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Mechanical Preparation of Samples For Profilometric Erosion Reserch

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Abstract
Objectives*This research aims at determining the existence and the type of influence of the different mechanical sample preparations on the dental erosion research results. The research also includes the influence caused by the protection used to isolate parts of the sample surface.*
Methods*This research involves fourteen dentin and fourteen enamel samples made of extracted intact human teeth, prepared mechanically in two different ways. We used lemon acid, as the erosion agent, diluted in artificial saliva in concentration of 0.1% with pH of 6.8. In addition, this research also analyzes the effectiveness of the fluoride preparations (a total of five different ones) on the progression of the previously caused erosion. The lemon acid and fluoride preparation treatment is cyclical, for a period of five days. For the purposes of this research, we protected parts of the sample surface using nail polish. The erosive surface changes were measured using the profilometer Surf test model No. SJ-410 (Mitutoyo make).*
Results*The changes to the sample surface conditions as a result of the influence from the lemon acid and the fluoride preparations were analyzed at a macro and micro level. The macro level analysis looked at the changes to the sample surface shape, and at the micro level it looked at the changes to the values of the roughness parameters* Rz, Rp, Rv, Ra *and* RSm*. The micro and macro level measurement results were processed and systematized graphically and in table form. We used the Analysis of Variance (ANOVA) method to statistically analyze the data.*
Significance*The results from the research showed that the sample mechanical preparation can significantly influence the dental erosion research results. The influence can be so significant as to suggest that casual mechanical preparation of the sample can lead to inaccurate conclusions regarding dental erosions.*
Keywords*: mechanical preparation, dental erosions, roughness parameter, lemon acid, fluoride preparations*

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I. Introduction

The quality of dental protection and the modern dental science achievements strongly depend on the characteristics of the teeth and the basic principles and mechanisms involved in their interaction with the surrounding media. The test erosion phenomenon results from the interaction of the teeth with erosive acids found, in greater or smaller quantities, in the food and drink that we ingest every day. Significant research [1-15] focuses precisely of the study of dental erosions. Having in mind that the erosion phenomenon occurs on the surface and changes its structure, the dental erosion research employs numerous methods and techniques to determine and measure those changes to the surface. The most frequently used techniques include contactless measuring techniques [10, 11, 13, 14] and contact profilometers for measuring surface roughness [7, 11, 12, 14, 15, 16].Dental erosion in vitro research involves various sample preparations during the research. Usually, the way researchers prepare their samples depends on their knowledge, the available equipment and the needs of the employed measuring technique. During the sample preparation, especially mechanical preparation, researchers pay very little attention to whether the preparation can impact the results of the dental erosion research. This poses a justified dilemma, since the mechanical preparation of the teeth creates the sample surface together with its roughness, waviness and final shape, which the researchers will subsequently erode and measure. Hypothetically, if, as a result of the mechanical preparation, we create an excessively rough surface or a surface with varying geometric characteristics (eg. roughness) in multiple directions, then the impact caused by erosions can be less than the differences of the geometric characteristics present on the sample surface measured in

multiple directions. This indubitably leads to misleading interpretations and conclusions regarding the impacts of erosion treatments. In addition, the surface condition can impact the accurate surface measurements. Therefore, this research pays special attention to the mechanical preparation of the samples during dental erosion research and the possible influences of the mechanical preparation on the obtained results.

II. Materials And Methods

2.1 Mechanical preparation of samples

Fig. 1 shows a schematic of the sequence of activities for the mechanical preparation of the samples, the erosion cycles, the placement and the measuring of the samples. This research includes enamel (E-sample) and dentin (D-sample) samples prepared from intact teeth. We stored the fourteen extracted teeth in a 0.1% timolol solution at 4˚C until their mechanical preparation for the purposes of this research.The mechanical preparation begins by cutting the teeth using a dental cutting disc Superflex TURBO 505.504.160 at a speed of 25.000 rpm (Fig. 2). Each tooth is cut along its entire length (the corona and the root) and the cutting line is near the periphery of the tooth, from the vestibular side. This provides us with both a dentin and an enamel sample. After the cutting, the teeth are manually sanded using sandpaper. Fig. 1 clearly shows subsequently, the mechanical preparation of the teeth follows two distinct paths, marked as 4.A and 4.B. One difference between paths 4.A and 4.B. refers to the way samples move along the sandpaper. Fig. 3 shows sanding where the samples move on the sanding paper following a linear repetitive pattern corresponding to step 4.B of Fig. 1. The different movement patterns of the samples along the sanding paper result in different shapes of the longitudinal and transversal total profile and the roughness profile of the tested sample surface, as shown on Fig. 5. Fig. 5(a) shows the longitudinal and transversal roughness profiles obtained using step 4.A. This suggests that longitudinally and transversally the sample surface profiles have almost identical shapes. Fig. 5(b) shows the longitudinal and transversal roughness profiles obtained using step 4.B. The shape of the longitudinal roughness profile differs from the shape of the transversal roughness profile.Path 4.A involves another sanding phase, using sanding paper with granulation P600. The sample movement on the sanding paper is the same as on Fig. 3. Polishing of the sample surface follows the sanding (Fig. 6). The polishing of the sample surfaces involves using a rotating nonabrasive disc. After the polishing we place the samples for measuring. Fig. 7 shows the placement of the samples.

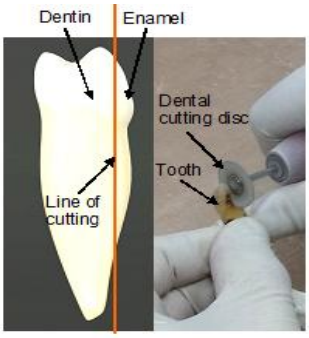


Fig. 2 - Cutting of the teeth

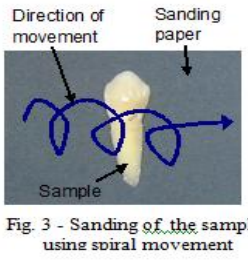


Fig. 3 - Sanding of the samples using spiral movement

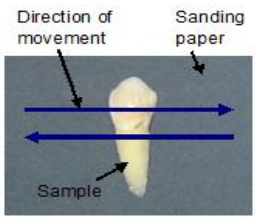
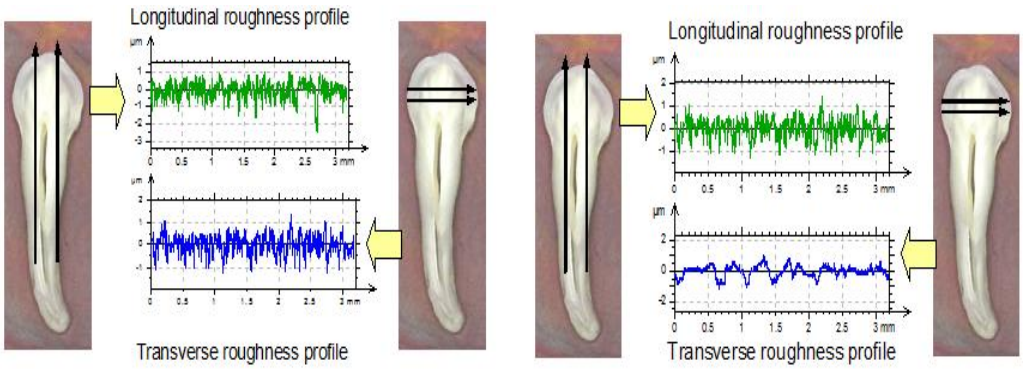


