



# Effect of laboratory and chairside polishing methods on the surface topography of occlusal splint materials manufactured using conventional, subtractive and additive digital technologies

Efeito de métodos de polimento em laboratório e em consultório na topografia de superfície de materiais de *splint* oclusal fabricados usando tecnologias digitais convencionais, subtrativas e aditivas

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## ABSTRACT

**Objective:** This study evaluated the polishing properties of the occlusal splint materials obtained using subtractive and additive manufacturing methods with the laboratory-type polishing (LP) and chairside-type polishing (CP) procedures. **Material and Methods:** Specimens (N=180, n=60 each group) were manufactured using one of the following methods: subtractive manufacturing method (SMM) (M-PM Disc, Merz Dental GmbH), additive manufacturing method (AMM) (Freeprint Splint 2.0, DETAX GmbH & Co. KG), and the conventional manufacturing method (CMM) (Promolux HC, Merz Dental GmbH). Following LP and CP procedures, surface roughness of the specimens was measured using a digital surface profilometer. One representative specimen was selected from each group, and a scanning electron microscope (SEM) image was made. **Results:** Both the manufacturing method and the polishing procedures significantly affected the results ( $P < 0.01$ ). Interaction terms were also significant ( $P < 0.001$ ). **Conclusion:** With both polishing methods, surface roughness of the AMM group was the highest and the CMM group the least. Although the CP procedure was more effective than LP with both methods, surface roughness was below the  $0.2 \mu\text{m}$  threshold after both polishing procedures tested.

## KEYWORDS

CAD-CAM; Dental materials; Occlusal splints; PMMA; Surface properties.

## RESUMO

**Objetivo:** Este estudo avaliou as propriedades de polimento dos materiais de splint oclusal obtidos usando métodos de fabricação subtrativos e aditivos com os procedimentos de polimento laboratorial (LP) e polimento em consultório (CP). **Material e Métodos:** As amostras (N=180, n=60 para cada grupo) foram fabricadas usando um dos seguintes métodos: método de fabricação subtrativo (SMM) (M-PM Disc, Merz Dental GmbH), método de fabricação aditivo (AMM) (Freeprint Splint 2.0, DETAX GmbH & Co. KG) e o método de fabricação convencional (CMM) (Promolux HC, Merz Dental GmbH). Seguindo os procedimentos de LP e CP, a rugosidade da superfície dos espécimes foi medida usando um perfilômetro de superfície digital. Um espécime representativo foi selecionado de cada grupo, e uma imagem de microscópio eletrônico de varredura (SEM) foi obtida. **Resultados:** Tanto o método de fabricação quanto os procedimentos de polimento afetaram significativamente os resultados ( $P < 0,01$ ). Os termos de interação também foram significativos ( $P < 0,001$ ). **Conclusão:** Com ambos os métodos de polimento, a rugosidade superficial do grupo AMM foi a maior e a do grupo CMM a menor. Embora o procedimento CP tenha sido mais eficaz do que LP com ambos os métodos, a rugosidade da superfície ficou abaixo do limite de  $0,2 \mu\text{m}$  após ambos os procedimentos de polimento testados.

## PALAVRAS-CHAVE

CAD-CAM; Materiais dentários; PMMA; Propriedades de superfícies; *splints* oclusais.

## INTRODUCTION

Temporomandibular disorder (TMD) is a general term that encompasses a variety of clinical complaints involving the masticatory muscles, temporomandibular joint (TMJ), or associated orofacial structures [1]. The etiology is multifactorial and involves a large number of indirect and direct causal factors such as parafunction, trauma, and the causes of increased joint friction, alone or together [2-5]. Although the relationship between bruxism and TMD is still controversial, a relationship between sleep bruxism (SB) and TMD was found in self-reported studies evaluating the presence of SB [6]. The use of occlusal splints as a non-invasive procedure for known bruxism is quite a common treatment [7,8].

The compression molding technique and vacuum thermoforming are commonly applied in manufacturing occlusal splints. Porosity, polymerization shrinkage, and residual monomer content are factors encountered in these conventional methods that have an adverse effect on the quality of occlusal splints [9-11]. The increasing use of new technologies in dentistry has replaced conventional manufacturing with digital workflows in various processes [12]. In computer-aided design and computer-aided manufacturing (CAD/CAM) technology, the subtractive manufacturing method (SMM) and additive manufacturing method (AMM) are also implemented in manufacturing occlusal splints [13,14].

