Santos, M; Soo, S; Petridis, H; (2013) The effect of Parylene coating on the surface roughness of PMMA after brushing. **Journal of Dentistry** , 41 (9) 802- 808. 10.1016/j.jdent.2013.06.011.

ARTICLE

The effect of Parylene coating on the surface roughness of PMMA after brushing

Short Title: Parylene coating affects PMMA surface roughness

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Abstract
Objectives
Acrylic resins, used in the manufacturing of different types of intra-oral prostheses, are vulnerable to colonization by microorganisms which potentially endanger the general health of the prostheses wearers. The aim of this study was to investigate the influence of a novel coating (Parylene) on surface roughness of Poly-methyl-methacrylate (PMMA) samples after simulated cleansing using an electric toothbrush and two brushing media (paste and pumice).

Methods
Fifty six square PMMA samples were fabricated. Half of the samples were coated with a uniform 10µm coating of Parylene. All samples were subjected to simulated brushing with either paste or pumice. Changes in surface roughness were measured with a laser non-contact profilometer and compared between groups. Scanning electron microscopy (SEM) and Raman spectroscopy were utilized for surface visualization and analysis.

Results
In the coated samples, the mean surface roughness remained the same before and after brushing in the Paste group 2.69µm (SD=0.92 and SD=0.87 respectively), and increased from 3.73µm (SD=1.25) to 5.05µm (SD=1.40) in the Pumice Group. In the uncoated samples, the mean surface roughness increased from 4.45µm (SD=0.92) to 6.73µm (SD=1.73) in the Paste group, and from 3.67µm (SD=0.74) to 7.50µm (SD=2.25) in the Pumice Group. Differences between the coated and uncoated groups were statistically significant ($p<0.05$). The surface analyses revealed that the coating remained adhered to the PMMA, although signs of partial detachment were noticed in the Pumice Group.

Conclusion
The Parylene coating resulted in a reduction of surface roughness of PMMA after brushing procedures.

Clinical Significance
Parylene appears to maintain a low surface roughness of PMMA after abrasion by brushing.

Keywords: Parylene, Acrylic, Roughness, Coating, Brushing, PMMA

Introduction

Acrylic resins are used widely in fixed and removable prosthetic rehabilitations. Poly-methyl-methacrylate (PMMA) is an acrylic resin commonly used in the fabrication of denture bases. PMMA surfaces are susceptible to indentation by hard objects and easy to abrade. These phenomena contribute to an increase in surface roughness and wear of the acrylic, leading to a favourable environment for biofilms to accumulate. Colonization of dentures by microorganisms like Candida albicans and Streptococcus oralis in the form of biofilms has been associated with denture stomatitis [1, 2] and frequently associated with general disease in diabetic patients, hospitalised

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populations and other immunocompromised patients. Methicillin-resistant Staphylococcus aureus (MRSA) has also been reported as present in dentures and its difficult erradication may facilitate the spread of infection in hospital environments [3].

In 1997 a clinical study by Bollen et al. [4] established that the threshold roughness (Ra) for plaque retention of intra oral materials was 0.2µm. Several studies have shown that brushing action causes increased surface roughness in acrylic materials [5, 6, 7, 8, 9]. The subsequent colonisation of a rough surface by microorganisms is also a well-documented fact [10, 11, 12] as the micro crevices created by the surface degradation serve as a shelter and a reservoir for microorganisms.

Roughness parameters can be analyzed either in two (2D) or in three (3D) dimensions with 3D profilometers gaining popularity as they can provide a broader and more comprehensive image of a material's surface characteristics and support specific imaging software [13]. Various measurements of surface roughness can be obtained, with Ra, which is the arithmetic average height measurement, being frequently used as a universal roughness parameter for general quality control [13]. Profilometers can be used to compare the same surface in different times and after different levels of wear and are classified either as contact or non-contact. The latter will not involve any part of the instrument physically touching the surface being analyzed, whereas the former involve a stylus running in direct contact with the surface of the sample while recording its texture [14].