Fig. 4 - Sanding of the samples using linear repetitive



а) б)

Fig. 5 - Shape of the longitudinal and transversal roughness profile of the samples
a) according to path 4.A; b) according to path 4.B.

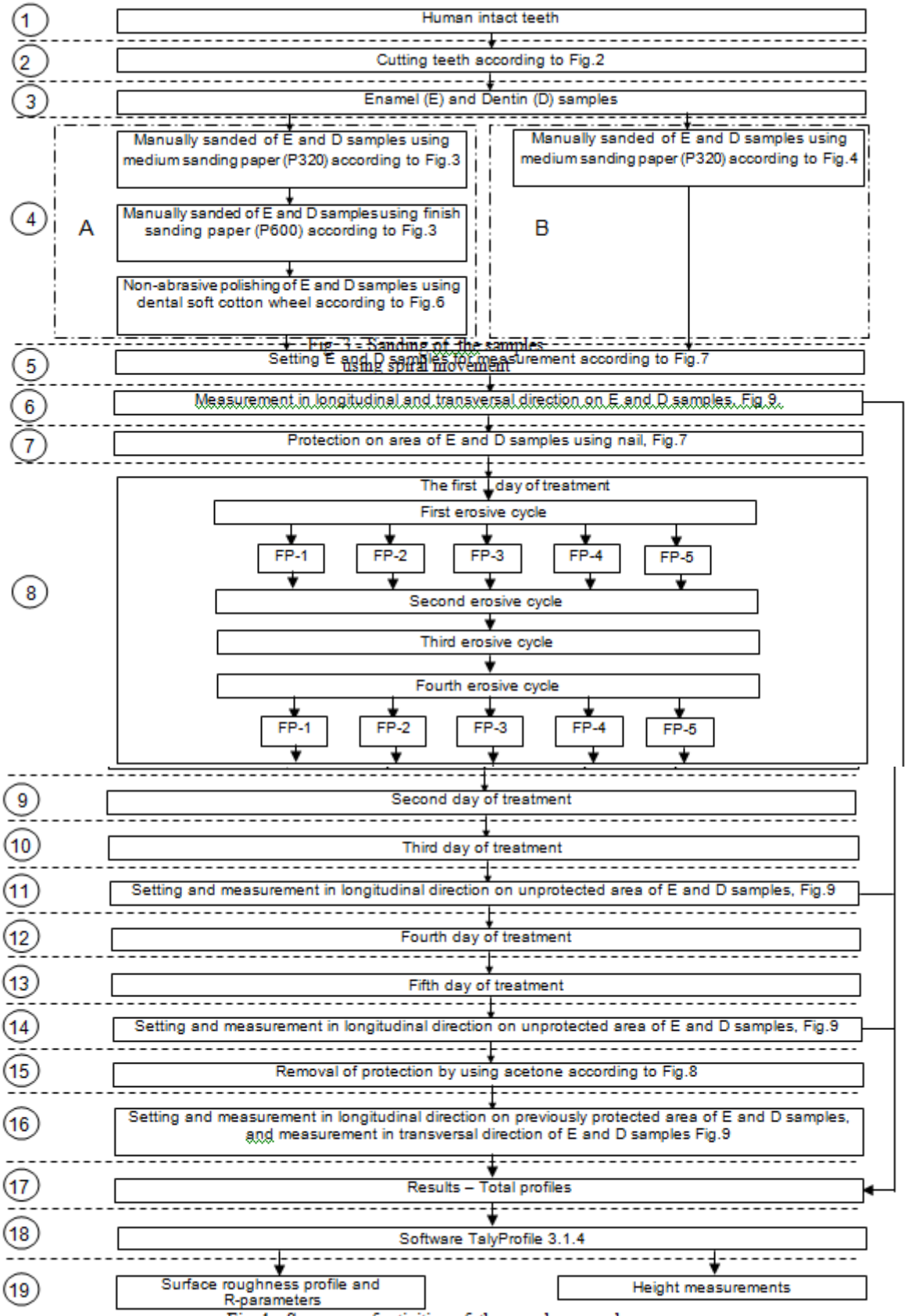


Fig. 1 - Sequence of activities of the sample research

Fig. 1 - Sequence of activities of the sample research

In order to measure the samples we place them in three metal boxes filled with plasticine mass. All samples from one box are simultaneously impressed in the plasticine mass. Section 2.3 of this paper provides detailed explanation of the measurement procedure.

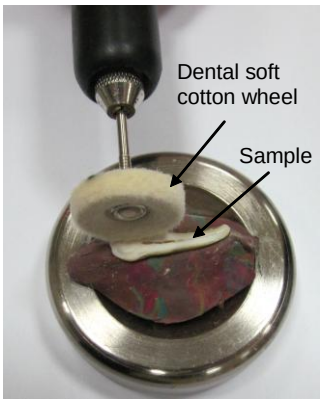


Fig. 6 - Nonabrasive polishing

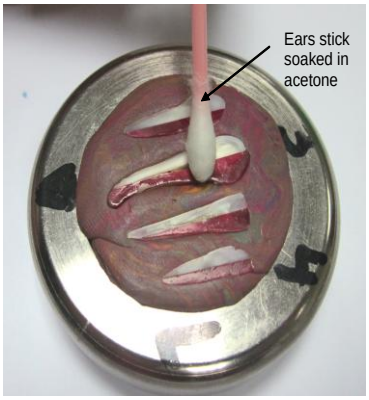


Fig. 8 - Removal of the protection from the samples

The part of the sample surface not subjected to erosion treatment, is protected using nail polish. Dental erosion research (in vitro) usually applies such protection methods [1, 2, 3]. In this research, one half of the sample surface has been protected using polish (Fig. 7). The polish is removed using acetone, as shown on Fig. 8.