The SMM is based on manufacturing occlusal splints by milling a polymer disc. The advantage of this method is that an industrial polymethylmethacrylate (PMMA) disc is used which provides a better passive fit in the mouth due to the high degree of double bond conversion and the absence of polymerization shrinkage. The disadvantage however is the significant amount of unusable material waste created by milling the industrial disc [14]. The AMM, on the other hand enables the manufacturing of more complex objects yielding to less waste material and without applying excessive force as the manufacturing takes place layer by layer in a three-dimensional (3D) printer from 3D model data [13].

Numerous AMMs are currently available according to the material used and the method of application. The methods used to provide

polymerization with ultraviolet light for polymeric resins are stereolithography (SLA), PolyJet, and digital light processing (DLP) [15,16].

It is crucial for the surfaces of occlusal splints to have smooth surfaces. An ideally polished surface prevents discoloration and bacterial adhesion and does not irritate the mucosa [17,18]. In previous studies, it has been reported that the tip of the tongue can detect a roughness up to 50  $\mu\text{m}$  [19,20]. The clinically acceptable polishing threshold of an appliance to be placed in the mouth is below 0.2  $\mu\text{m}$  [21,22]. There is no consensus regarding the polishing procedure for occlusal splints as a function of LP and CP procedures. In the literature, polishing procedures are generally divided into two types: two-body wear abrasion and three-body wear abrasion [23]. During the polishing of resin occlusal splints, standard polishing procedures (i.e. polishing burs, pumice, and high-shine polishing protocols) are followed similar to polymethylmethacrylate based other dental appliances [24]. However, the suitability of these methods for occlusal splints produced using the SMM or AMM is unknown.

The aim of this study therefore was to evaluate the polishing properties for the occlusal splints obtained using three manufacturing methods and LP and CP polishing procedures. The first null hypothesis of the present study was that there would be no significant difference in the surface topography of the splints produced with the three manufacturing methods. The second null hypothesis was that there would be no significant difference between LP and CP procedures in the polishing of occlusal splints.

## MATERIALS AND METHODS

The specimens were manufactured using three methods: SMM (n=60), AMM (n=60), and CMM (n=60). The details of the materials used in all three production methods are shown in Table I. The manufacturing of the specimens and polishing procedures were performed by a single researcher (H.B.) in order to prevent the effect of differences between practitioners on the outcomes who was blinded to the groups.

### Specimen preparation

The discs (diameter: 15 mm; thickness; 3 mm) were designed in the CAD program

**Table 1** - The brands, types, manufacturers, chemical compositions, and batch numbers of the materials used in the current study

Brand	Type	Manufacturer	Chemical Composition	Batch number
M-PM Disc Clear	Clear disc suitable for production by milling method	Merz Dental GmbH, Lütjenburg, Germany	Polymethylmethacrylate (PMMA) and cross-linked polymers based on methacrylic acid esters, dibenzoyl peroxide, residual monomer <1%	21118
Freeprint Splint 2.0	Photo-polymerized liquid resin	DETAX GmbH & Co. KG, Ettlingen, Germany	Acrylate resin, aliphatic urethane acrylate, tripropylene glycol diacrylate (TPGDA), tetrahydrofurfuryl methacrylate (THFMA), thermoplastic polyolefins (TPO)	230101
Promolux HC	Heat-polymerized resin (powder, liquid)	Merz Dental GmbH	Powder: PMMA copolymer, dibenzoylperoxide, organic colorants, inorganic pigments. Liquid: MMA, dimethylmethacrylate	1020003

(SolidWorks 3D CAD, Dassault Systèmes SolidWorks Corporation, Waltham, Canada). The data were used as a master standard tessellation language (STL) file to fabricate all the milling and printing specimens. For the SMM group, PMMA discs (M-PM Disc, Merz Dental GmbH, Lütjenburg, Germany) were milled using a 5-axis milling machine (M30, CAMCube, Montreal, Canada). The specimens of the AMM group were printed at 50  $\mu\text{m}$  layer thickness by curing the photopolymerized acrylic resin liquid (Freeprint Splint 2.0, DETAX GmbH & Co. KG, Ettlingen, Germany) using a DLP 3D printer (D20+, Dental Wings Inc, Montreal, Canada) with the wavelength of 385 nm, at a building angle of 0°. The specimens were rinsed in isopropanol (99%) twice for 2 minutes in order to prevent the formation of non-polymerized monomer residues. The polymerization of the specimens was completed by curing them for 10 minutes in an ultraviolet polymerization device (SHERA flash-light Plus, Shera Material Technology GmbH & Co. KG, Lemförde, Germany) twice with 2000 flashes and a 5-minute break.

