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RESEARCH ARTICLE

IN-VITRO EVALUATION OF SURFACE PROPERTIES AND EARLY BACTERIAL ADHESION IN CAD/CAM RESIN MATERIALS FOR PROVISIONAL RESTORATIONS

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ABSTRACT

Objectives: The aim of this study was to determine the effects of surface roughness and material type on bacterial adhesion to provisional resin materials. **Materials and Methods:** Standardized specimens of six different materials were fabricated using a stainless-steel mould/computer-aided design/computer-aided manufacturing (CAD/CAM) system. A profilometer was used to measure surface roughness. Each group was divided into two subgroups, which were treated with *Streptococcus mutans* and *Candida albicans* suspensions, respectively. Bacterial adhesion was assessed using a confocal laser microscope. Differences in surface roughness in independent groups were evaluated with the Kruskal–Wallis test, and multiple comparisons were performed using Wilcoxon rank sum tests. Bacterial data were analysed using the analysis of variance F test, with multiple comparisons using Tukey's honestly significant difference test. **Results:** The main result can be briefed as; surface roughness differed significantly among groups. Composite resin CAD/CAM blocks showed greater surface roughness and vital bacterial adhesion than did the other materials. **Conclusions:** Surface smoothness is important for provisional restorations, as plaque accumulation and diffusion of allergenic elements to these restorations is greater than that to natural tooth surfaces. If provisional crowns are to be used in the long term, this should be taken into consideration.

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INTRODUCTION

Provisional restorations (PR) are used during various stages of fixed prosthodontic treatment with the aims of achieving protection, stabilization, and functionality of prepared teeth and surrounding tissues (Burns, 2003). They help to maintain gingival health and protect teeth from physical, chemical, bacterial and thermal damage. PR should thus have appropriate marginal adaptation, fracture resistance, and low thermal conductivity and be non-irritating to the dental pulp and neighboring tissues (Burns, 2003; Wang, 1989) In recent years; while implant being a popular treatment option; sometimes implant therapy continues to be a challenge for many clinicians because of an excellent soft and hard tissue healing is required to have a good aesthetic outcome (Roe *et al*., 2012).

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Clinicians need to wait for this tissue healing with a provisional crown (Siqueira, 2019). PR should also have smooth surfaces, as rough surfaces lead to plaque accumulation on restorations, which may cause caries and inflammation of the gingiva, tempering the final result of the final restoration (Combe *et al*., 2004; Jin, 2004; Silva, 2008). Different materials have been introduced for use in provisional fixed prosthetics; methacrylate-based and bis-acryl composite materials are among the most popular. These materials can be polymerized chemically and/or by light. Recently advances in computer-aided design/computer-aided manufacturing (CAD/CAM) techniques have provided popularity in use of restorative dentistry during the last few decades. Besides; many restorative materials with different purposes have been produced. Recently, resin blocks for use with digital technology were introduced; they are currently preferred as a time-saving, aesthetic, and long-lasting PR material. Furthermore, these materials are easy to repair intra-orally in case of minor defects (Ruse, 2014). Composite resin CAD/CAM blocks, composed of a polymeric matrix reinforced by organic, inorganic, or composite filler, are fabricated under controlled conditions using an industrial polymerization process with high temperature and pressure.[9] Among the foreseen advantages of CAD/CAM blocks are; better polymerization, less porosity, greater homogeneity and the avoidance of operator-related variables.[10] Many in-vitro studies have indicated; physical properties of CAD/CAM resin blocks are superior then the conventionally polymerized resins. (Alt *et al*., 2011; Stawarczky, 2012). Surface roughness is thought to be an important factor determining the amount of plaque accumulation, (Jin *et al*., 2004; Kawai *et al*., 2000) as it influences the adhesion of Candida albicans to dental materials. [14]Microscopic evaluation of early plaque adhesion on solid-substrate surfaces has demonstrated that bacteria colonies initially along cracks and pits and that these microorganisms are difficult to remove by mechanical or chemical cleaning (Radford, 1999; Bollen, 1997) Surface roughness has been argued to be vital in the entrapment of bacteria in scratches and surface irregularities, and it protects restorations against shear forces (Radford, 1999; Bollen, 1997) In addition to surface characteristics, material components and polymerization may influence bacterial adherence.