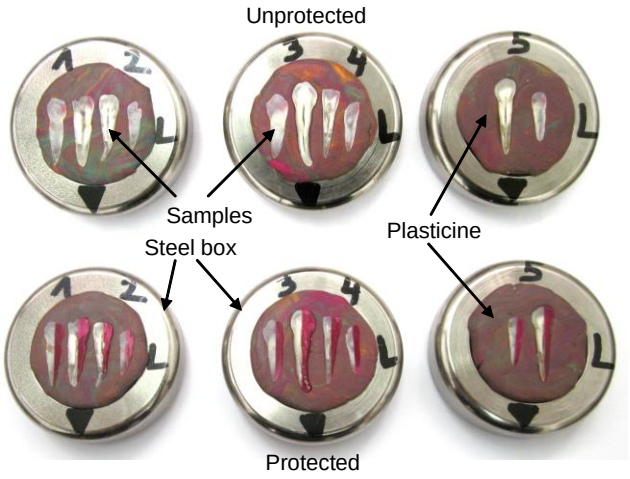


Fig. 7 - Placement of samples (unprotected and protected) for measurement

Path 4.B does not involve sanding using sanding paper P600 and nonabrasive polishing. As a result, the roughness parameters of the samples machined using path 4.B had higher values compared to the roughness parameters of the samples machined using path 4.A. Thus, the mean value of the Ra parameter for all samples prepared with 4.A. is 0.344 □ m,while for the samples prepared using 4.B, Ra is 0.547□ □ m.

Therefore, the different mechanical preparations of the sample will result in obtaining two different groups of samples. The group of samples (E and D-sample) obtained using path 4.A will result in a surface condition with identical longitudinal and transversal roughness profile and uniform roughness parameter values. The group of samples obtained using path 4.B will result in a surface condition with different longitudinal and transversal roughness profile and different roughness parameter values, higher than those obtained for path 4.A. Following the mechanical preparation, we store the samples in artificial saliva for a period of 24 hours in order to avoid dehydration of the samples.

2.2. Dental erosion of samples

As previously stated, this research, among other things, aims at analyzing the changes to the surface condition of the E and D samples (sample surface erosion) under the influence of lemon acid and the effectiveness of fluoride preparations on the progression of previously caused erosion. The lemon acid is diluted in 0.1% artificial saliva solution. The artificial saliva has a pH value of 6.8. The chemical composition of the

artificial saliva comprises NaCl-0.50 g/l, $NaHCO_3$-4.20g/l, $NaNO_3$-0.03g/l, KCl 0.20g/l, prepared on the Faculty of Veterinary Medicine Skopje.The fluoride preparations used in this research include: Crest toothpaste NaF (1500 ppm F, pH 6.9, Procter & Gamble), on Fig. 1 and hereinafter in the text marked as FP-1, a placebo toothpaste without fluorine Parodontax, on Fig. 1 and hereinafter in the text marked as FP-2, toothpaste White Glo (1450 ppm F), on Fig. 1 and hereinafter in the text marked as FP-3, 4 % TiF_4 (1450 ppm F) solution, on Fig. 1 and hereinafter in the text marked as FP-4 and 1% NaF (1450 ppm F) solution, on Fig. 1 and hereinafter in the text marked as FP-5. After having mechanically prepared the samples, measured them in accordance with step 6 on Fig. 1 and protected parts of their surface according to step 7, we cyclically treat the samples with lemon acid and fluoride preparations. The cyclical treatment lasts five days and step 8 of Fig. 1 shows the treatment procedure for each of the five days. First we submerge the samples in lemon acid for a period of 90 seconds. Then we remove the lemon acid by submerging the samples in deionized water for 10 seconds. This procedure is repeated five times a day. The fluoride preparation treatment follows after the first erosion cycle and the rinsing of the samples using deionized water. One D and one E sample, mechanically prepared using path 4.A are always treated with only one fluoride preparation. This also applies for the samples obtained using the path 4.B.

The samples treated with the fluoride preparation FP-1, are hereafter designated as 1D and 1E- sample, the samples treated with the fluoride preparation FP-2 are hereafter designated as 2D and 2E- sample, the samples treated with the fluoride preparation FP-3 are hereafter designated as 3D and 3E- sample, the samples treated with the fluoride preparation FP-4 are hereafter designated as 4D and 4E- sample and the samples treated with the fluoride preparation FP-5 are hereafter designated as 5D and 5E- sample. The treatment involves dissolving the fluoride preparation in 0.5 ml of deionized water and applying the solution onto the sample surface for a period of 20 seconds. We dissolve the fluoride preparations in deionized water in order to avoid friction between the fluoride preparations and the sample surface. After the fifth erosion cycle we repeat the fluoride preparation treatment in the same way as during the first cycle.

2.3. Measurement of dental erosion

We analyzed the influence of the lemon acid and the fluoride preparations using the change of the sample surface condition on two levels, macro and micro level. The macro changes to the surface condition are obtained by making transversal measurements of the samples, while the micro changes are determined by making longitudinal measurements (Fig. 9). We measured the micro and macro measurements using the profilometer Surf test model No. SJ-410 (Mitutoyo make) (Fig. 10). We used a measuring needle with a 60 degree tip angle and a 2 □ m tip radius The measuring force with which the measuring needle presses onto the surface amounts to 0.75 mN. The process uses a skidless pickup. During the measuring, we mechanically levelled the profilometer in reference to the measured surface. The measuring system was calibrated using a type *C* etalon with Ra=2.97□ m, and additionally verified using a type *C* etalon with Ra=6 □ m. These calibration etalons have a measurement uncertainty of 5%. We used the Surf test model No. SJ-410 device to obtain the coordinates of the total profile. The total profile contains the information about the micro and macro changes. The Laboratory for Metrology of Geometric Characteristics and Quality Research, at the Faculty of Mechanical Engineering in Skopje then analyzed the data and obtained the primary and the roughness profiles using the professional software TalyProfile 3.1.4. Fig. 11 shows the method for measuring the surface condition on a macro level. After having measured and obtained the total profile, the process involves software levelling of the total profile using the least squares method (Fig. 11a). Then, using a Gaussian profile filter of 0.8 mm, a mean line is drawn through the total profile (Fig. 11b). Finally, we measure the height difference between the mean lines of both segments (protected and unprotected) of the sample (Fig. 11c).

