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Abstract: Statement of the Problem. The colonization of micro-organisms on acrylic resin dentures may result in denture-induced stomatitis. No efficient coating has yet been proposed to address this issue.
Purpose. The purpose of this in vitro study was to assess the effect of various initial surface finishes and different Parylene (P) coating thicknesses on the surface roughness (Ra) and surface free energy (SFE) of Parylene coated polymethyl methacrylate (PMMA).
Material and Methods. One hundred and sixty PMMA specimens were produced and divided into 8 groups as follows: Group A: uncoated-1000grit finish; group Ap1: 10 μm P coated -1000grit finish; group B: uncoated - 1200grit finish; group Bp: 10 μm P coated - 1200grit finish; group C: uncoated - 2400grit finish; group Cp: 10 μm P coated - 2400grit finish; group Ap2: 20 μm P coated - 1000grit finish; group Ap3: 30 μm P coated - 1000grit finish. The Ra of all specimens was measured with a noncontact profilometer. To calculate the SFE, the Owens-Wendt approach was applied after measuring the contact angles with a goniometer. The topography of the specimens was observed by scanning electron microscope.
Results. Groups Ap1 and Bp presented significantly lower Ra values compared with their respective uncoated groups A and B ($P<.001$). No statistical difference was found between the Ra values of groups C and Cp, between A and Ap3, and between Ap2 and Ap3. The SFE values of the coated groups were significantly higher than the SFE values of the uncoated groups with the same initial finish ($P<.001$).
Conclusions. Coating with a 10-μm layer of Parylene C resulted in lower Ra values for the rougher groups and increased SFE values. Increasing the coating thickness resulted in an increase of the Ra.
CLINICAL IMPLICATIONS.
The coating of removable prostheses with Parylene C alters the surface properties in a way which may reduce microorganism colonization of the fitting surfaces. More experiments are needed to verify this approach.

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The effect of different initial finishes and Parylene coating thickness on the surface properties of coated PMMA

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Results. Groups Ap1 and Bp presented significantly lower Ra values compared with their respective uncoated groups A and B ($P<.001$). No statistical difference was found between the Ra values of groups C and Cp, between A and Ap3, and between Ap2 and Ap3. The SFE values of the coated groups were significantly higher than the SFE values of the uncoated groups with the same initial finish ($P<.001$).

Conclusions. Coating with a 10-μm layer of Parylene C resulted in lower Ra values for the rougher groups and increased SFE values. Increasing the coating thickness resulted in an increase of the Ra.

CLINICAL IMPLICATIONS.

The coating of removable prostheses with Parylene C alters the surface properties in a way which may reduce microorganism colonization of the fitting surfaces. More experiments are needed to verify this approach.

INTRODUCTION

Although widely used in dentistry, polymethyl methacrylate (PMMA) possesses a number of shortcomings regarding its material properties that may manifest after long-term intraoral use.[1] These include discoloration, wear, and surface adhesion and accumulation of microorganisms. The last is attributed to its porous surface and has been associated with oral infections such as denture-induced stomatitis.[2,3] *Candida* species are dominant in this denture biofilm,[4] but another pathogen, methicillin-resistant *Staphylococcus aureus* (MRSA) has also been isolated from the oral cavity of elderly or hospitalized patients, and its presence could have implications in regard to cross infection and systemic infection.[5,6]

The initial adhesion of microorganisms on surfaces depends on their physical and chemical properties along with those of the substrates and environmental solutions.[7] Two material properties that affect initial adhesion are surface roughness (Ra) and surface free energy (SFE).[8,9] Restorative materials with increased surface roughness serve as a favorable substrate for the attachment of microorganisms,[10] and a surface roughness of 0.2 μm has been established as the threshold for bacterial adhesion, above which the aggregation of bacteria increases

dramatically.[9,11] Surface free energy also plays a significant role in the initial phase of the adhesion of microorganisms[9,12] and may be affected by some surface characteristics such as the surface charge, topography, and chemical composition.[13] Bacteria with low free energy tend to attach to surfaces with low SFE, and the exact opposite applies to microbes with high SFE.[14,15] However, the SFE of a substrate is a weaker determinant of bacterial adhesion compared with its surface roughness,[16] and these properties are also affected by the acquired salivary pellicle.[17,18]

Although various studies[19-21] have shown that laboratory and chairside polishing techniques can achieve a surface roughness below the 0.2-μm threshold for the polishing surfaces of intraoral removable prostheses made of PMMA, this does not hold true for the fitting surfaces. Denture base materials processed under ideal laboratory conditions after wax pattern investment still show Ra measurements between 3.4 and 7.6 μm.[22] That fitting surface is the one vulnerable to microbial colonization. In order to overcome this issue a number of researchers developed techniques and applied different coating materials, such as titanium dioxide, oils, monomers, or high polymerized glaze.[23-26] All of these coatings presented issues such as discoloration of the denture material, limited short-term improvement of its mechanical properties, or questionable longevity in the oral environment, preventing them from becoming established.[23-26]

Parylene, the trade name of poly para-xylylene, refers to a family of polymers used as coating materials because of their unique ability to create a protective layer on various surfaces.[27] Parylene is synthesized by vapor deposition polymerization[28,29] and can form a film of uniform thickness, which is almost impermeable to moisture,[30-32] biocompatible, chemically inert, and thermally stable. In addition, it possesses a high level of crystallinity and good mechanical properties.[28] Parylene is used in the medical field,[30] but the application in dentistry remains

limited, although experimental work has shown a number of advantages,[33] including a decrease of the microbial adhesion on coated acrylic resin and silicone specimens.[34] Parylene-coated PMMA has also demonstrated a significantly higher abrasion resistance to mechanical wear (brushing with toothpaste and pumice) compared wth the uncoated specimens.[35] Therefore, the potential use of Parylene as a coating material for PMMA intraoral prostheses warrants further investigation.

The purpose of this study was to assess the effect of various initial PMMA surface finishes and different Parylene coating thicknesses on the surface properties (Ra and SFE) of PMMA. It is part of a series of experimental studies assessing the potential use of Parylene as a coating material for PMMA intraoral prostheses.

MATERIAL AND METHODS

Two different experiments were designed as part of this investigation; one evaluating the effect of the initial surface finishing, and the second examining the effect of different Parylene coating thicknesses on the surface properties of PMMA.

Heat-polymerized PMMA (C&J De-luxe; Chaperlin & Jacobs Ltd) was used for the fabrication of the specimens in a ratio of 1:3 (30 mL monomer, 90 cc powder) according to the manufacturer's instructions. A stone mold was fabricated in a large, rectangular custom-made metal flask, which enabled the production of identical rectangular PMMA specimens of 15×15×3 mm in dimension. Wax (Associated Dental Products Ltd) was used to form the stone mold. The extended polymerization took place in a boiler unit (Acrydig 10, Manfredi). The temperature was first increased to 70°C within an hour and held at this temperature for 4 hours. It was then raised to 100°C over the next hour and maintained at this temperature for 4 more hours. After cooling, the acrylic resin pieces were removed from the mold, and their periphery was

made smooth with an acrylic bur (Acrylic Trimmer Crosscut, Komet). Following this, the specimens were labeled on 1 surface and stored in airtight and moisture sealed containers.