Parylene is the commercial patented name of a family of organic polymers called poly(para-xylylenes) formed by means of Vapour Deposition first observed by Szwarc, in 1947 [15]. The production of Parylene films starts with the pyrolytic decomposition at 600ºC of p-cyclophane, a dimer composed of two p-xylylene molecules, into its respective molecules [16, 17]. The substance obtained this way is a vapour of monomer that, if injected into a deposition chamber at room temperature, rather than going back to its original dimeric powder form, produces instead a transparent, strong, highly crystalline polymer film.

Parylenes exhibit a very high level of biocompatibility and very low toxicity and are therefore, indicated for medical uses [18, 19]. They are nowadays widely used in medical sciences as a coating material for metallic implants [20], for covering biological micro-electromechanical systems (BioMEMS) [21] and encasing implantable neural electronic devices [22]. Its use in oral appliances has shown some possible advantages, such as the creation of a beneficial microbial ecological shift, protection against corrosion [23] and the increase in the fracture resistance of dental ceramics [24]. Although its adhesion to some materials may not be easily achieved, several methods can be used to bypass this and achieve a reliable adhesion [18, 22].

The aim of this study was to compare the changes in surface roughness after brushing cycles with pumice or paste between PMMA samples coated with Parylene and control uncoated samples. The null hypothesis was that no statistically significant difference in surface roughness change would be detected after brushing between samples coated with Parylene compared to uncoated samples.

Materials and Methods

Sample Size determination:

Sample size was calculated using nQueryAdvisor® (Statistical Solutions, Cork, Ireland) and data from previous published studies. A final sample size of 14 samples per group was decided based on the calculation that it would allow for a 95% power to detect a difference in mean roughness of 1.5µm assuming that the common standard deviation is 1.00, using a two-group t-test with a 0.05 two-sided significance level.

Sample Fabrication:

Square samples of PMMA (Diamond D® Heat Cure Denture Resin, Keystone Industries, New Jersey, USA) measuring 15x15mm were fabricated after being processed in a large two-part metal flask. Every square was analysed under an optical microscope (Mantis Elite® Vision Engineering, Surrey, England) at 10x magnification and any samples showing gross surface defects were rejected. Fifty six samples were randomly divided into 4 equal stacks and attributed a group number. The first and second groups (test groups) were coated with Parylene whereas the third and fourth groups (control groups) were left uncoated. The samples in the test and control groups were subsequently brushed with either paste or pumice. The samples in the test groups were handled as follows: The samples were packaged in moisture tight sealed bags and sent to be coated with Parylene to Specialty Coating Systems Ltd (SCS), Surrey, England. The target thickness of the Parylene coating was 10µm on all sides. Control of the coating thickness achieved after the 2 cycles was ascertained by using coupons placed inside the chamber.

Experimental Testing:

A custom-made brushing apparatus was designed and built for this experiment. An electric toothbrush (Oral B Triumph 5000®, Procter and Gamble, Surrey, England) was fixed in a horizontal position over a plastic holder and secured to a flat surface. The brush operated at 48,800 oscillations per minute. A weight of 200 g was placed on top of the brush's head. A small gypsum well was used to retain the samples and prevent pumice or paste escaping during brushing. The brush was fixed so that the brush head position was consistent between samples and was fully charged each time. The brush head was replaced for a new one every 5 samples within the same group. Two different brushing media were used: a pumice slurry (Skillbond®, Skillbond Direct, High Wycombe, England) and a commercially available Toothpaste (Oral B Pro-Expert®, Procter and Gamble, Surrey, England). To simulate one year of wear, a total of 20 minutes brushing per sample was undertaken [25]. Two milliliters of each slurry were placed on the well over the sample with a plastic pipette before the brushing commenced. Every 2 minutes the slurries were replenished while the brush kept rotating to compensate for the slurry lost with the oscillation/vibration of the brush head. The slurry used was always taken from the same depth in the container after agitation so that the average amount of abrasive particles was

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similar in every cycle. At the end of the brushing cycle the samples were rinsed under running water, dabbed dry using a soft paper tissue and left to dry at room temperature.