On a macro level, we analyzed the impacts of the lemon acid and the fluoride preparations by looking and the changes to shape of the roughness profile and the values of the roughness parameters. This research investigates the changes to the shape of the roughness profile using the parameters Rz, Rp, Rv, Ra and RSm. Usually, dental erosion researches [11, 12, 16] involve the parameters Rz, Ra and RSm. This research involves the additional parameters Rp and Rv because they can be directly measured (they are not averaged parameters) and depend on the roughness profile mean line. The change of the shape of the roughness profile will result in a change of the position of the roughness profile mean line which will, in turn, impact the values of Rp and Rv.Since the roughness profile is a derived quantity, it was measured and obtained in accordance with the recommendations from the ISO standards [17-21] и ASME B46.1-2009 standard [21] i.e. in accordance with the procedure for obtaining the primary profile, the roughness profile and waviness profile presented on [22]. The λ_c profile filter used involves a Gaussian filter of size equal to the sampling length, i.e. 0.8 mm. The evaluation length involves five sampling lengths.

This research makes 15 total profile measurements for each sample. After the mechanical preparation and before applying the nail polish protection and the erosion treatment, we made five longitudinal measurements for each of the two halves of the sample (the treated half and the protected half, Fig. 9) and five transversal measurements of the sample, step 6 of Fig. 1. The third day of the sample treatment we made five longitudinal measurements on the treated side, step 11. The fifth day of the treatment, we made a total of 15 measurements for each sample. First, before removing the polish with acetone, we made five longitudinal measurements on the treated side, step 14. After removing the polish, we made five longitudinal measurements on the protected part of the surface, as well as five transversal measurements, step 16 of Fig. 1.

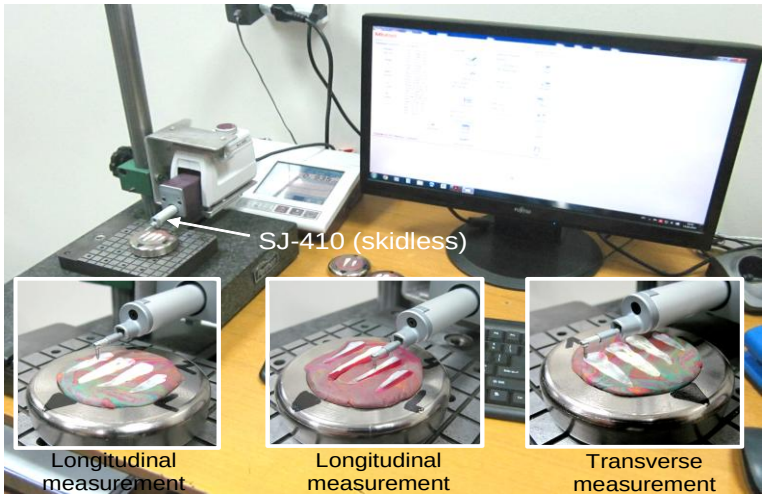


Fig. 10 - Surf test model No. SJ-410 (Mitutoyo make)

Here we need to emphasize the specificity of the measurements and the process for obtaining the roughness profiles of the samples. During measuring, the measuring stylus needs to move perpendicularly to the mechanical treatment traces (lay direction). This does not have an influence on the measured values of the samples, mechanically prepared according path 4.A, with uniform roughness in all directions of the surface. This is not the case for the samples which have been mechanically prepared using path 4.B and which have different shapes of the profile for different directions on the surface. Thus, in this case, the human factor, by properly placing the samples for measurement, will have some influence on the measurement results.

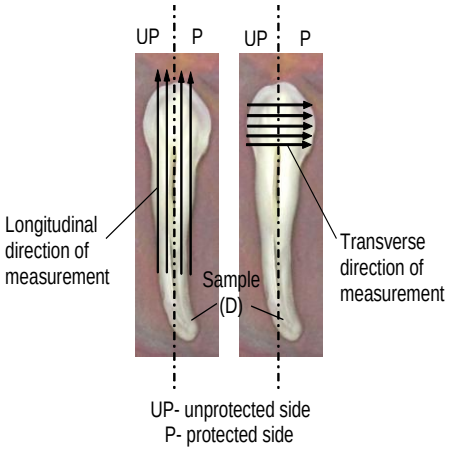


Fig. 9 - Sample measurement directions

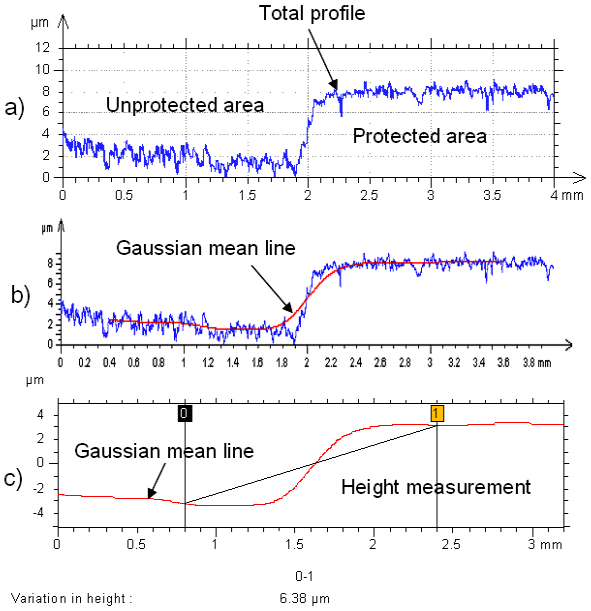


Fig. 11 - Measurement of the macro changes to the

III. Results And Discussion

3.1. Macro changes to the sample surface

Table 1 systematizes the five measured values at the vertical distance (H) according to Fig. 11c, the highest and lowest value, as well as the mean values for the samples prepared using both path 4.A and 4.B.