One hundred and sixty PMMA specimens were produced and divided to form the groups of the 2 experiments (Table 1). Groups A and Ap1 were common to both experiments. The sample size per group (20 specimens) was determined from data from previous publications[34,35] and software (Sample Size Tables for Clinical Studies v1.0; National Cancer Center). All 160 specimens produced were finished with abrasive paper (Waterproof silicon carbide paper; Struers) of different grits in a polishing machine (Laboforce-1, Labopol-5; Struers). All the groups of experiment 2 (A, Ap1, Ap2, Ap3) were finished with 1000 grit abrasive paper for 45 seconds. Groups B and Bp were finished with 1200 grit abrasive paper for 60 seconds, and groups C and Cp were finished with 1200 grit abrasive paper for 60 seconds initially, followed by 2400 grit abrasive paper for 30 seconds. Only 1 surface of the specimens was polished. Finishing was carried out at 300 rpm under constant pressure and water irrigation. The abrasive paper was cleaned under running tap water after each cycle and was replaced after 5 polishing cycles (equivalent to 15 specimens polished).

Of the total specimens prepared, 100 (groups Ap1, Bp, Cp, Ap2, and Ap3) were sent to Specialty Coating Systems Ltd. to be coated with Parylene C according to the following protocol: Groups Ap1, Bp, and Cp were coated with a thickness of 10 μm, group Ap2 with a thickness of 20 μm, and group Ap3 with a thickness of 30 μm. The specimens were initially rinsed with de-ionized water and isopropanol and underwent plasma treatment. An adhesion promoter, Silane A174 – (3-[Methacryloyloxy]propyl) trimethoxysilane - was subsequently applied as the specimens were suspended with special clips in the coating chamber for vapor deposition of Parylene C. After the coating procedure, the specimens were mailed back

individually wrapped in vacuum sealed bags. The specimens were handled with nitrile gloves to avoid contamination of the surfaces before, during, and after testing.

A laser noncontact profilometer (ProScan 1000; Scantron) was used to calculate the arithmetic average height (Ra) of the specimens.[36-38] The program was set to operate at a height of 200 μm. The Ra value calculated for each scanned point was the average of 4 readings. Three areas measuring 2×2 mm and situated 3 mm apart from each other to avoid any overlap were scanned on each specimen. A total of 30 readings per specimen were obtained and the mean Ra value was calculated.

For the SFE calculation, 3 liquids (deionized water, glycerol, and di-iodomethane) with well-established polar and dispersive components of surface tension were used with the sessile drop technique on the surface of the specimens. The contact angle measurements were obtained with a goniometer (Cam 200; KSV Instruments Ltd.) (Fig. 1).[39] A droplet of 10 μL size was allowed to drop onto the specimens, and the camera was set to capture frames of 1 second intervals for 10 seconds. The Cam2008 software (KSV Instruments Ltd) calculated the right and left static contact angles of the droplet for all the 10 frames, producing 20 readings for the calculation of the mean value of each specimen. The same process was followed for all 3 liquids. After completion of the contact angle experiment, the data were transferred to the SFECam2008 software (KSV Instruments Ltd), and the surface free energy of each specimen was calculated by applying the Owens-Wendt approach.[40]

A scanning electron microscope (SEM) (XL 30; Philips) was used to visualize the surface topography of 2 arbitrarily selected specimens from each group under ×500, ×1000 and ×2000 magnification.

The data obtained after calculating the Ra and the SFE of the specimens were analyzed with software (SPSS v20; IBM Corp). Two-way analysis of variance (ANOVA) was applied individually for the 2 variables (Ra and SFE) of the groups of the first experiment (α=.05). For the groups of the second experiment, the statistical difference of the mean Ra values was investigated with a Kruskal-Wallis test, whereas a 1-way ANOVA was used to compare the mean SFE values. A post hoc Bonferonni test was carried out to compensate for the multiple comparisons.

RESULTS

Table 2 provides a synopsis of the mean surface roughness (Ra) values of all experimental groups. The average Ra values for the 10-μm coated groups with 1000 and 1200 grit surface finish (groups Ap1 and Bp) were significantly lower (P<.001) than their uncoated counterparts (groups A and B), with a decrease of 55% and 49% . No statistically significant difference (P=.869) was found, however, between the mean Ra values of the coated and uncoated groups with 2400 grit initial finish (groups C and Cp) with an equal mean Ra of 0.61 μm.

Comparison of the data of the 4 groups with 1000 grit initial finish (A, Ap1, Ap2, and Ap3) revealed a significant decrease (P<.001) of the mean Ra after coating with 10 μm of Parylene (group Ap1, 55% reduction), which became less apparent as the coating increased to 20 μm (group Ap2, 33% reduction) and 30 μm (group Ap3, 27% reduction). The difference between the groups A and Ap2 (P=.012) and between the groups Ap1 and Ap2 (P=.001) was statistically significant, whereas the groups Ap2 and Ap3 (P=1) and the groups A and Ap3 (P=.189) were not statistically different (Fig. 2).

Tables 3 and 4 summarize the mean values of total SFE along with its dispersive and polar components in mN/m for the different groups. In the first experiment, the mean SFE values of the uncoated groups (A, B, C) were lower than the mean values of the respective coated groups (Ap1, Bp, Cp). Statistical analysis showed a statistically significant difference at the 1% level ($P<.01$).

Comparing the groups of the second experiment revealed an increase of SFE as a result of the increase in the thickness of the coating. A statistically significant difference was found among all the sample groups ($P=.001$), except for between the Ap2 and Ap3 coated groups, where the difference in average SFE was not statistically significant ($P=.6$).

The scans of the uncoated specimens with 1000 and 1200 grit finish (groups A and B) revealed a significant number of irregularities, with the group A displaying a greater proportion of these (Fig. 3, A), (Fig. 4, A). Parallel, unidirectional indentation grooves from the polishing procedure were visible in both specimens. Although still present, these imperfections have been filled with Parylene, leaving a more uniform appearance for the groups Ap1 and Bp (Fig. 3, B), (Fig. 4, B). The uncoated and coated 2400 grit finish groups (C and Cp) both illustrate a smooth surface with only shallow indentations present across the specimen surfaces (Fig. 4, C,D). Coating of the PMMA specimens with 20 and 30 μm of Parylene (groups Ap2 and Ap3) created a surface with more prominent areas of convexity compared with the 10 μm coated groups (Fig. 3, C,D).

DISCUSSION

This study investigated how the different initial finishing and different Parylene C coating thicknesses of PMMA specimens would affect surface roughness and SFE. The results

demonstrated a decrease in Ra after the coating for almost all the groups. This finding was similar to the results of a previous study[35] with a similar experimental protocol in which lower Ra values were achieved as a result of the coating. Interestingly, the coating of specimens finished with 2400 grit initial finish had no effect on the surface roughness. A possible explanation could be that the beneficial smoothing effect of Parylene coating was limited on surfaces with already low roughness levels because of the lack of deep crevices and grooves on a micrometer scale that needed to be filled. Another finding was that the reduction in surface roughness after coating was not proportional to the initial Ra, even though the same thickness of coating was applied, a possible indication that Parylene C deposition was not of absolute consistent thickness and entirely uniform everywhere. A study on Parylene coated metallic substrates[32] supported this finding but direct comparison is limited because of the difference in the substrates and in the instrumentation used for the Ra measurement. The results of this study also showed that the smoothest surfaces were produced when the coating thickness was 10 μm. The increase in the Ra values as the thickness of the Parylene coating increased is also supported by a study[41] which demonstrated that the top surface was getting rougher with increasing thickness and the deposition of Parylene nuclei was getting denser and nanoparticles were visible. The results of the current study also showed that, even though Parylene C deposition resulted in smoother surfaces, it failed to produce surfaces with Ra values less than 0.2 μm, the threshold for microbial colonization.[11] Parylene coating, it seems, cannot replace the traditional finishing and polishing procedure of PMMA[19-21] in terms of initial surface roughness. However, as the fitting surface cannot be exposed to finishing treatment and constitutes the main area of bacterial accumulation and growth, Parylene coating can still be beneficial by producing smoother surfaces, which may also be more abrasion-resistant.[35]