Roughness Measurements:
The initial (before brushing) and final (after brushing) surface roughness measurements (arithmetic mean height - Ra) were taken from each sample using a Laser Profilometer (ProScan 1000® Scantron, Sommerset, England). Surface roughness measurements were also obtained for seven uncoated PMMA samples, before coating in order to analyze the effect of Parylene coating on initial surface roughness.

Two areas of 3 x 3mm (9mm2) on each sample were chosen where abrasion was predicted (initial Ra) or observed (final Ra) and an average roughness value of each area was measured and calculated. For each area the Profilometer scanned 600 lines, 0.005mm away from each other to obtain the final 3 x 3mm area. The Ra measurements were obtained by choosing 5 points diagonally down the area and recording the Ra of the 2 lines that intercepted at each point. Obvious aberrations in surface topography in the 9mm2 area scanned were ignored and no point with lines crossing those areas was read except when impossible to avoid in highly roughened areas after testing

Visual Surface Analysis:
A Scanning Electron Microscope (SEM) (JEOL JSM 5410 LVSEM, JEOL Ltd, Herts, England) was used to visualize the surface topography of control and experimental samples. One sample from each group and 2 untested samples (one coated and one uncoated) were coated with sputtered gold and visualised at x50, x200 and x1000 magnification. An attempt was made to focus on a representative area and increase the magnification while remaining on the same area.

Surface Molecular Analysis:
After the brushing procedure all samples from the Test Groups were analysed using a Laser Raman Spectroscope (Labram 300, Horiba Jobin Yvon) to detect the presence of the Parylene coating. This spectroscope uses a HeNe (633nm) laser. Additionally, one sample that had not been subjected to abrasion cycles was also analysed to serve as control. In each sample an area measuring 100 x 100µm was chosen in the most abraded surface present.

Statistical Analysis:
The mean roughness values before and after the Parylene coating before brushing, were analysed using the Paired t-test. Multivariable Linear Regression analysis was undertaken analyze the effect of coating and brushing medium on the final Ra after adjusting for the initial Ra. The computer software SPSS® (Version 20, IBM, Portsmouth, UK) was used for the statistical analysis and production of graphs. The interaction between coating and brushing medium was included as a co-variant in the regression analysis. The assumptions underlying the regression analysis were checked by study of the residuals. A significance level of 0.05 was used for the hypothesis testing.

Results

Roughness measurements:
The mean roughness values, before and after brushing, in the 4 experimental groups are shown in Table 1.

In the Control Groups (28 samples of uncoated PMMA) there was an increase in mean final roughness of 51.24% compared to the initial Ra in the Paste Group (Fig.1) and an increase of 104.36% in the Pumice Group. All samples in the Control

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Groups showed an increase in average surface roughness after the brushing procedure. In the Test Groups (28 samples of Parylene coated PMMA) there was no change in mean final Ra scores compared to the initial Ra in the Paste Group (Fig.1) and there was a 34.67% increase in the Pumice Group. Seven samples in the Test Group showed a decrease in mean surface roughness (4 in the Paste group and 3 in the Pumice group) after the brushing cycle.

Seven samples from the Test Group were scanned before being sent for coating. This was done to analyse the change in roughness granted by the Parylene coating alone. Surface roughness decreased in all the samples after coating from a mean Ra of 3.830µm before (SD=1.132) to 2.750µm after (SD=1.084).

