fabricated discs

Following this, the prepared discs were subjected to fine polishing using three polishing agents, Rouge, Polishing paste and Pumice. Among the 20 discs prepared, 5 were polished with rouge, 5 with polishing pasteand 5 with pumice. The remaining 5 were control discs, not subjected to polishing.

### Culture of *S. mutans* and introduction of discs into culture

All the discs post polishing,were disinfected using surgical spirit, to prevent contamination. Parallelly, a 200 ml liquid culture of S. mutans in trypticase soy broth was prepared and incubated at 37°C for 24 hours. The following day, the disinfected discs were placed in sterile containers, each with 10 ml of the S. mutans broth. These containers were incubated again, at 37°C for 24 hours. The next day, the incubated discs were retrieved and cleaned with saline. The discs were vortexed for obtaining bacterial colonies formed on the discs as biofilm, which were then swabbed with sterile cotton swabs and streaked onto agar plates, and were labelled accordingly. The streaked plated were then incubated at 37°C for 48 hours. Post-incubation, the plates that showed microcolonies of S. mutanswere observed and colonies were counted (Fig.1). The results obtained were recorded and tabulated. They were also statistically analysed using SPSS v26 (IBM. inc., USA) with the One-way ANOVA and Tukey HSD Post Hoc Tests performed.

## Results

The results pertaining to the microbial colonisation with respect to the polishing agent used on provisional crown material is presented here. Table 1 indicates the mean values of the microbial colonisation for each group, including the control group, on performing the One-way ANOVA test. For discs polished with rouge, a mean of 5.00 x $10^3$ CFU was obtained. Discs polished with polishing paste showed a mean value of 8.00 x $10^3$ CFU. Further, a mean value of 13.00 x $10^3$ CFU was found with discs polished with pumice. The control discs gave a mean of 18.00 x $10^3$ CFU microbial colonies. It is also observed that there is a significant difference between the mean values for each group of polishing agents, which is indicated by a p-value of 0.001 (where $p<0.05$). It is also noted that the least number of microbial colonies was observed on polishing discs with rouge, which is followed by polishing paste, and then pumice (Fig. 2). The control group, being unpolished, showed the greatest number of microbial colonies.

Table 2 is indicative of the comparative mean differences between each polishing agent obtained on performing the Tukey HSD Post Hoc Test. The mean differences were calculated from the mean values of the microbial colonies after polishing the discs with the specific polishing agent. A mean difference of 3.00, which is also significant ($p=0.015$), is observed between the groups containing discs that were polished with polishing paste and rouge. Discs polished with pumice and rouge gave a mean difference of 8.00, which is also observed to be significant ($p=0.001$). Further, discs polished with polishing paste and pumice gave a mean difference of 5.00, which again is significant ($p=0.001$). Thereby, the results pertaining to the effect of polishing the discs with each agent, was compared with one another.

## Discussion

On counting the colonies in the petri dishes after incubation, each of the samples showed different results. This complies with the

**Figure 1. Culture on plates post incubation.**

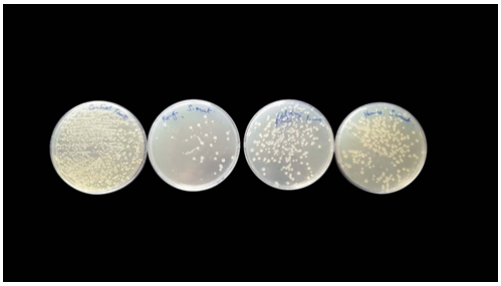


**Table 1.**

| Group | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | F | p-value |
|---|---|---|---|---|---|---|---|
| | | | | Lower Bound | Upper Bound | | |
| Rouge | 5.00 | 0.000 | 0.000 | 5.00 | 5.00 | 87.111 | 0.001[a] |
| Polishing Paste | 8.00 | 1.581 | 0.707 | 6.04 | 9.96 | | |
| Pumice | 13.00 | 1.581 | 0.707 | 11.04 | 14.96 | | |
| Control | 18.00 | 1.581 | 0.707 | 16.04 | 19.96 | | |

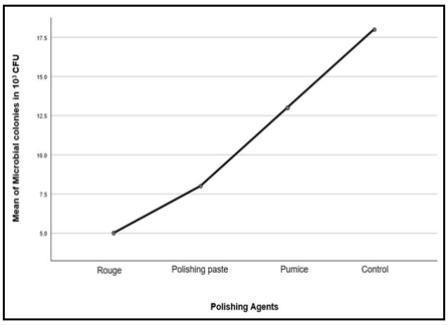
p-value derived from One-way ANOVA test; asignificant at $p < 0.05$.