Table 1 - Vertical distance of the samples

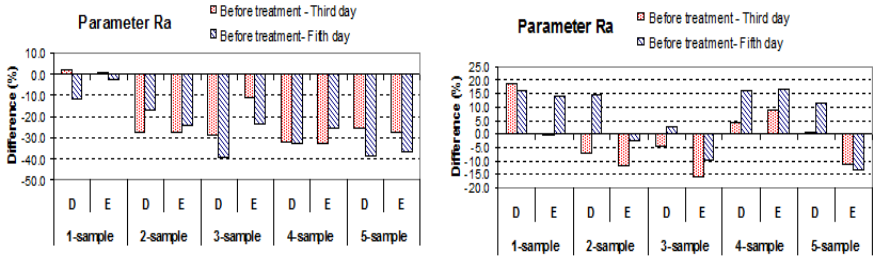
Samples		Vertical distance H (□ m)															
		4.A								4.B							
		1	2	3	4	5	Max.	Min.	Mean	1	2	3	4	5	Max.	Min.	Mean
1	D	2.46	2.22	1.92	2.05	1.83	1.83	2.46	2.096	0.32	0.58	0.78	0.22	0.47	0.78	0.22	0.474
	E	0.98	0.83	0.94	0.75	0.87	0.75	0.98	0.873	0.87	0.32	0.75	0.54	0.68	0.87	0.32	0.631
2	D	1.92	1.57	1.53	1.83	1.63	1.53	1.92	1.696	2.05	2.21	2.91	2.54	2.68	2.91	2.05	2.478
	E	0.24	0.52	0.14	0.35	0.47	0.14	0.52	0.343	0.87	0.68	0.68	0.54	0.72	0.87	0.54	0.698
3	D	0.74	0.42	0.76	0.85	0.63	0.42	0.85	0.680	0.18	0.66	0.44	0.33	0.52	0.66	0.18	0.427
	E	0.37	0.31	0.69	0.45	0.44	0.31	0.69	0.450	1.17	0.47	0.24	0.84	0.59	1.17	0.24	0.662
4	D	1.07	1.93	1.96	1.38	1.56	1.07	1.96	1.580	0.68	1.23	1.38	0.98	1.14	1.38	0.68	1.081
	E	0.81	0.38	0.88	0.58	0.68	0.38	0.88	0.665	1.84	0.43	1.66	1.24	0.98	1.84	0.43	1.230
5	D	1.61	1.19	1.64	1.38	1.45	1.19	1.64	1.454	0.13	0.58	1.64	0.87	0.95	1.64	0.13	0.834
	E	0.93	1.09	0.80	0.86	0.94	0.80	1.09	0.923	0.32	0.12	0.55	0.44	0.38	0.55	0.12	0.360

The results in Table 1 for the samples prepared mechanically using path 4.A, suggest that a lesser mean value of the H distance for all E-samples than the H distance for the D-samples. Out of all the D-samples, sample 1D has the highest H value while sample 3D has the lowest. Out of all the E-samples, sample 5E has the highest H value while sample 2E has the lowest.

For the samples prepared mechanically using path 4.B the results do not suggest a lesser H value for all E-samples than the H value for all the D-samples. For samples 1E, 3E and 4E-the H value is higher than that for samples 1D, 3D and 4D. If we analyze the differences between the maximal and the minimal values measured for H, i.e. the data distribution, we will come to the conclusion that greater differences and greater data distribution exists for samples prepared mechanically using path 4.B.

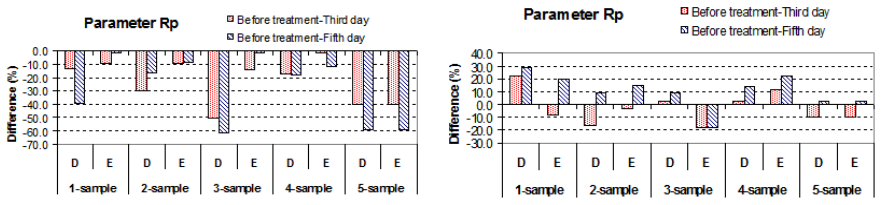
3.2. Micro changes

We analyzed the micro changes to the sample surface by comparing the measured values for the Rz, Rp, Rv, Ra and RSm parameters. Fig. 12-16 show the percentage differences resulting from the comparison between the parameters measured before the erosion treatment and the third day, as well as before the erosion treatment and the fifth day, for the mechanically prepared samples using both path 4.A and 4.B. The figure shows the percentage comparison and the ensuing differences between the five mean values from the five measurements of the surface. The negative sign in front of the percentage difference suggests a smaller value of the parameter during the previous measurement.



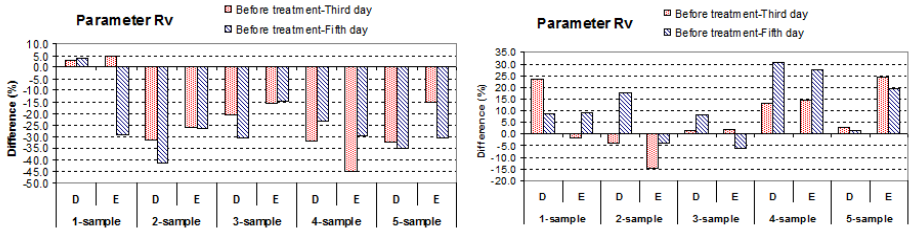
a) Mechanical preparation using path 4.A b) Mechanical preparation using path 4.B

Fig. 12 - Percentage differences between the mean values of the Ra parameter measured before treatment, on the third day and on the fifth day



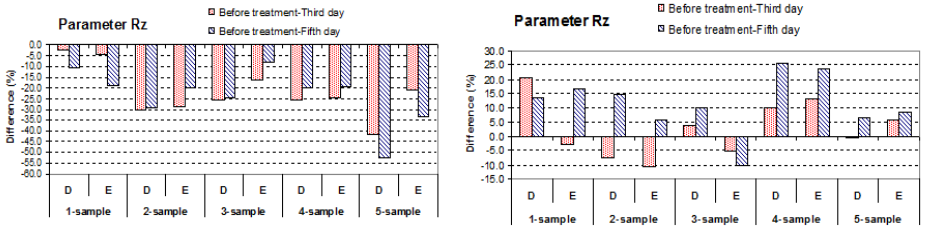
a) Mechanical preparation using path 4.A b) Mechanical preparation using path 4.B

Fig. 13 - Percentage differences between the mean values of the Rp parameter measured before treatment, on the third day and on the fifth day



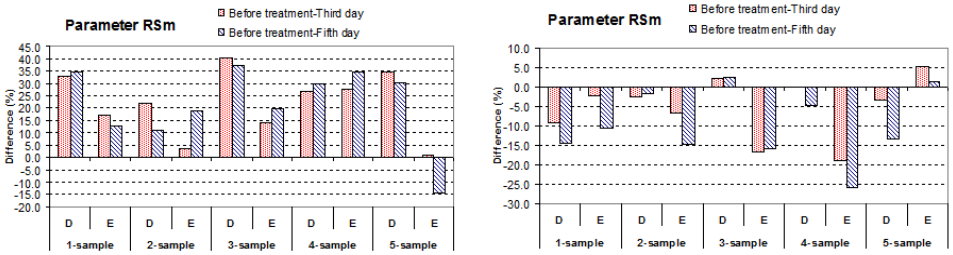
a) Mechanical preparation using path 4.A b) Mechanical preparation using path 4.B