The specimens in the CMM group were fabricated using the compression molding technique. First, disc-shaped patterns (diameter: 15 mm; thickness; 3 mm) were milled from wax discs in accordance with the data prepared in the CAD program. The disc-shaped waxes were then placed in a flask with hard dental plaster type IV (Fujirock EP, GC Europe, Leuven, Belgium). The wax patterns were eliminated in 70°C water for 20 minutes. Thereafter, the flask was opened, and wax elimination was completed by washing the wax residues with water at the same temperature. The monomer and polymer of the heat-polymerized PMMA (Promolux HC, Merz Dental GmbH, Lütjenburg, Germany) were mixed

according to the manufacturer's instructions. The mixture of PMMA was tapped in the place of the wax patterns. The flask, tightened with a clamp, was placed in the polymerization device (C-11, Ermetal Dental, Ankara, Turkey) with water at room temperature. After reaching 100°C, polymerization began at this temperature for 30 minutes. At the end of this period, the flask was removed from the polymerization device and left to reach room temperature. The specimens were then stored in water at room temperature for 48 hours. They were pre-polished for 2 minutes at a contact pressure of 0.3 MPa under continuous water cooling in a polishing machine (Polishing Machine, Mecatech 334 SPC, Presi France, Eybens, France) with 400-, 800-, 1200-, and 1500-grit silicon carbide papers (Silicon Carbide Grinding Paper, Struers ApS, Ballerup, Denmark) [17]. Twenty pre-polished specimens from each group were used as the CG (CG).

### Labside polishing

A platform was designed in the CAD program (SolidWorks 3D CAD) by curing the photopolymerized acrylic resin liquid (Freeprint Splint 2.0) using a DLP 3D printer (D20+, Dental Wings Inc.) to apply the standard polishing procedure to all specimens. A round-shaped area with the diameter of the specimens and a depth of 2 mm was arranged in the middle of the platform. The height of the platform was adjusted so that the specimen surfaces to be polished were in full contact with the polishing heads attached to the LP device. This platform was supported by the practitioner (H.B.) to prevent the movement of the specimens during the polishing steps. A lathe bristle brush (Polishing Brushes Chungking White, Bredent GmbH & Co. KG, Senden, Germany) was attached to the LP

device (Poliereinheit PE5, Degussa AG, Hanau, Germany), and 20 specimens from each group were brushed with laboratory-type pumice slurry (Pumice Fine Grits, Kerr Corp., Orange, CA, USA). Then, pumice slurry application was continued with a rag wheel (Abraso-sil Acrylic, Bredent GmbH & Co. KG, Senden, Germany). After the specimens were washed, polishing paste (Universal Polishing Paste, Ivoclar Vivadent Inc., Schaan, Liechtenstein) was applied with a soft cloth wheel (High Luster Buff Acrylic, Bredent GmbH & Co. KG, Senden, Germany) to obtain a glossy surface. All the polishing steps were applied to each specimen for 2 minutes at 3000 rpm. Finally, the specimens were ultrasonically cleaned (Eurosonic Energy, Euronda SpA, Vicenza, Italy) for 1 minute and left to dry.

### Chairside polishing

In order to ensure standardization in the CP procedure, a stabilizing unit for a micromotor-coupled handpiece (STRONG 206, SAESHIN, Daegu, Korea) and a platform where the specimens would be placed were designed in the CAD program (SolidWorks 3D CAD) and printed. The height of the platform and the depth for placing the specimens were adjusted as specified in the LP procedure. Twenty specimens from each group underwent CP with a polishing kit (EVE Denture Polishing Set, EVE Ernst Vetter GmbH, Keltern, Germany). For that, the green, gray, and yellow rubber polishers included in the set were used. After that, polishing was continued with the brown rag wheel followed by the pink rag wheel included in the set. After the specimens were washed, polishing paste (Universal Polishing Paste, Ivoclar Vivadent Inc.) was applied with a soft cloth wheel to obtain a glossy surface. All polishing steps were applied to each specimen for 2 minutes at 5000 rpm. Finally, the specimens were ultrasonically cleaned (Eurosonic Energy) for 1 minute and left to dry.