No available data about the surface roughness of CAD/CAM composite blocks or their effects on bacterial adhesion are available in the literature. Thus, the aim of this study was to evaluate the surface roughness of six commonly used PR materials and their sensitivity to C. albicans and Streptococcus mutans adhesion. The authors hypothesized that bacterial adhesion and surface roughness would differ significantly among groups, with the composite resin CAD/CAM blocks demonstrating the most favorable properties.

MATERIALS AND METHODS

Six temporary crown and bridge materials were assessed in the present study. Material composition and manufacturers' information are presented in Table 1. Under the assumption of a difference of one standard deviation with respect to the primary end point between the groups, an alpha level of 5 % and power goal of 80 %, ten samples per group were necessary. Sixty temporary material discs (*n*=10/group) were prepared. A custom stainless-steel mould with calibrated circular holes (10×2 mm) was used to prepare the specimens composed of self-curing restorative materials for Groups A,B,C. The polymethyl methacrylate (PMMA) material for specimens in Group A (Temdent, Schütz Dental GmbH, Rosbach, Germany) were hand mixed at a 2:1 powder:liquid ratio, according to the manufacturer's instructions. The composite materials for specimens in GroupB (Luxatemp Plus, DMG GmbH, Hamburg, Germany) and Group C (Luxatemp Plus+Glaze&Bond, DMG GmbH, Hamburg, Germany)were mixed using an automix dispenser and automix tips from the same manufacturer. The mixed resin material for each specimen was packed into the mould, and both sides of the mould were clamped with a glass slide. After 10 min, the specimen was removed from the mould. To prepare specimens made of CAD/CAM-fabricated temporary materials (groups D–F), resin discs (Pattern Resin LS, GC Corporation, Tokyo, Japan) of the same size were prepared using the steel mould. Both sides of each disc were smoothed using agrinding and polishing machine (DAP-U, Struers, Ballerup, Denmark) with wet abrasive paper (Carbi Met-Micro Cut Abrasive Discs, Buehler, Lake Bluff, IL, USA: 1000, 2000, and 4000 grit) for 60 s. A digital image of each polished disc specimen, in STL file format, was then created using an optical scanner (S600ARTI, Zirkonzahn GmbH, Gais, Italy). Using the STL file, the triangulated geometry of the specimen was imported into the software module of the CAD/CAM system (Zirkonzahn. module, Zirkonzahn GmbH). The test specimens were fabricated using millable discs (Milling Unit M4, Zirkonzahn GmbH). All specimens were smoothed with wet abrasive paper, as described above, and then polished with a white stone (Dura White Stone, Shofu Inc., Kyoto, Japan) for 60 s by a single operator. Specimens in group C were coated only with surface sealer (Luxetemp-Glaze & Bond, DMG GmbH, Hamburg, Germany) according to the manufacturer's instructions. All specimens were stored in distilled water before further processing.

A profilometer (Surtronic 25, Taylor Hobson Ltd., Leicester, England) was used to measure surface roughness in three regions (middle and both sides) of each specimen. Mean surface roughness (Ra) values were then calculated. All specimens were cleaned for 15 min with an ultrasonic cleaner (SONICA S2, Soltec, Milan, Italy) and then sterilizedin an autoclave (Lisa, W&H GmbH, Bürmoos, Austria) at 121°C for 15 min before the assessment of bacterial adhesion. The specimens were covered with artificial saliva and a mucin suspension, and left for 1 h to allow the production of a pellicle. *C. albicans*(ATCC 18804) and *S. mutans* (ATCC 25175) were cultured at 37°C overnight in brain heart infusion(BHI) broth media (Oxoid, Basingstoke, UK). After incubation, the samples were rinsed three times with sterile phosphate-buffered salinesolution, then placed in 96-well culture plates in BHI, and incubated separately under aerobic conditions with bacterial suspension for 24 h (1×10^9 cfu/ml for *S. mutans*, 1×10^8 cfu/ml for *C. albicans*). Bacteria adhering to the specimens were stained with SYTO59 (Invitrogen, Carlsbad, CA, USA) and examined under a confocal laser scanning microscope (LSM 700, Carl Zeiss GmbH, Göttingen, Germany).