Fig. 14 - Percentage differences between the mean values of the Rv parameter measured before treatment, on the third day and on the fifth day



a) Mechanical preparation using path 4.A b) Mechanical preparation using path 4.B

Fig. 15 - Percentage differences between the mean values of the Rz parameter measured before treatment, on the third day and on the fifth day



a) Mechanical preparation using path 4.A b) Mechanical preparation using path 4.B

Fig. 16 - Percentage differences between the mean values of the RSm parameter measured before treatment, on the third day and on the fifth day

This research statistically compares the measured macro results using the Analysis of Variance (ANOVA) method. Thus, Tables 3 and 4 provide the *p*-values obtained from the ANOVA analysis fir the comparison of the Rz, Rp, Rv, Ra and RSm parameters measured before the erosion treatment of the samples and the third day of the treatment, for the samples obtained using paths 4.A and 4.B respectively. Similarly as Tables 3 and 4, Tables 5, 6, 7 and 8 provide the results of the comparisons between the parameters measured before treatment and the fifth day and the comparisons between the third and the fifth day.

Table 3. *p*-values (ANOVA) from the comparison of the R-parameters measured before sample treatment (4.A) and the third day.

Samples		Ra	Rp	Rv	Rz	RSm
		p				
1	D	0.0007*	0.0164	0.0029*	0.0017*	0.1087
	E	0.0001*	0.0063*	0.0012*	0.0015*	0.1873
2	D	0,0423	0,1390	0,0348	0,0785	0,0380
	E	0,1025	0,0060*	0,0291	0,0300	0,0051*
3	D	0.2517	0.0281	0.0230	0.0304	0.2778
	E	0.0106	0.0045*	0.0071*	0.0613	0.0319
4	D	0.1301	0.0051*	0.0256	0.0222	0.0289
	E	0.3835	0.0001*	0.0652	0.0767	0.6623
5	D	0.0403	0.0836	0.0318	0.0727	0.0365
	E	0.0997	0.0958	0.0051*	0.0251	0.0001*
*- Non significant differences (p<0.01)						

Table 4. *p*-values (ANOVA) from the comparison of the R-parameters measured before sample treatment (4.b) and the third day.

Samples		Ra	Rp	Rv	Rz	RSm
		p				
1	D	0.1264	0.0362	0.1508	0.2015	0.0242
	E	0.0001*	0.0025*	0.0011*	0.0015*	0.0027*
2	D	0.0755	0.0308	0.0010*	0.0061*	0.0021*
	E	0.0377	0.0023*	0.0398	0.1462	0.0114
3	D	0.0063*	0.0013*	0.0001*	0.0018*	0.0006*
	E	0.1816	0.0496	0.0010*	0.0180	0.0519
4	D	0.0073*	0.0031*	0.0232	0.0390	0.0001*
	E	0.0226	0.0066*	0.0477	0.0265	0.0221
5	D	0.0001*	0.0176	0.0020*	0.0003*	0.0035*
	E	0.0384	0.1244	0.0224	0.0030*	0.0019*
*- Non significant differences (p<0.01)						

Table 5. *p*-values (ANOVA) from the comparison of the R-parameters measured before sample treatment (4.A) and the fifth day.

Samples		Ra	Rp	Rv	Rz	RSm
		p				
1	D	0.0269	0.0473	0.0069*	0.0130	0.1352
	E	0.0009*	0.0003*	0.0332	0.0287	0.0158
2	D	0.0861	0.0315	0.0520	0.1763	0.0123
	E	0.0873	0.0082*	0.1887	0.0983	0.0334
3	D	0.0351	0.0590	0.0144	0.0124	0.1106
	E	0.1559	0.0001*	0.0556	0.0179	0.0688
4	D	0.2388	0.0052*	0.0495	0.0163	0.0664
	E	0.3079	0.0054*	0.0547	0.0471	1.1651
5	D	0.5145	0.0578	0.1390	0.1511	0.0283
	E	0.1890	0.1522	0.0282	0.0772	0.0632

*- Non significant differences (p<0.01)

Table 6. *p*-values (ANOVA) from the comparison of the R-parameters measured before sample treatment (4.B) and the fifth day.

Samples		Ra	Rp	Rv	Rz	RSm
		p				
1	D	0.0169	0.0385	0.0057*	0.2015	0.0116
	E	0.0232	0.0489	0.0058*	0.0015*	0.0349
2	D	0.0707	0.0308	0.0414	0.0533	0.0002*
	E	0.0027*	0.0023*	0.0027*	0.0395	0.0263
3	D	0.0022*	0.0144	0.0033*	0.0101	0.0022*
	E	0.0082*	0.0156	0.0052*	0.0286	0.0322
4	D	0.2858	0.0358	0.1822	0.2587	0.0046*
	E	0.0938	0.0361	0.1592	0.0936	0.2957
5	D	0.1783	0.0010*	0.0001*	0.0044*	0.0201
	E	0.0634	0.0038*	0.0209	0.0064*	0.0005*

*- Non significant differences (p<0.01)

Table 7. p-values (ANOVA) from the comparison of the R-parameters measured on the third and the fifth day of sample treatment (4.A).

Samples		Ra	Rp	Rv	Rz	RSm
		p				
1	D	0.0591	0.0155	0.0005*	0.0066*	0.1789
	E	0.0040*	0.0062*	0.0489	0.0468	0.0020*
2	D	0.0066*	0.0126	0.0016*	0.0001*	0.0140
	E	0.0023*	0.0001*	0.0001*	0.0026*	0.0280
3	D	0.0024*	0.0008*	0.0013*	0.0001*	0.0011*
	E	0.0121	0.0046*	0.0001*	0.0291	0.0254
4	D	0.0002*	0.0001*	0.0022*	0.0018*	0.0005*
	E	0.0366	0.0098*	0.0067*	0.0036*	0.0592
5	D	0.0123	0.0051*	0.0002*	0.0030*	0.0097*
	E	0.0246	0.0163	0.0048*	0.0089*	0.0225

*- Non significant differences (p<0.01)

Table 8. p-values (ANOVA) from the comparison of the R-parameters measured on the third and the fifth day of sample treatment (4.B).