**Table 2.**

| Group | Mean Difference | Std. Error | p-value |
|---|---|---|---|
| Rouge vs Polishing Paste | -3.000[b] | 0.866 | 0.015[a] |
| Rouge vs Pumice | -8.000[b] | 0.866 | 0.001[a] |
| Polishing Paste vs Pumice | -5.000[b] | 0.866 | 0.001[a] |

[b]Mean difference is significant at the 0.05 level; p-value derived from Tukey HSD Post Hoc Test; [a]significant at $p < 0.05$

**Figure 2. Mean values for microbial colonies with respect to polishing agents in homogeneous subsets are displayed.**




hypothesis that microbial colonisation on the provisional crown material varies with the polishing agent used on it. The mean number of colonies observed in the petri dishes with samples from the discs polished with rouge was 5.00 x $10^3$ CFU. For the discs polished with polishing paste, the mean number of colonies counted from these petri dishes was 8.00 x $10^3$ CFU. The mean number of colonies counted from the petri plated smeared with samples from the discs polished with pumice was 13.00 x $10^3$ CFU. Finally, the control plates showed a mean value of 18.00 x $10^3$ CFU. The observed results were represented graphically and were statistically analysed using the One-way ANOVA test to obtain a p value < 0.05, indicating that there is a significant difference in the method of polishing employed and the technique dependant bacterial adherence and colonisation on the surface. Besides, the mean difference between the microbial colonisation observed after polishing the discs with a polishing agent, with respect to another polishing agents was parallelly compared using the Tukey HSD Post Hoc Test.

There have been several studies [19], which previously established that the adhesion of oral commensals on the surface of structures introduced into the oral cavity leads to deposition of dental plaque, thus posing as a primary etiological factor to a variety of oral diseases including denture stomatitis, gingival inflammation, and secondary caries. Microscopic examination by Ionescu et al., [20], in his study, showed that microbial colonization begins in the crevices, grooves, or pits on the surface.An occlusal surface with many pits and grooves also promotes greater bacterial colonisation, corresponding to the high surface free energy in such cases. A study by Dantas et al., [21], found that surface roughness and

bacterial adherence were influenced by manufacturing techniques and finishing/polishing protocols. Besides, in his study, Nestor et al., [22] demonstrated metabolically active bacterial settlements in polished bis-acrylic resin surface areas with surface imperfectionswhich, after polishing, yielded a much more regular surface with only few microorganisms, when observed on an electron microscope.

In fact, considering the material chosen for the present study, Protemp, other specific studies [23] conclude that Protemp allows for exemption from polishing. Instead, rubbing with alcohol after polymerization is sufficient to provide a smooth surface with the oxygen inhibition layer removed. However, the same study also states that, with the use of the material for provisional crowns, polishing becomes a critical step.

From the results, it can be inferred that different polishing agents prevent the colonisation of bacteria on the discs to different degrees. Among all the discs, the ones polished with rouge showed minimum bacterial colonies on sub-culturing. Rouge, composed of Fe2O3, varies from bright red to a sandy colour, with varying hardness based on the intensity of the colour. Generally, it is used for gross polishing of metals, glass, and stones, and for fine polishing of gold, silver, brass, and steel [24].

In the current study, the second set of discs were polished with Smile-N-Shine polishing paste, with the help of a dental polishing brush attached to a hand piece. Generally, these are prophylactic pastes [25], preferred to be used on teeth and restorations on it. They often contain particulate zirconium silicate, rouge, cuttle, tripoli, cuttle, emery, coarse pumice to prevent roughening surfaces.

The third polishing agent used was pumice powder. Pumice, a light coloured, siliceous material produced by volcanic activity, is used generally for the polishing of tooth enamel, gold foil, dental amalgam and acrylic resins. On comparison of results obtained for each of the polishing agents, it was observed that the maximum bacterial colonisation was on the discs polished with pumice, followed by polishing paste. Hence, rouge was observed to be more effective among the three agents, as it resulted in least microbial colonisation. Besides, when observing the unpolished control discs, there was a manifold increase in biofilm formation. This indicates the importance of polishing as a final procedure that attributes to the success of treatment. However, the study is limited by the fact that the findings are confined to in-vitro conditions.

## Conclusion

From the study, it is determined that rouge is a better polishing agent, followed by polishing paste, and then pumice, which is inferred from the microbial colonisation on the discs polished with respective polishing agents. Thus, it can be concluded that the efficacy of the polishing agent is a major consideration for a dentist when it comes exercising control over the extent of biofilm accumulation on the surface of the prosthesis, thereby ultimately ensuring better treatment.

## Clinical Significance

The accumulation of biofilm on the surface of provisional restorations is associated with and dependant on the roughness of its surface. Moreover, the longer the period that the prosthesis is placed in the oral cavity, the greater is the need for preventing plaque accumulation. Thus, prior to the temporary cementation of the provisional prosthesis it is necessary to render the surface smoothto ensure less bacterial adherence, and thereby, minimize the probabilityof development of caries and periodontitis lesions, and prevent any discoloration.Thus, with respect toa pathological and aestheticview,identifying how finishing and polishing procedures can be made more effective, is a mandate.

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