Differences in Ra values among groups were assessed using non-parametric methods. Independent groups were first evaluated using the Kruskal–Wallis test, and multiple comparisons were then performed using Wilcoxon rank sum tests. The association of bacterial adhesion with material type was assessed using analysis of variance (F test). Multiple comparisons were performed using Tukey's honestly significant difference test, with the significance level set at $p < 0.05$. For all statistical tests, the standard SPSS program 15.0 for Windows (SPSS, Chicago, II, USA) was used.

RESULTS

Surface roughness differed significantly among materials (Table 2a-2b). Ra values were lower for bis-acryl composite materials (0.302 and 0.350 μm) than for the methacrylate-based material (0.376 μm) and the CAD/CAM resin blocks (0.476 and 0.556 μm). Ra values were highest for the CAD/CAM blocks and lowest in group C (Luxatemp Plus+Glaze&Bond, DMG GmbH, Hamburg, Germany) for which liquid polishing was used. Adhesion of *C. albicans*, but not *S. mutans*, differed significantly among restorative materials($p<0.001$; Tables 3–5, Figs. 1, 2). The number of viable bacteria detected increased with surface roughness.

Table 1. Characteristics of the materials used in this study

	Group	Composition	Manufacturer
A	Temdent	Polymethyl methacrylate	Temdent, Schütz Dental GmbH, Rosbach, Germany
B	Luxatemp	Bisacryl	Luxatemp Plus, DMG GmbH, Hamburg, Germany
C	Luxatemp A	Bisacryl	Luxatemp Plus+Glaze&Bond, DMG GmbH, Hamburg, Germany
D	Vita	Cross-linked acrylate polymer with microfiller	CAD-Temp MonoC, VITA Zahnfabrik, Bad Sæckingen, Germany
E	WielandZeno	cross-linked PMMA	Telio CAD, Wieland Dental GmbH, Pforzheim,Germany
F	ZirkonZahn	cross-linked PMMA	TEMP PREMIUM CAD, Zirkonzahn GmbH, Gais, Italy

Table 2a. Surface roughness Ra (mean and standard deviation µm) for the groups

Group Name	Mean	SD±
Luxatemp	0.350	0.020
Luxatemp A	0.302	0.021
Temdent	0.376	0.043
Vita	0.408	0.020
Wieland Zeno	0.556	0.011
Zirkon Zahn	0.476	0.092

Table 2b: Multiple-comparisons (Wilcoxon Rank Sum Tests) to assess the group differences (statistically significant)**

Group	Group	W	p
LuxatempA	Luxatemp	5.0	0.006036328 **
LuxatempA	Temdent	5.5	0.006036328 **
LuxatempA	Vita	0.0	0.002348482 **
LuxatempA	Zirkonzahn	2.0	0.003264344 **
LuxatempA	WielandZeno	0.0	0.002348482 **
Luxatemp	Temdent	25.5	0.135929161
Luxatemp	Vita	3.5	0.004448676 **
Luxatemp	Zirkonzahn	10.0	0.016839373
Luxatemp	WielandZeno	0.0	0.002348482 **
Temdent	Vita	26.0	0.135929161
Temdent	Zirkonzahn	14.0	0.035549207
Temdent	WielandZeno	0.0	0.002348482 **
Vita	Zirkonzahn	16.0	0.045072987
Vita	WielandZeno	0.0	0.002348482 **
Zirkonzahn	WielandZeno	20.0	0.073601275

Table 3. Mean and standard deviations of the number of vital bacteria.