Samples		Ra	Rp	Rv	Rz	RSm
		p				
1	D	0.0003*	0.0025*	0.0199	0.0042*	0.0015*
	E	0.0223	0.0322	0.0079*	0.0348	0.0376
2	D	0.1641	0.0769	0.0600	0.0812	0.0001*
	E	0.0144	0.1566	0.0618	0.7506	0.0067*
3	D	0.0639	0.0067*	0.0050*	0.0062*	0.0001*
	E	0.0029*	0.0001*	0.0126	0.0085*	0.0001*
4	D	0.0666	0.0308	0.0826	0.0919	0.0023*
	E	0.0274	0.0097*	0.0297	0.0959	0.0029*
5	D	0.0869	0.0225	0.0001*	0.0050*	0.0112
	E	0.0225	0.0545	0.0545	0.0003*	0.0009*

*- Non significant differences (p<0.01)

For the samples, prepared mechanically using the path 4.A, we can say that the surface changes occurring under the influence of the lemon acid and the fluoride preparations produce higher values of the Rz, Rp, Rv, Ra parameters and smaller value for the RSm parameter for all five samples. This conclusion does not apply to the mechanically prepared samples using the path 4.B. In that case, for some samples (eg. 3E), the values of some of the considered roughness parameters increase and for some they decrease (eg. 1D, 4E). In some cases, the sign in front of the percentage differences between the measured values before treatment, on the third day and on the fifth day, changes. One such example involves sample 2D where the values of the Ra, Rp, Rv and Rz parameters measured after the third day of treatment increase, and those measured after the fifth day of treatment decrease in comparison to the measured values of the parameters before the erosion treatment.

The percentage differences for the mechanically prepared samples using path 4.B are smaller than the samples using path 4.A.

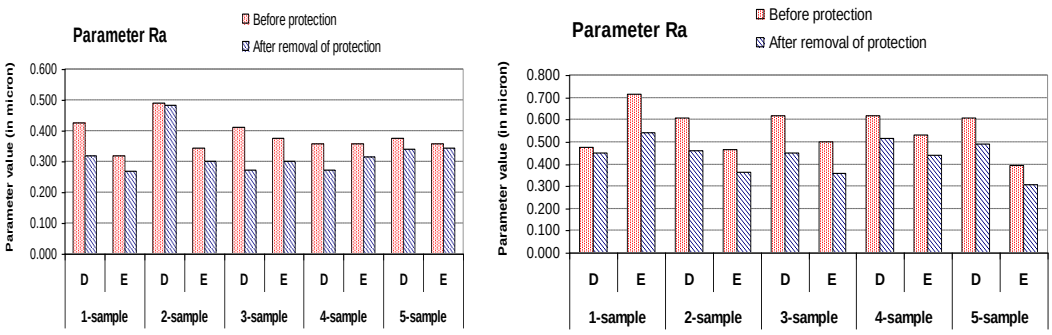
The conducted statistical analysis shows that the differences between the measured parameter values are greater between the time before the erosion treatment and the fifth day, in comparison to the value before the erosion treatment and the third day. The comparison between the third and the fifth day do not indicate significant differences. These conclusions are heightened for the samples prepared using the path 4.A.

Here we have to emphasize that the small percentage differences (eg. up to 5%) should not necessarily be viewed as parameter differences caused by the erosion cycles. Such small differences could appear as a result

of the measurement uncertainty of the parameter values deriving from the measuring equipment used and its calibration.

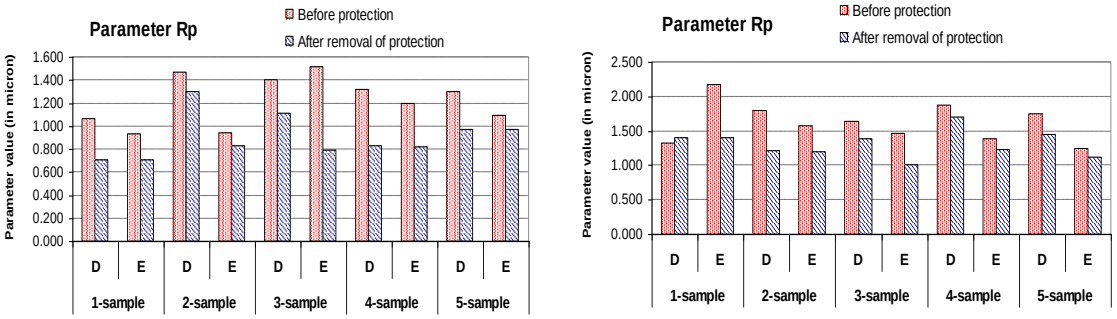
3.3. Changes to the condition of the sample surface as a result of the used protection

Fig. 17-21 show the values for the Rz, Rp, Rv, Ra and RSm parameters measured before applying surface protection and after the removal of the protection for both groups of samples (4A and 4B). Tables 9 and 10 provide the *p*-values from the comparison between the two.



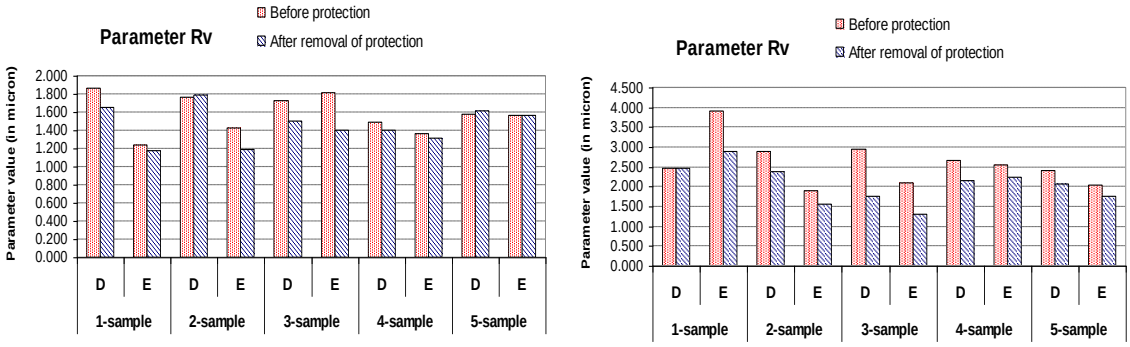
a) Mechanical preparation using path 4.A b) Mechanical preparation using path 4.B

Fig. 17 - Mean values of the Ra parameter before the application of surface protection and after the removal of the protection from the samples