### Measurement of surface roughness and SEM Analysis

The surface roughness (Ra) was measured immediately after the production of the specimens, after pre-polishing, and after applying the LP and CP procedures using a digital surface profilometer (Perthometer M2, Mahr GmbH, Gottingen, Germany). The highest point of the diamond stylus of the profilometer was measured at a constant speed of 1 mm/s and the

measurement length of 2 mm on each specimen's surface. The surface roughness was calculated by measuring 3 vertical and 3 horizontal lines for each specimen and taking the average of these 6 lines [18]. The profilometer was calibrated before measuring each specimen.

After the specimens were produced and after all the polishing procedures had been carried out, one representative specimen was selected from each group, and scanning electron microscopy (SEM) (Carl Zeiss EVO LS 10, Carl Zeiss NTS GmbH, Aalen, Germany) images were obtained at x600, 1500, and 2500 magnification.

### Statistical analysis

A sample size of 12 in each group was estimated with 95% confidence ( $1-\alpha$ ), 95% test power ( $1-\beta$ ), and  $f=0.40$  effect size (PASS 15 Power Analysis and Sample Size Software-2017, NCSS LLC., Kaysville, Utah, USA). The number of specimens was determined to be 20 in each group to increase the power of the study and to account for the possibility of damage occurring in any of the specimens. Data were analyzed with SPSS version 23 (IBM). The assumption of normality was tested using the Kolmogorov-Smirnov test. Two-way ANOVA and Tukey's tests were used to compare the Ra values according to polishing procedures and manufacturing methods. Data were presented as mean and standard error.  $P<0.05$  was considered as statistically significant in all tests.

## RESULTS

Both the manufacturing method and the polishing procedures significantly affected the results ( $P<0.01$ ). Interaction terms were also significant ( $P<0.001$ ).

There was a significant difference between the surface roughness values according to the polishing types ( $P=0.001$ ), where the specimens that underwent CP showed the lowest surface roughness values (Table II). The mean Ra values of the CG, the CP group, and the LP group were  $0.195\pm 0.005$ ,  $0.064\pm 0.005$ , and  $0.099\pm 0.004$   $\mu\text{m}$ , respectively (Table III, Figure 1).

Regarding manufacturing methods, the lowest mean Ra value was obtained in the CMM group ( $P=0.001$ ). The mean Ra values of the AMM, CMM p, and the SMM groups were  $0.168\pm 0.006$ ,



$0.083 \pm 0.006$ , and  $0.099 \pm 0.006 \mu\text{m}$ , respectively (Table III, Figure 1).

The mean Ra values of the splints produced via the AMM did not show a significant difference compared to those produced using CP and LP ( $P=0.700$ ). Similarly, the specimens produced using the CMM and SMM did not show a significant difference in the CG ( $P=0.811$ ) (Table III, Figure 1).

According to the means of the total Ra values before-polishing, the CP group, and the LP group were  $1.073 \pm 0.091$ ,  $0.087 \pm 0.012$ ,

and  $0.118 \pm 0.005 \mu\text{m}$ , respectively (Table IV, Figure 2). In the AMM group, CP and LP did not show a significant difference (Table IV, Figure 2).

SEM images from specimens immediately after production showed more irregular and rough areas in the CMM group among all three manufacturing methods, while the surface topography was smoother in the SMM group (Figure 3). After the surfaces were treated with silicon carbide papers (CG), lines and slight indentations were observed in the SMM and CMM groups, while the traces of grooves in the AMM group were more prominent. After the CP and LP procedures roughness decreased and smooth, homogeneous surfaces became evident in all groups, but there were more grooves present in the AMM group than in other groups.