The use of abrasive paper as the finishing medium allowed for standardization as a polishing machine was used to eliminate the potential errors of manual handling. The mean surface roughness (2.69 μm) achieved by the use of 1000 grit abrasive paper (Group A) represented more closely the expected roughness of a fitting surface of a removable dental prosthesis, which can vary between 3.4 and 7.6 μm.[22,35] A noncontact laser profilometer was used to measure surface roughness, and the Ra, arithmetic average height was calculated. Ra is the most common amplitude parameter used to characterize surface roughness[10,19,35] and was preferred over measuring the Rz (ten point height), which is more sensitive in cases of high peaks and deep valleys or the Rq (root mean square roughness), which is appropriate when a large deviation from the mean line is present.[36] A noncontact profilometer was selected in contrast with a number of studies, in which measurement of the surface topography of acrylic resin specimens after different polishing procedures was performed by a contact stylus-type profilometer.[19-21] The use of a diamond or ruby stylus is related to potential damage of the specimen while it is moving on its surface. In addition, the stylus usually measures the deviations in the vertical direction without taking into account the average gradient of the surface roughness[37] and involves a more complicated procedure.[38]

The values resulting from the SFE calculation indicated an increase of the surface free energy after coating with Parylene C for all the groups, which was statistically significant. The increase in SFE may be attributed to the change in roughness of the specimens, as altering the Ra can affect the contact angle and the SFE.[12,42] Another explanation could be that the baseline SFE of Parylene C itself was higher than that of PMMA and the overall chemical interactions on the Parylene surface layer brought about the change in SFE. To the authors' best knowledge, the Zhou et al[34] were the first to investigate the effect of Parylene coating on the SFE of acrylic resin.

The approach used for the SFE calculation was the same as in the present experiment, but the specimens were coated with 5 μm of a different variety of Parylene N.[34] The results[34] showed no statistically significant change of the SFE after coating, although the values obtained were higher for the coated specimens, confirming the increasing trend after coating observed in the present experiment.

The calculation of surface free energy (SFE) was carried out with the Owens-Wendt method, which is well supported in the literature.[25,34,43] A limitation of most of the methods used for the SFE calculation though is that the Young equation assumes that the solid surface is rigid, chemically homogemous, and smooth on an atomic scale.[39] The selection of the probe liquids was also based on the experimental protocols of relevant studies.[7,17,34] A further limitation to this study was that all measurements were made under laboratory conditions. The presence of a salivary pellicle formed on the surface of the specimens might have simulated the oral environment to a certain degree, providing more accurate SFE values of these materials in situ.[17] However, this view is disputed by a number of studies in the literature[9,18] which support the idea that the SFE properties of a material can be transferred through the salivary proteins and are not affected by them.

The impact of SFE on bacterial adhesion is also an ambiguous issue. It appears that the SFE of the material is important at the initial stages of bacterial adhesion, whereas its influence decreases with prolonged biofilm formation.[12] Although surfaces with low SFE promote less microbial retention,[8] bacteria also tend to adhere to surfaces that have a similar SFE.[14,17] Taking into account these findings and the knowledge that the roughness of the material plays a substantial role in plaque formation, assessing the clinical significance of the increased SFE of

the PMMA specimens after coating is difficult. Previous work,[34] however, has demonstrated that the increased SFE of Parylene coated specimens results in lower Candida albicans adhesion.

More studies are needed to explore how the surface properties of Parylene C can affect the microbial adhesion and also investigate the effect of Parylene C on the retentive, mechanical, and chemical properties as well as color stability of removable prostheses. Until clinical trials produce favorable results, its success as a coating material against biofilm formation can only be assumed.

CONCLUSIONS

Within the limitations of this in vitro study, the following conclusions can be drawn:

1. Coating with a 10-μm layer of Parylene C had a smoothing effect on surfaces which resembles the fitting surface of denture (Ra>3.4 μm) but did not achieve Ra values below the 0.2-μm threshold of microbial colonization.
2. The use of a 10-μm coating produced favorable results in comparison with the 20-μm and 30-μm coating regarding the surface roughness of acrylic resin.
3. Coating with Parylene C of all thicknesses resulted in specimens with statistically significantly higher SFE values than their uncoated counterparts.

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Table 1. Description of experimental groups

	Experimental Groups	Finishing Grit (µ)	Parylene C Coating (µ)
Experiment 1	**A**	**1000**	**-**
	Ap1	**1000**	**10**
	B	**1200**	**-**
	Bp	**1200**	**10**
	C	**2400**	**-**
	Cp	**2400**	**10**
Experiment 2	**A**	**1000**	**-**
	Ap1	**1000**	**10**
	Ap2	**1000**	**20**
	Ap3	**1000**	**30**

Group A: uncoated-1000grit finish
Group Ap1: 10µm P coated-1000grit finish
Group B: uncoated-1200grit finish
Group Bp: 10µm P coated-1200grit finish
Group C: uncoated-2400grit finish
Group Cp: 10µm P coated-2400grit finish
Group Ap2: 20µm P coated-1000grit finish
Group Ap3: 30µm P coated-1000grit finish

Table 2. Surface roughness values in experiments 1 and 2

Experiment 1	Mean Ra (SD)	Sig	Experiment 2	Mean Ra (SD)
A	2.69µm (0.80)	<0.001	A	2.69µm (0.80)
Ap1	1.21µm (0.20)		Ap1	1.21µm (0.20)
B	1.95µm (0.70)	<0.001	Ap2	1.79µm (0.36)
Bp	1.00µm (0.22)		Ap3	1.97µm (0.40)
C	0.61µm (0.16)	0.869		
Cp	0.61µm (0.15)			

Group A: uncoated-1000-grit finish
Group Ap1: 10µm P coated-1000-grit finish
Group B: uncoated-1200-grit finish
Group Bp: 10µm P coated-1200-grit finish
Group C: uncoated-2400-grit finish
Group Cp: 10µm P coated-2400-grit finish
Group Ap2: 20µm P coated-1000grit finish
Group Ap3: 30µm P coated-1000grit finish

Table 3. Surface free energy values in experiment 1

Experimental Group	Mean DSFE	Mean PSFE	Mean TSFE (SD)
A	29.86 mN/m	3.81 mN/m	33.27 mN/m (3.31)
Ap1	39.00 mN/m	2.02 mN/m	38.02 mN/m (3.07)
B	27.84 mN/m	2.25 mN/m	30.09 mN/m (2.87)
Bp	32.34 mN/m	1.31 mN/m	33.65 mN/m (1.99)
C	24.98 mN/m	3.90 mN/m	28.88 mN/m (1.66)
Cp	29.57 mN/m	1.75 mN/m	31.32 mN/m (1.61)

DSFE: Surface Free Energy – Dispersive Component
PSFE: Surface Free Energy - Polar Component
TSFE: Total Surface Free Energy
SD: Standard Deviation

Table 4. Surface free energy values in experiment 2

Experimental Group	Mean DSFE	Mean PSFE	Mean TSFE (SD)
A	29.86 mN/m	3.81 mN/m	33.27 mN/m (3.31)
Ap1	39.00 mN/m	2.02 mN/m	38.02 mN/m (3.07)
Ap2	47.05 mN/m	2.39 mN/m	49.44 mN/m (2.27)
Ap3	45.28 mN/m	2.28 mN/m	47.56 mN/m (5.01)

DSFE: Surface Free Energy – Dispersive Component
PSFE: Surface Free Energy - Polar Component
TSFE: Total Surface Free Energy
SD: Standard Deviation

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Figure 1: Image of goniometer (Cam 200; KSV Instruments Ltd).