	Candida albicans		*S. mutans*	
Group Name	Mean	SD	Mean	SD
Luxatemp	65290	80241.82	185300	91571.53
Luxatemp A	54270	62342.96	178000	44171.38
Temdent	77340	77446.47	202000	52662.45
Vita	103500	65291.23	173000	53758.72
WielandZeno	199000	59338.95	176000	60037.03
ZirkonZahn	198200	103346.02	102600	84748.25

Table 4. Test for group differences (ANOVA) for *Candida albicans*

	Sum of Squares	*df*	Mean Square	F	*p*
Restorative material	2.168e+11	5	4.336e+10	7.478	2.16e-05

Table 5. Test for group differences (ANOVA) for *S. mutans*

	Sum of Squares	*df*	Mean Square	F	*p*
Restorative material	5.908e+10	5	1.182e+10	2.647	0.0327

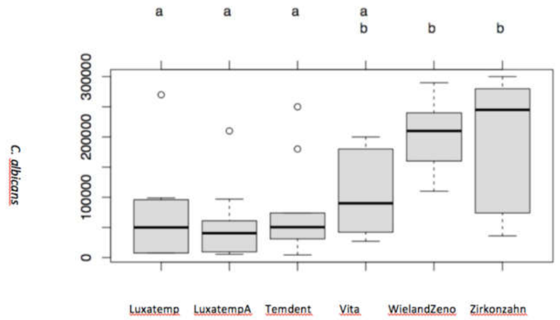

Fig. 1 Group differences in *C. albicans* accumulation, as determined by Tukey's HSD test. Groups with the same letter do not differ significantly.

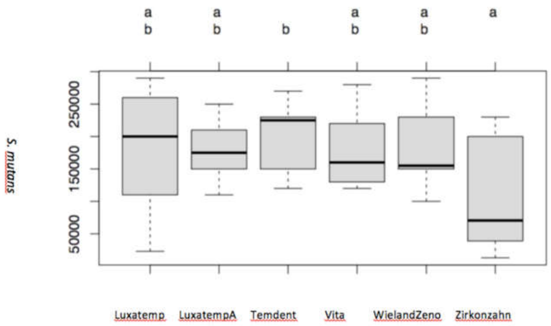

Fig. 2 Group differences in *S. mutans* accumulation, as determined by Tukey's HSD test. Groups with the same letter do not differ significantly.

DISCUSSION

Based on the results of the current investigation, the hypothesis that bacterial adhesion and surface roughness would differ significantly among groups, with CAD/CAM resin blocks producing the most favorable results, was partly rejected. Significant differences were detected among groups (Table 2b), but composite resin CAD/CAM blocks had higher Ra values and more vital bacterial adhesion than did specimens in the other groups. Surface smoothness is important for PR, as plaque accumulation and diffusion of allergenic elements to these restorations are greater than that to natural tooth surfaces (Luthardt, 2000). The matrix - type of the material and the polishing method used to influence surface roughness. Bis-acryl composites have been shown to have smoother surfaces than dimethacrylate-based materials, in this study and previous studies (Haselton, 2004; Maalhagh- Fard, 2003).