**Table II** - ANOVA results comparing the Ra values with Robust ANOVA according to polishing type and manufacturing methods

	Ra	
	Test statistics	P
Polishing method	731.4	0.001
Manufacturing methods	292.7	0.001
Polishing method x Manufacturing methods	58.1	0.001

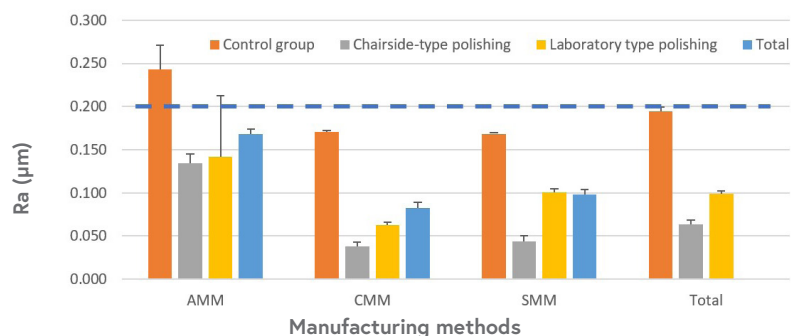
## DISCUSSION

In the present study, the surface topography of occlusal splint materials obtained using three manufacturing methods were evaluated after two polishing procedures. The splints manufactured using the CMM had the best topography, and

**Table III** - Mean Ra values of AMM, CMM and SMM groups as a function of polishing procedures and multiple comparison of polishing methods and manufacturing methods

	Manufacturing methods			Total
	AMM	CMM	SMM	
Polishing method				
Control group ( $\mu\text{m}$ )	$0.243 (\pm 0.011)^c$	$0.171 (\pm 0.005)^a$	$0.169 (\pm 0.007)^a$	$0.195 (\pm 0.005)^b$
Chairside-type polishing ( $\mu\text{m}$ )	$0.135 (\pm 0.003)^b$	$0.038 (\pm 0.002)^d$	$0.044 (\pm 0.001)^f$	$0.064 (\pm 0.0048)^a$
Laboratory-type polishing ( $\mu\text{m}$ )	$0.142 (\pm 0.007)^b$	$0.063 (\pm 0.003)^e$	$0.101 (\pm 0.004)^g$	$0.099 (\pm 0.004)^c$
Total	$0.168 (\pm 0.001)^a$	$0.083 (\pm 0.006)^b$	$0.099 (\pm 0.001)^c$	

<sup>a-c</sup> There is no difference between polishing types/manufacturing methods with the same letter. <sup>A-G</sup> No difference between polishing methods and manufacturing method interactions with the same letter.

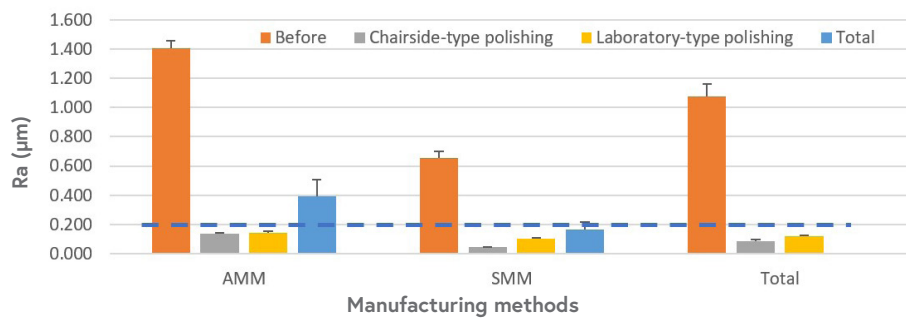
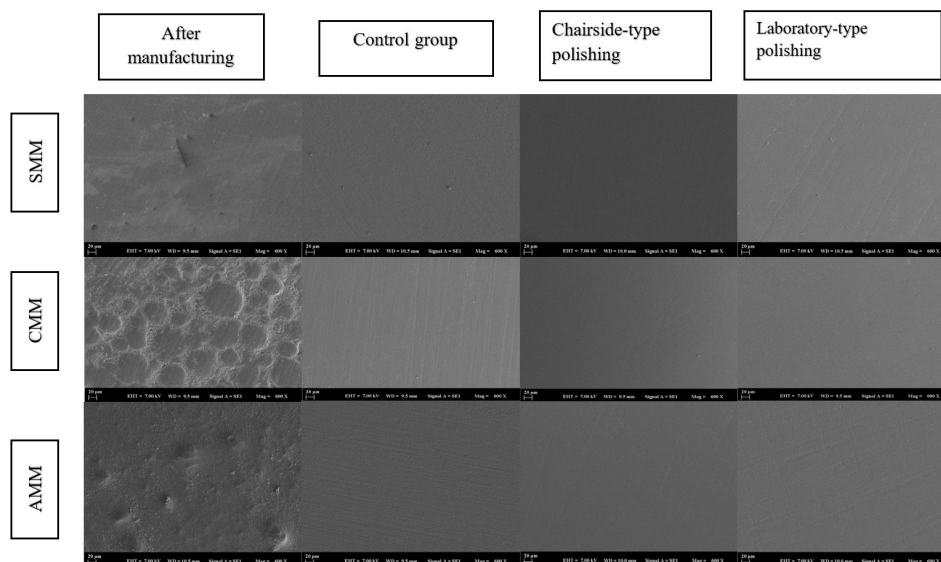