Figure 2: Box-plots of Ra values of groups in experiment 2.
*: statistically significant difference
**: nonstatistically significant difference

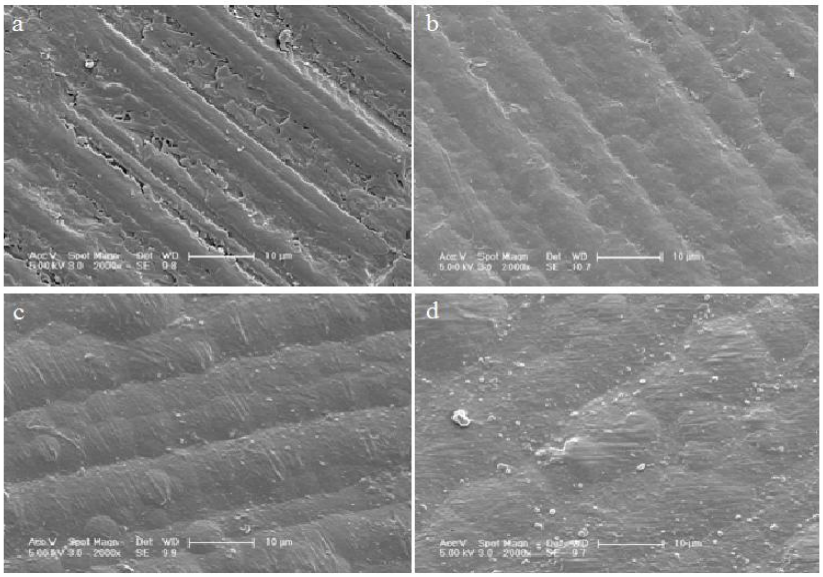

Figure 3: SEM images (×2000 magnification) of PMMA and Parylene-coated PMMA specimens. A, PMMA specimen with 1000 grit finishing. B, 10 μm Parylene-coated PMMA specimen with 1000 grit finishing. C, 20 μm Parylene-coated PMMA specimen with 1000 grit finishing. D, 30 μm Parylene-coated PMMA specimen with 1000 grit finishing.

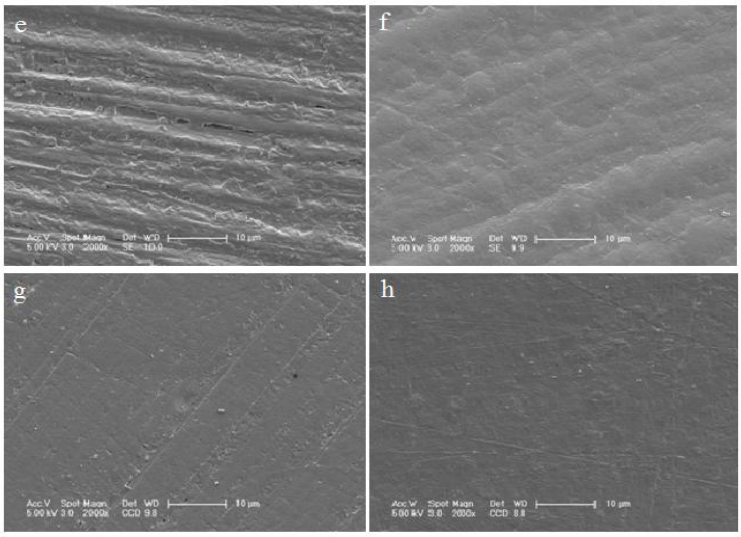

Figure 4: SEM images (×2000 magnification) of PMMA and Parylene-coated PMMA specimens. A, PMMA specimen with 1200 grit finishing. B, 10 μm Parylene-coated PMMA specimen with 1200 grit finishing. C, PMMA specimen with 2400 grit finishing. D, 10 μm Parylene-coated PMMA specimen with 2400 grit finishing.

Figure 1

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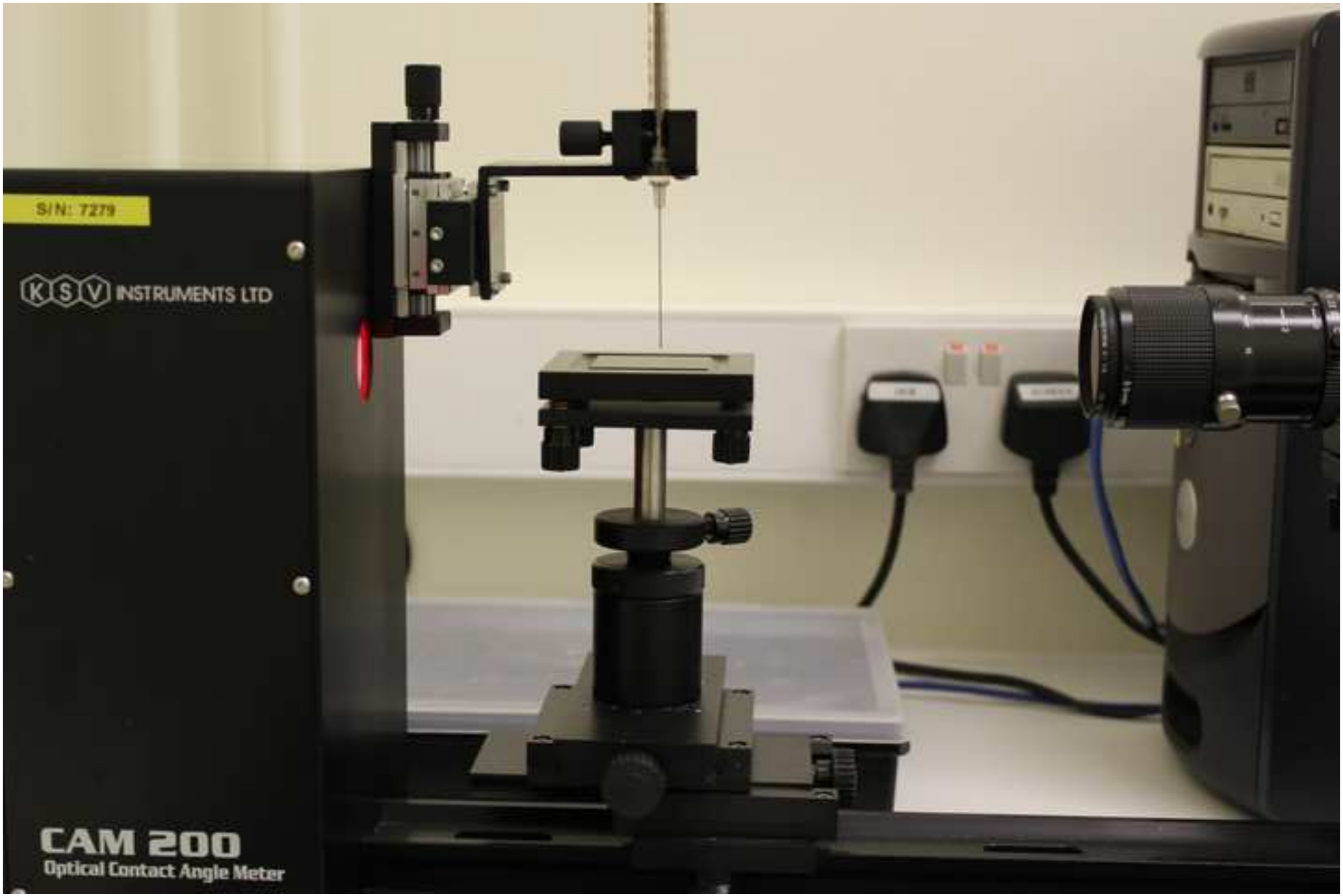