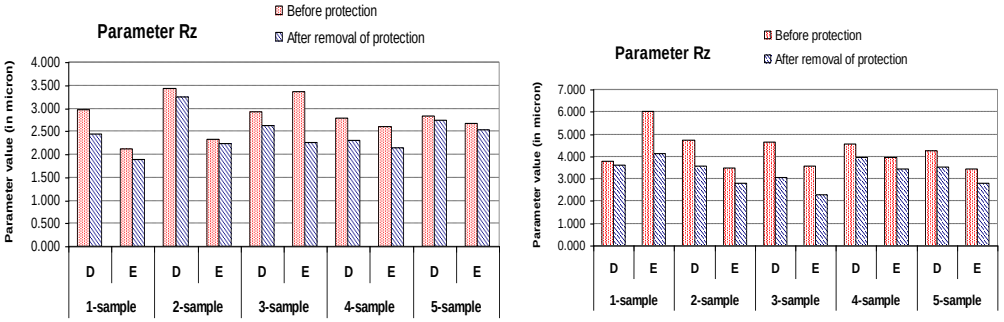
a) Mechanical preparation using path 4.A b) Mechanical preparation using path 4.B

Fig. 18 - Mean values of the Rp parameter before the application of surface protection and after the removal of the protection from the samples



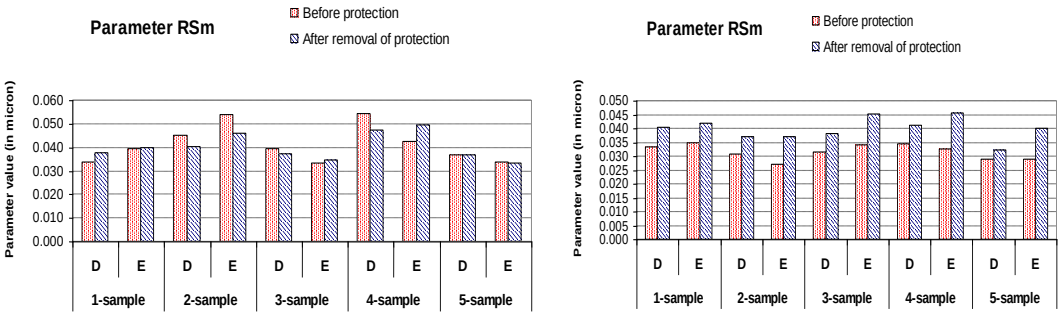
a) Mechanical preparation using path 4.A b) Mechanical preparation using path 4.B

Fig. 19 - Mean values of the Rv parameter before the application of surface protection and after the removal of the protection from the samples



a) Mechanical preparation using path 4.A b) Mechanical preparation using path 4.B

Fig. 20 - Mean values of the Rz parameter before the application of surface protection and after the removal of the protection from the samples



a) Mechanical preparation using path 4.A b) Mechanical preparation using path 4.B

Fig. 21 - Mean values of the RSm parameter before the application of surface protection and after the removal of the protection from the samples

The diagrams shown on Fig. 17-21 suggest that the values of the considered R-parameters decrease after the removal of the protection (nail polish). This conclusion applies for the mechanically prepared samples using both paths 4.A and 4.B. One exemption from this conclusion refers to the parameter *RSm*. For the mechanically prepared samples using the path 4.B.the values increase after the removal of the protection, while for the samples using the path 4.A, the values sometimes increase and sometimes decrease. This suggests that the use of nail polish to protect parts of the sample surface cause micro changes to the surface of the samples, i.e. changes to the shape of the roughness profile. These changes to thee roughness surface can be also seen from the statistical comparison of the parameters, Tables 9 and 10. The *p*-values demonstrate significant differences between the parameter values measured before applying protection and after the removal of the protection from the samples. The changes to the shape of the roughness profile are more emphasized for the vertical characteristics of the profile.

Table 9. *p*-values (ANOVA) from the comparison of the R-parameters measured before applying protection and after removing the protection from the samples (4.A).

Samples		Ra	Rp	Rv	Rz	RSm
		p				
1	D	0.1540	0.1026	0.0184	0.1791	0.0430
	E	0.0511	0.0452	0.0012*	0.0108	0.0001*
2	D	0.0003*	0.0057*	0.0001*	0.0029*	0.0079*
	E	0.0885	0.0105	0.0024*	0.0047*	0.0484
3	D	0.0096*	0.0102	0.0118	0.0109	0.0013*
	E	0.0985	0.1352	0.0481	0.0880	0.0017*
4	D	0.0558	0.0177	0.0018*	0.0092*	0.0063*
	E	0.0071*	0.0411	0.0009*	0.0365	0.0317
5	D	0.0516	0.0174	0.0006*	0.0011*	0.0001*
	E	0.0021*	0.0176	0.0001*	0.0024*	0.0006*

*- Non significant (p<0.01)

Samples	Ra	Rp	Rv	Rz	RSm

Table 10. *p*-values (ANOVA) from the comparison of the R-parameters measured before applying protection and after removing the protection from the samples (4.B).

Samples		Ra	Rp	Rv	Rz	RSm
		p				
1	D	0.0021*	0.0112	0.0001*	0.0013*	0.1186
	E	0.2499	0.2160	0.1060	0.2842	0.1392
2	D	0.2206	0.1856	0.0907	0.2426	0.1064
	E	0.1682	0.0298	0.0306	0.1798	0.0854
3	D	0.1999	0.0374	0.0528	0.0823	0.1350
	E	0.1489	0.2220	0.3718	0.6893	0.2646
4	D	0.1535	0.0074*	0.0395	0.0288	0.0287
	E	0.0664	0.0303	0.0245	0.0565	0.1147
5	D	0.1933	0.1186	0.0297	0.1496	0.0207
	E	0.1745	0.0090*	0.0031*	0.1570	0.3076

*- Non significant (p<0.01)

Since we have changes to the shape of the roughness profile caused by the protection of the surface, we should expect a shift of the mean line of the profile, section 3.1 of the paper, for determining the vertical distance at the macro level. The shift of the mean line will have a direct impact on the measured values for the vertical distance provided in Table 1.

IV. Conclusion

This research shows that the mechanical preparation of the samples, when researching dental erosion, has a key and a big influence on the obtained results and their interpretation. This is especially significant when the erosion agents are weak (or with small concentrations) acids for erosion abrasion of the samples, which are expected to cause small changes to the surface. Using polish to protect parts of the surface causes changes to the surfaces and other protection methods should be sought out in the future. This research did not show a clear picture of the influences caused by the fluoride preparations, with a view of showing which fluoride preparation has a greater impact on the effectiveness of the progression of the previously caused erosion.

The results suggest that in the future it might be necessary to establish standard procedures for sample preparation when investigating dental erosion. This helps eliminate the possibility of wrong conclusions, but also comparing the results obtained from different dental erosion investigations.

When mechanically preparing samples for the purposes of researching dental erosion, one should always strive to obtain a uniform surface condition in all directions, both on macro and on micro level. This is important primarily for conducting proper measurements.

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