The roughness of CAD/CAM block surfaces observed in this study can be explained by the superior mechanical properties of these materials. The standard chair-side polishing procedure was not sufficient to obtain smooth surfaces on these blocks; thus, these materials should be polished in a laboratory or by laboratory conditions. These composite CAD/CAM blocks were introduced also as long-term provisional restorations. The use of resin has considerable advantages (Kramer *et al*., 2006; Kunzelmann, 2001) CAD/CAM production opened up a completely new area of research in restorative dentistry. Great success has been gained with CAD/CAM ceramics, but the process has just begun for CAD/CAM composite resins. CAD/CAM technology is here to stay, but research on these materials is still in its infancy. Especially; if the PR will be used long-term to reach the predictability of the esthetic outcome for implant treatment; the material type of the provisional is important for the implant-supported provisional prostheses (Martin, 2014). Many provisional crowns are fabricated chairside with adjustment made using fine-grained diamond rotary cutting instruments, followed by polishing (Burns, 2003). Sandpaper discs, rubber wheels, and polishing liquids are used commonly for polishing. Chair-side polishing is important not only for aesthetic appearance but also to produce smooth surfaces allowing limited plaque accumulation. Previous studies indicate that; surface-free energy and roughness are two factors that affect bacterial adhesion the most.[22]Microscopic examination has revealed that microbial colonization initializes in the crevices, grooves, or pits on the surface. [23] Also in this study; liquid polishing group (Luxatemp A) achieved the greatest surface smoothness and less S. mutans and C. Albicans adhesion than the unused group (Table 2b). These polishing liquids have become popular in recent years. This product imparts a high-luster surface to bis-acryl restorations and reduces polishing time. In an in-vitro study; Davidi *et al*. (2007) demonstrated that liquid-polished PMMA surfaces allowed less plaque accumulation by flowing over the small grooves and pits at the surface. However, this result and the results of the current study cannot be extrapolated directly to clinical usage, as dietary habits and tooth brushing can affect the surface roughness of restorative materials. However, the resistance of liquid polish to toothbrush abrasion and the effect of masticatory forces should be investigated before any clinical recommendation is made. No study has evaluated the resistance of liquid polish in the oral environment. S. mutans and C. albicans were selected to determine the microbial adhesion on different PR because; S. mutans is the primary progenitor pathogen in the formation of intraoral caries, and C. albicans is the most common opportunistic intraoral pathogen isolated from the oral cavity. (2006) C. albicans was isolated from oral cavities of 25% of healthy individuals in previous studies, and the percentage increased to 50 - 90% in cases of immunosuppression.[26] Many studies have demonstrated linear relationships between surface roughness and C. albicans adhesion (19,27), and Ra values >0.2 μm have been found to promote bacterial adhesion (Bollen, 1997) The results of the current study support the findings reported in the dental literature; all Ra values exceeded 0.2 μm (Table 2a), and the amount of bacterial accumulation increased with these values. Given the bacterial accumulation observed in the CAD/CAM groups, great attention should be given to polishing procedures when using these materials. PR prevents leakage into dentinal tubules by covering the prepared dental tissue until the preparation of fixed permanent prostheses. So it's vital that these in-vitro results should be supported within-vivo research, especially long-term studies. PR is also used for arranging occlusal plans, increasing vertical dimension and also contouring soft tissue, expected to improve the patient's conditions and achieve the final esthetic and functional outcomes (Gough, 1994) Because of the possible surface roughness on PR and their low marginal adaptation, bacterial colonization on PR is higher than that on permanent prosthetic materials. This is a significant factor in determining the life span of permanent prostheses and the health of the supporting teeth and periodontal tissues (Buergers, 2007). Within the limitations of this in vitro study; the aim was to evaluate the effects of material composition alone on microbial adhesion, but further extensive analysis, mimicking clinical situations should be performed to investigate bacterial adhesion to PR. In the oral cavity, dental materials are exposed to thermal and pH changes and aging may affect bacterial adhesion. The effects of permanent humidity and liquid changes, as well as chewing forces, should be taken into account. Thus, a study protocol simulating the various oral aging conditions would generate more detailed data about bacterial adhesion to polished surfaces.

Conclusion

The results of the study presented that the available provisional restorative materials exhibited surface roughness above the level that would prevent bacterial accumulation. Bis-acryl composite materials demonstrated the least surface roughness and bacterial adhesion, whereas CAD/CAM resins had the highest values. If the new generation CAD/CAM blocks will be used especially for a long-term provisional restoration; these materials should be polished in a laboratory or by laboratory conditions.

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