Figure 2

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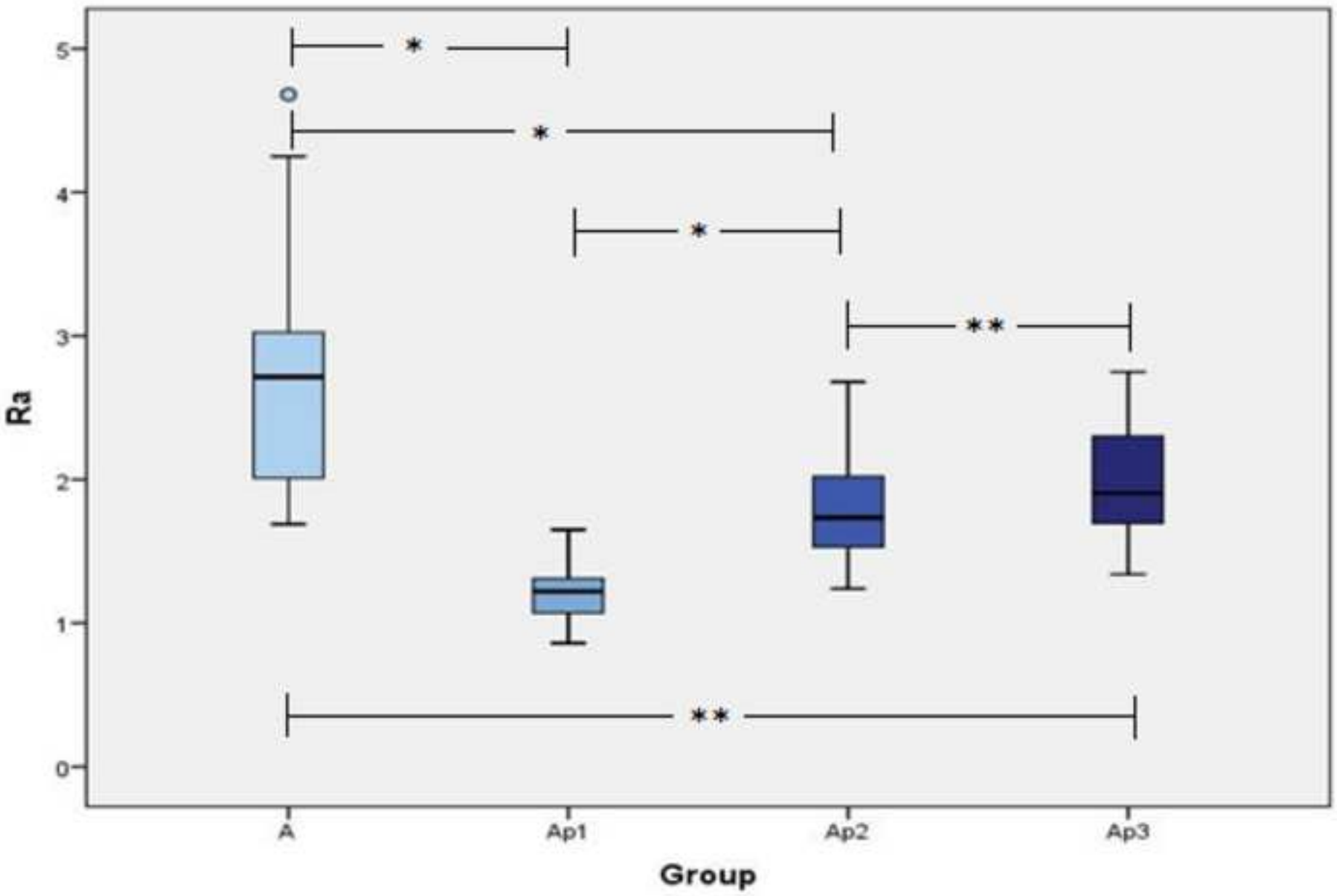

Figure 3a

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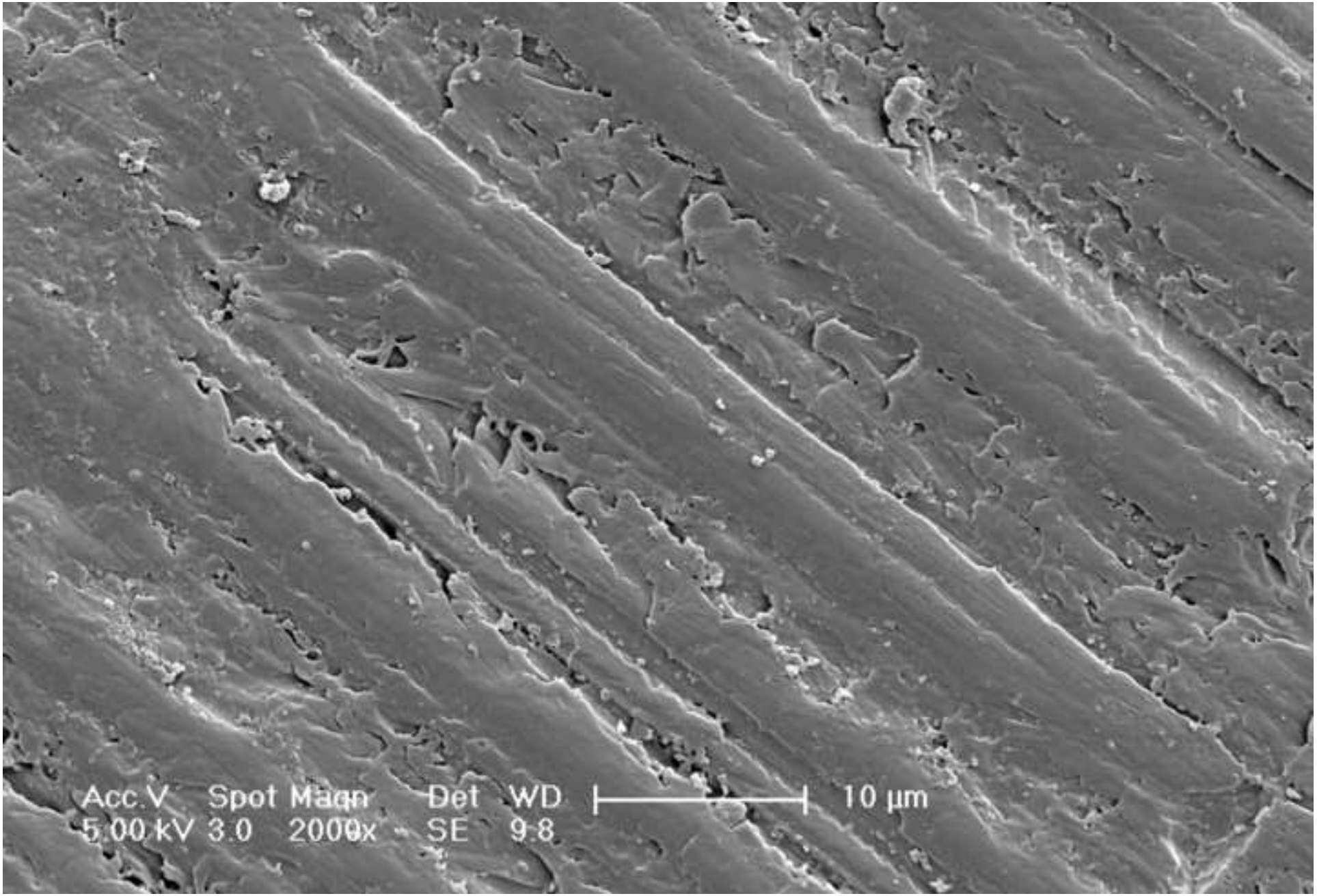