**Figure 1** - Surface roughness (Ra) of AMM, CMM, SMM groups with applied polishing procedures. The blue dashed line represents the clinical threshold for surface roughness ( $0.2 \mu\text{m}$ ).

**Table IV** - Mean Ra ( $\mu\text{m}$ ) values of the AMM, CMM and SMM groups before and after applying polishing procedures and multiple comparison of polishing methods and manufacturing methods

Polishing method	Manufacturing Methods		Total
	AMM	SMM	
Before	1.407 ( $\pm 0.048$ ) <sup>c</sup>	0.654 ( $\pm 0.047$ ) <sup>d</sup>	1.073 ( $\pm 0.091$ ) <sup>a</sup>
Chairside-type polishing	0.136 ( $\pm 0.004$ ) <sup>a</sup>	0.045 ( $\pm 0.002$ ) <sup>b</sup>	0.087 ( $\pm 0.012$ ) <sup>b</sup>
Laboratory-type polishing	0.145 ( $\pm 0.008$ ) <sup>a</sup>	0.104 ( $\pm 0.005$ ) <sup>e</sup>	0.118 ( $\pm 0.005$ ) <sup>c</sup>
Total	0.391 ( $\pm 0.117$ )	0.168 ( $\pm 0.049$ )	

<sup>a-c</sup> There is no difference between polishing types. <sup>A-E</sup> No difference between polishing types and manufacturing methods interactions with the same letter.

**Figure 2** - Surface roughness (Ra) of AMM, CMM, SMM groups with and without applying polishing procedures. The blue dashed line represents the clinical threshold for surface roughness ( $0.2 \mu\text{m}$ ).**Figure 3** - Representative SEM images of the SMM, CMM, AMM groups after manufacturing and polishing procedures (original magnification).

the splints manufactured by the AMM showed the worst polishing properties. Manufacturing method and polishing method significantly affected the results. Thus, based on the results, the first null hypothesis that there would be no significant difference in the polishing properties of the splints produced using three manufacturing

methods was rejected. The second null hypothesis that the LP and CP procedures would cause no significant difference in the polishing of occlusal splints was also rejected.

Occlusal splints are generally used at night, and the salivary flow rate decreases during sleep [25]. Without mechanical cleaning, mature

plaque on an occlusal splint can contribute to gingival diseases and caries [26]. In occlusal splints, the material type, surface roughness, and surface free energy are important in terms of plaque accumulation [27]. In a study by Quirynen et al. [22], it was reported that when the surface roughness of the titanium surface around the implant is less than  $0.2 \mu\text{m}$ , a biofilm layer is not present. This specific value is referred to as a threshold value for the smoothness of dental materials [21,22]. In the study of Schubert et al. [27], more *C. albicans* accumulation was observed on splint materials produced through digital methods compared to those produced using a conventional method. In the study by Freitas et al. [28] regarding denture bases, milled and conventional splints were found to be more successful than printed splints in terms of preventing *C. albicans* adhesion. In both studies, the surface roughness of the splints produced using additive method was the highest, which is in accordance with the current study. In the present study, the highest surface roughness was found in the AMM group compared to occlusal splints produced using other methods both before and after polishing. As the surface roughness values remained below  $0.2 \mu\text{m}$  after both polishing procedures, it is thought that splints produced using the AMM method will not pose a clinical problem. Yet, these findings need to be further observed clinically. In the measurements made immediately after manufacturing, the specimens in the CMM group were too rough to be measured with a digital surface profilometer, as seen in the SEM images. Although the SMM specimens were extremely smooth when viewed with the naked eye surface roughness values were above  $0.2 \mu\text{m}$ .