Statistical analysis of roughness results:
To check the assumptions underlying the regression analysis, the residual values were plotted and revealed a normal distribution and constant variation.The statistical analysis of the results revealed that the coating significantly affected the Final Ra ($p<0.005$), with its presence leading to reduced final Ra values by a mean of 2.8µm (95% CI:1.9-3.7µm) after adjusting for the brushing medium and initial Ra. The brushing medium also exhibited a statistically significant effect on the roughness with the pumice increasing the roughness by 1.5µm ($p<0.005$, 95% CI 0.6-2.4µm) when compared to paste, after adjusting for the presence of the coating and the Initial Ra. The mean roughness values before and after the Parylene coating were analysed using the Paired t-test and revealed a significant change in roughness ($p<0.05$).

SEM analysis:
The results of the SEM analysis are depicted in Figure 2. There were evident differences between the various groups, as explained in the legend.

Raman Analysis:
After the brushing procedure all samples from the Test Groups were analysed using the Raman Laser Spectroscope. Notable

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peaks at the regions of 308, 865, 1015, 1204, 1336 1441 and 1608 cm-1 are characteristic of the Parylene C film [26]. Parylene peaks were found in all the samples brushed with paste (Fig.3). In the samples brushed with pumice only one sample demonstrated an absence of Parylene, with only peaks representing PMMA visible on the spectrum analysis.

Discussion

Poly-methyl methacrylate is one of the oldest and most commonly used materials in dentistry. The inherent limitations of PMMA make it susceptible to bacterial and fungal colonization when used for the fabrication of intraoral prostheses [1-3]. The aim of this study was to investigate whether the coating of PMMA samples with Parylene would have an effect in reducing surface roughness after simulating 1 year of brushing with either paste or pumice. A reduction in roughness could possibly lead to a lower risk of microbial colonization [10, 11, 12]. Furthermore, the use of this coating in the manufacturing of removable or fixed PMMA protheses could potentially increase the life-span of these devices by delaying the loss of material and the increase in surface roughness due to abrasion from chewing and brushing procedures.

The results of this study showed that the Parylene coating of PMMA led to a statistically significant reduction in mean Ra values before and after the brushing cycles. Samples coated with Parylene showed a lower increase in mean surface roughness after brushing (no change in the Paste Group and 34.67% for the Pumice Group) when compared to the uncoated samples (51.24% in the Paste Group and 104.36% in the Pumice Group). The linear regression analysis established these differences as statistically significant with $p<0.05$. The null hypothesis was, therefore, rejected.

All Ra measurements presented with values above the 0.2µm described by Bollen et al. [4] as the threshold for bacterial adhesion, showing that even coated samples in this study could be susceptible to microbial colonization. This might have been due to the fact the samples did not undergo any polishing procedure before testing. Nevertheless, the beneficial effect of coating demonstrated could lead to further development and testing of different parameters, such as coating thickness and initial surface polishing, in order to approach the 0.2µm threshold. These parameters are currently being tested in other experimental designs.

The increase in surface roughness of PMMA after the brushing cycles is consistent with most of the literature on this subject [4, 8, 27, 28]. It is not possible to compare the absolute surface roughness values of this study with other studies as there are differences in methodology (processing and polishing methods) and surface analysis using different scanners. In this study a laser non-contact profilometer was used. Other types, such as contact profilometers could have been used, but the main disadvantage of the latter devices is the fact that the contact of a stylus against the easily abraded surface of the acrylic could cause the roughness measured to be skewed due to the plowing effect of the stylus [29]. Denture patients are advised to use some form of denture cleaning powder during cleansing procedures. Paste was used as a medium in this study as various surveys [30, 31] have reported its frequent use by patients. Pumice, although not regularly used for denture cleansing, was chosen as a second medium in order to subject the coating to extreme brushing and abrasion conditions. The Raman analysis showed the persistence of the Parylene coating after brushing, proving an efficient adhesion of the polymer to the acrylic substrate. The SEM analysis showed that the coating retained its integrity after brushing with paste, but showed signs of deterioration after the pumice treatment. This aggressive, irregular texture might also

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explain the higher Ra values obtained. Clinically, this would translate to the necessity for a less aggressive cleansing protocol. This should be the subject of a future investigation on the adequacy of cleansing procedures.