Figure 3b

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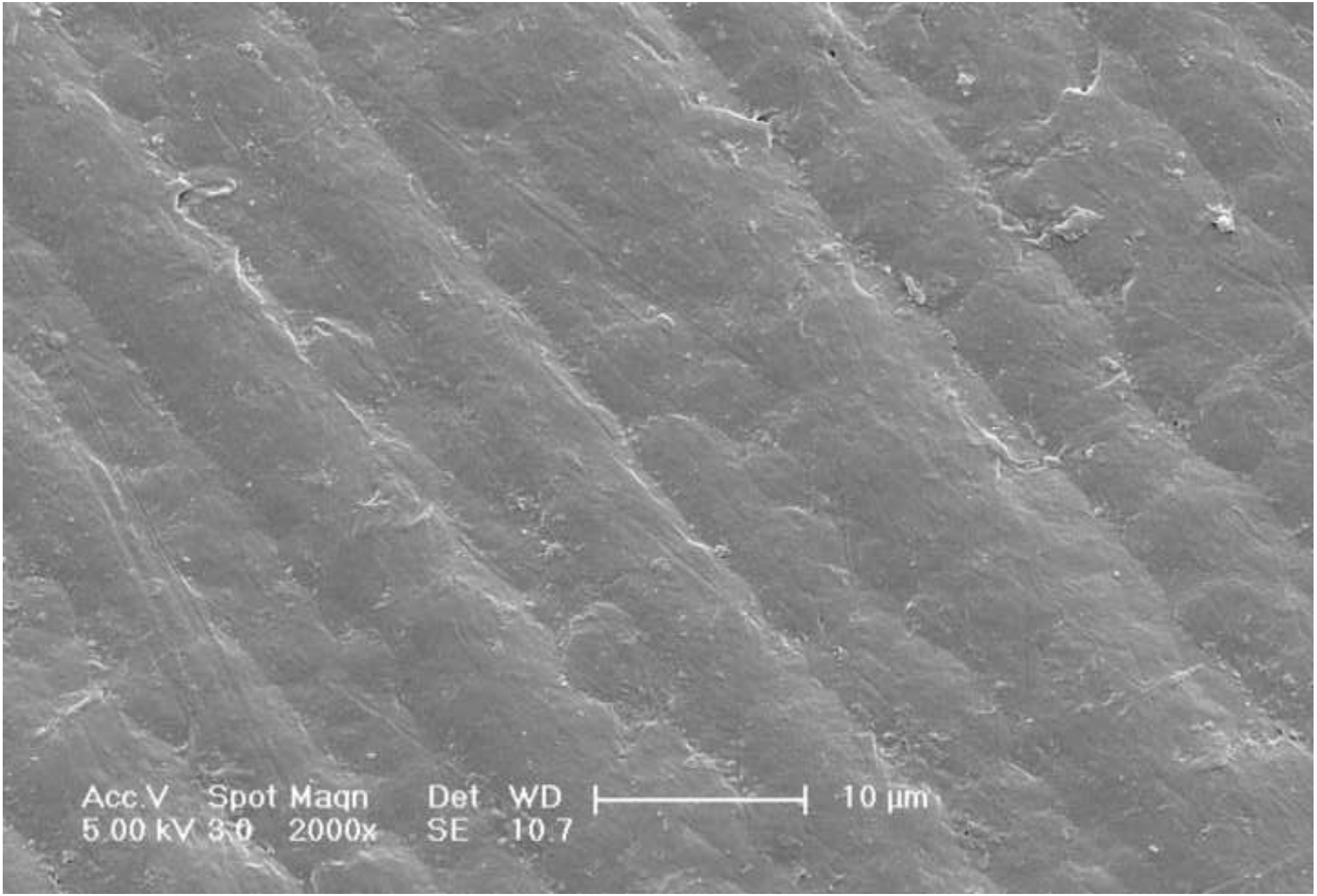

Figure 3c

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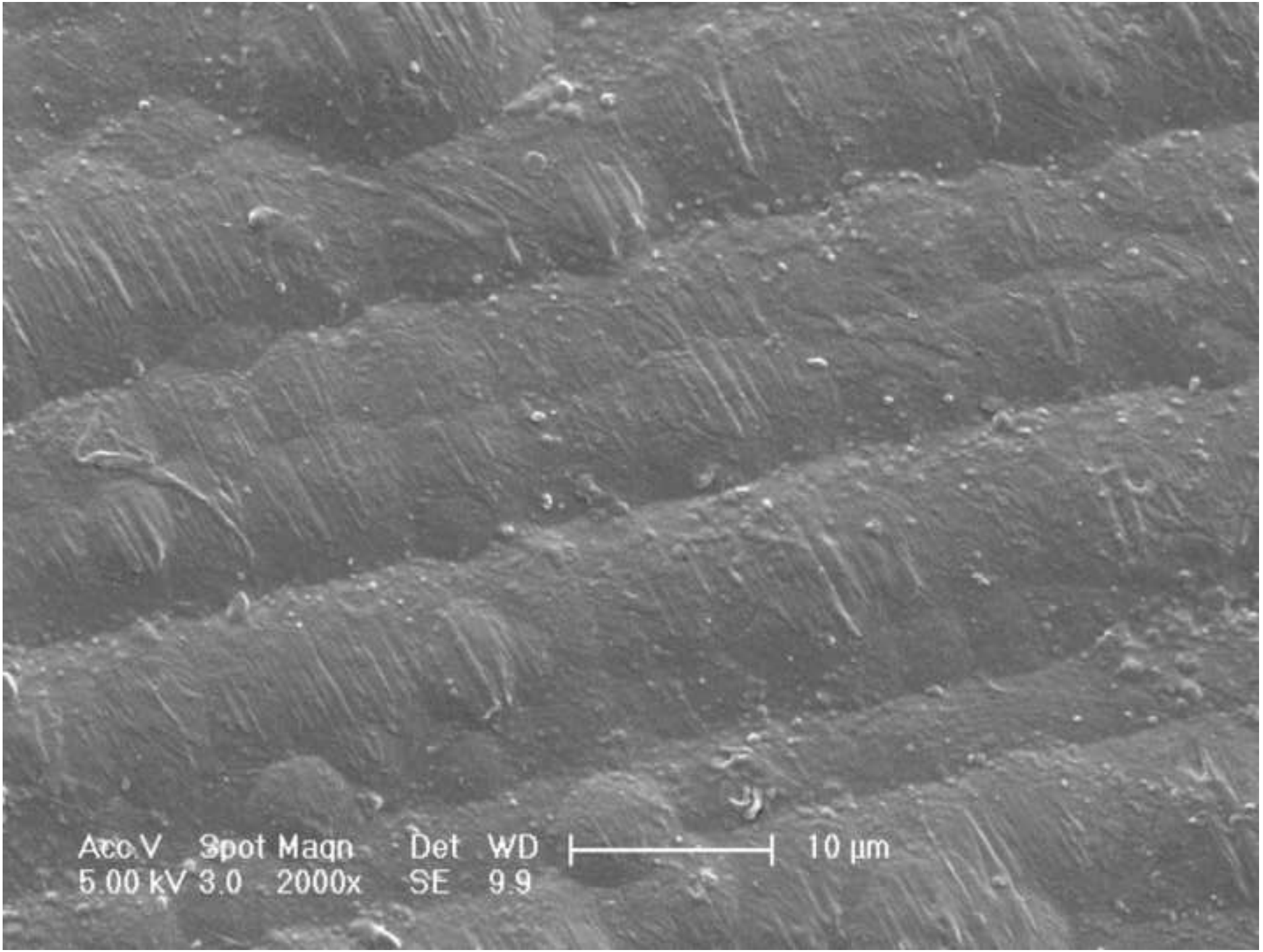