It is not easy to compare the results of current study with the literature due to the variability of many parameters about polishing studies. In this study, the surface topography of splints produced by conventional and subtractive methods were found to be more successful than those produced by the additive method. In the study of Grymak et al. [18], it was observed that the surface roughness of heat polymerized splints was less than the splints produced with the subtractive method when they were first produced. After they used various polishing methods, they stated that the splints produced with heat-polymerized and subtractive methods were better polished in consistence with the

current study. In addition, they stated that the production angles for splints produced by the additive manufacturing method are important for polishability. In another study [29], only polishing machine was used and the surface roughness was found to be the least in the splints produced by the additive method and the most with the subtractive method. The reasons for the difference with the present study may be the difference in the polishing device, the differences in the production angles in the additive group, and the material differences in the subtractive and conventional groups.

Previously it was shown that on the denture bases, manual and mechanical polishing procedures performed with a polishing kit demonstrated surface roughness being lower in manual polishing [30]. The same methods were applied in two different polishing procedures. The reason for the differences could be attributed to the pressure applied by the practitioner. In the current study, platforms were produced in accordance with the specimens and polishing methods in both LP and CP procedures in order to avoid deviations in polishing procedures.

In this study, CP was more effective than LP for polishing of occlusal splints. In the literature, the duration of occlusal splint treatment in TMDs varies between 1 and 12 months [31]. In this process, after the contacts of the occlusal splints are checked during the follow-ups of the patients, and the necessary procedures are carried out on the occlusal splints where CP is often preferred because it is practical and enables successful polishing.

Different 3D printing methods are implemented in several disciplines in dentistry of which SLA and DLP technologies are frequently used in the production of occlusal splints [32]. In the current study, DLP technology was used to obtain the specimens where rougher surfaces were observed compared to CMM and SMM technologies. In the review of Shaikh et al. [32], superior surface finish quality was advocated using the SLA method. Future studies should focus on SLA and DLP technologies for manufacturing occlusal splints and evaluate their surface texture.

The surface roughness of the splints produced with the AMM was affected by many parameters such as the resin type, the resolution of the printer, the polymerization duration and the shape, intensity of the laser, along with the

orientation of printing [33,34]. In the study of Campbell et al. [35], the lowest surface roughness was obtained at an orientation angle of 0 degrees. In addition, in the study of Grymak et al. [18] with 3D-printed occlusal splints, it was reported that the samples produced with a 0-degree angle had the lowest surface roughness compared to milled and heat-polymerized splints in the pre-polishing evaluation. Authors even claimed that there was even no need for polishing. Here, a 0-degree angle was chosen to produce the AMM specimens. The reason for choosing a 0-degree angle in the manufacturing in the AMM group was to produce specimens with as smooth surfaces as possible and compare them with other production methods.

The limitations of present study were that polishing procedures were employed in a controlled manner which may not be clinically always feasible. New AMM technologies that allow for lower layer thickness should be further investigated.

## CONCLUSIONS

From this study, the following could be drawn:

1. The chairside polishing procedure tested was more effective in obtaining a smooth surface for the splint materials manufactured using conventional, subtractive and additive digital methods.
2. After both chairside and labside polishing procedures, the surface roughness was the highest with the additive method and the least with the conventional method.
3. Both chairside and labside polishing procedures were sufficient to obtain a mean average roughness value of 0.2  $\mu\text{m}$  set as a threshold, with all splint materials and manufacturing methods.

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## Author's Contributions

HB: Conceptualization, Methodology, Data Curation, Writing – Original Draft Preparation.

SEE: Data Curation, Writing – Review & Editing.  
EK: Data Curation, Writing – Review & Editing.  
MÖ: Conceptualization, Methodology, Data Curation, Writing.  
NL: Data Curation, Writing – Review & Editing.

## Conflict of Interest

No conflicts of interest declared concerning the publication of this article.

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## Regulatory Statement

The study was waived of ethical approval because it did not include patients or animals.

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