There were some limitations to this study. Only the Ra values were recorded for each sample, a fact which limited the extent in which these results could be used to characterise the acrylic surface. Ra is the arithmetic average height measurement, and was used in this study as it is considered a universal roughness parameter for general quality control [13]. A different parameter like the Rz, the ten-point height measurement, could have also been used as it is considered more sensitive to high peaks and deep valleys in the surface of a material. The results, in Ra, were nonetheless comparable with other studies on this subject, including the study by Bollen et al [4]. Also the Ra values demonstrated a relatively high standard deviation (from 0.87 to 2.25 µm). This relates to the variation present in the initial roughness readings and the variability inherent to the fact that the acrylic samples, although made from the same 2 acrylic sheets, presented with microscopic irregularities that the processing procedure could not bypass. However, the sample size and randomization procedures were adequate to allow for valid conclusions to be drawn and the normal distribution of the data was demonstrated by the residuals plotted. Thermo cycling was not used in this study. Although this process produces an artificial ageing effect that may affect the bond to the substructure, previous studies have shown that Parylene coatings were very resistant to prolonged contact with physiological fluids and even sterilization procedures [32, 33]. Based on this literature, it was decided not to include this parameter in this experimental design in order to isolate the material's reaction to the brushing alone.

The results of this study show some positive effect of the use of Parylene as coating for intraoral prostheses made of PMMA, regarding surface roughness. More experiments are currently being conducted in order to further develop this technique.

Conclusions

The coated samples showed a statistically significant lower change in roughness after the brushing procedures compared to the uncoated samples.

The coated samples brushed with paste showed no change in roughness before and after the brushing procedures.

The samples brushed with pumice in both groups showed a higher roughness after brushing than the ones brushed with paste.

Acknowledgements
Based on a dissertation submitted to UCL as partial fulfillment of the requirements for the MSc degree in Conservative Dentistry

Mr Aaron Clark (SCS Coatings, Surrey, England) for the kind offer to coat the samples

Mr Colin Clark, Dr Nicola Mordan, Dr Graham Palmer for their technical advice, and Dr Aviva Petrie for the guidance in the statistical analysis (Eastman Dental Institute, UCL)

ORAL B, Procter and Gamble, Surrey, England for the kind provision of the electric toothbrush.

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Table 1 - Surface roughness values before and after brushing in the four experimental groups

Experimental Groups	Brushing Medium	Mean Initial R_a (SD)	Mean Final R_a (SD)
Control Group Uncoated PMMA	Paste	4.45 µm (0.92)	6.73 µm (1.73)
	Pumice	3.67 µm (0.74)	7.50 µm (2.25)
Test Group Parylene Coated PMMA	Paste	2.69 µm (0.92)	2.69 µm (0.87)
	Pumice	3.73 µm (1.25)	5.05 µm (1.40)

SD= Standard Deviation; Ra= Arithmetic Surface Roughness; PMMA= Poly methyl methacrylate

CAPTIONS

Table 1 - Surface roughness values before and after brushing in the four experimental groups
SD= Standard Deviation; Ra= Arithmetic Surface Roughness; PMMA= Poly methyl methacrylate

Figure 1 - Graph showing the roughness values before and after brushing with Paste in the Parylene and Control groups

Figure 2 - SEM images (x1000 magnification) of the samples after testing
a) a coated sample after brushing with Paste: a smooth surface is present with particles from the paste visible on the surface; b) an uncoated sample after brushing with Paste: a roughened surface with streaks and surface degradation is present; c) a coated sample after brushing with Pumice: a degraded surface is present with signs of flaking and detachment of the Parylene coat, no streaks are present; d) an uncoated sample after brushing with Pumice: the surface presents streaks and obvious surface degradation

Figure 3 - Raman analysis showing the spectrum obtained from a sample brushed with paste (lower line) and the control coated un-brushed sample (upper line)