Figure 3d

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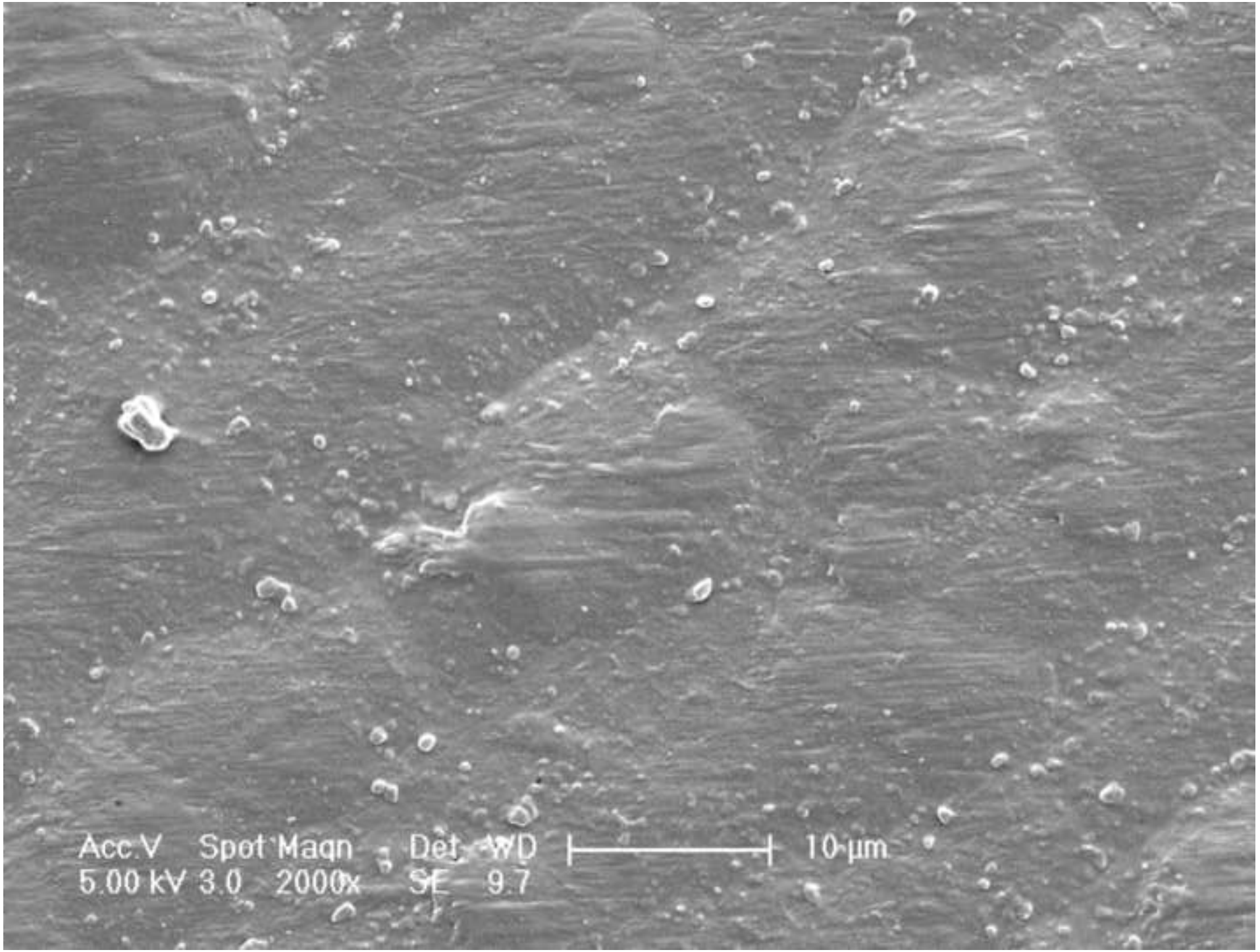

Figure 4e

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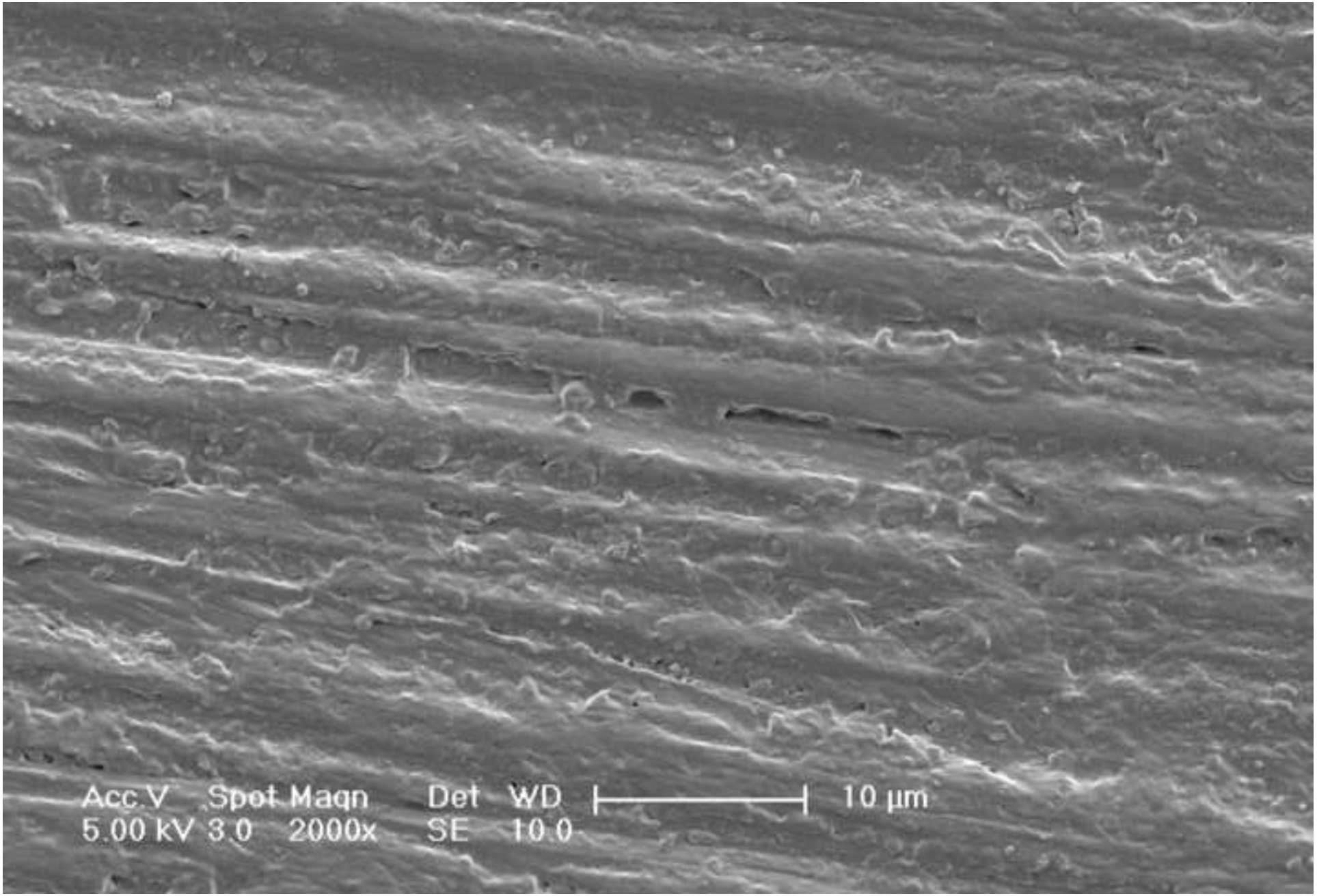

Figure 4f

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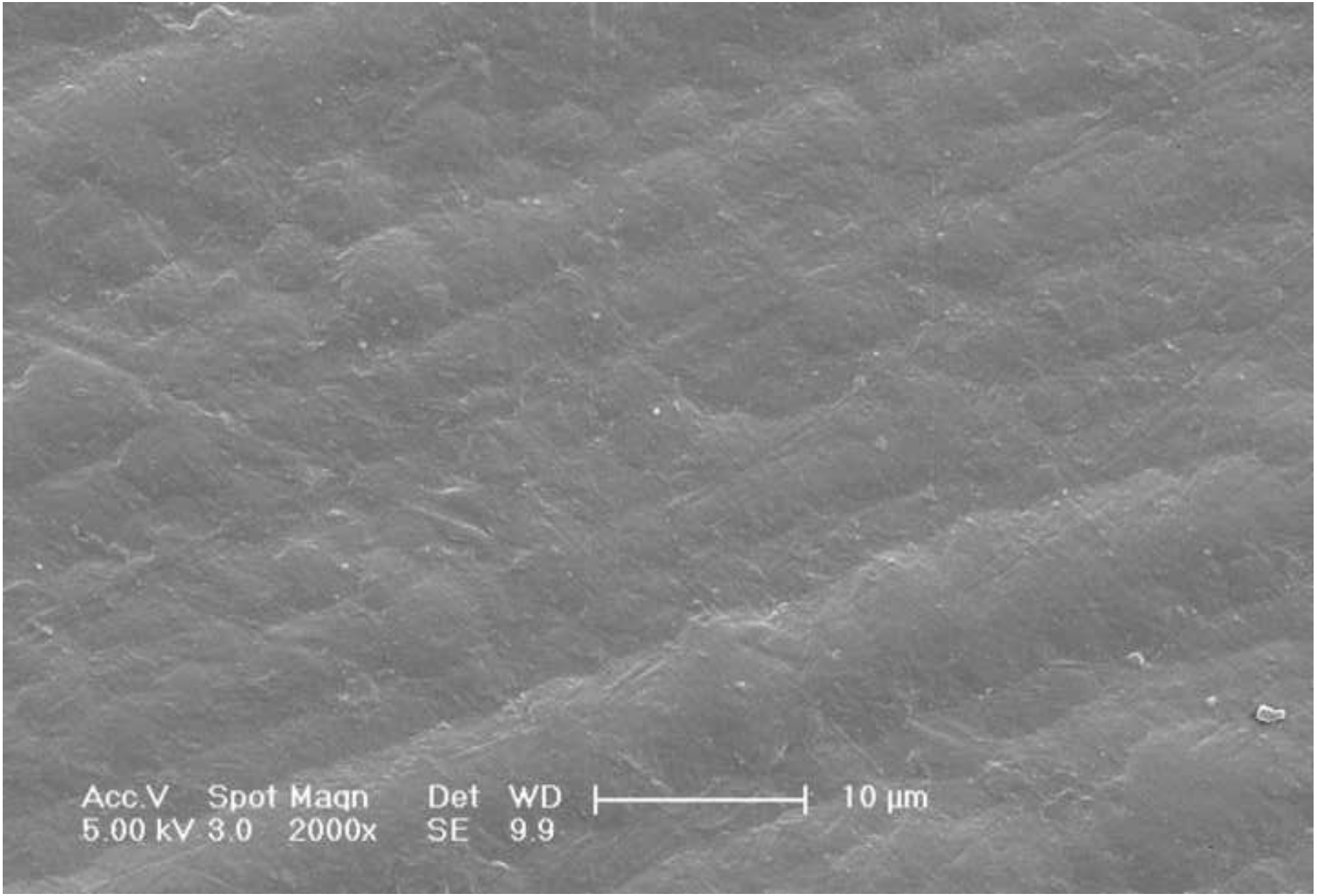

Figure 4g

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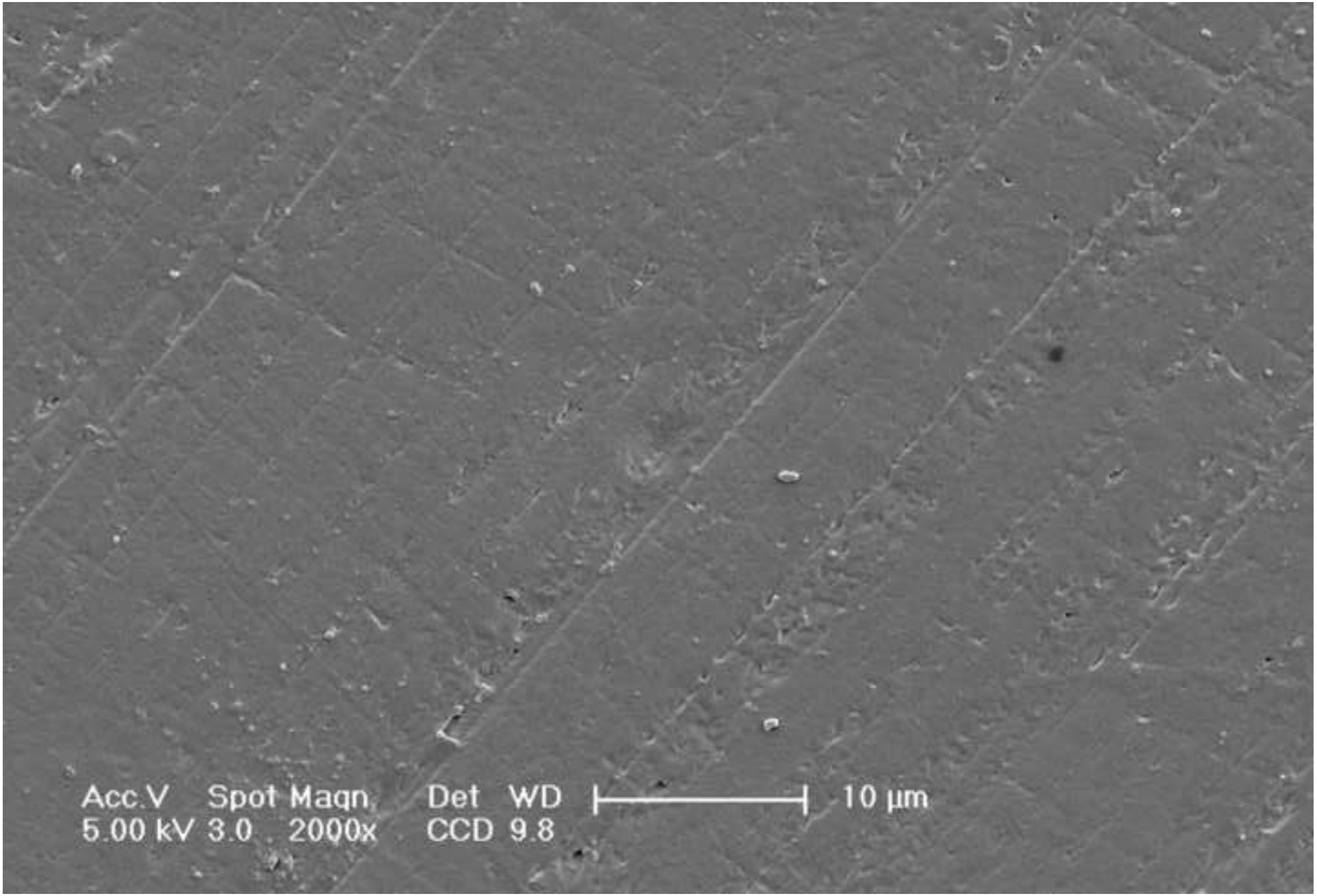

Figure 4h

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