Supporting Information

AFM for Nanomechanical Assessment of Polymer Overcoatings on Nanoparticle Decorated Biomaterials

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Table S1. Data were acquired using an NT-MDT NSG03 cantilever. Each overcoating measurement was performed using different cantilevers from the same batch as supplied by the manufacturers. Values for each cantilever calibration and setup are displayed in the supplementary table below. Free-air amplitude values at a magnitude of 10^{-15} are below the instrument's expected noise floor. However, the values presented are from instrumentation software.

Overcoating Thickness	0 Nm Overcoating	5 Nm Overcoating	10 Nm Overcoating	15 Nm Overcoating	20 Nm Overcoating
Sensitivity (nm/V)	29	30.5	33	24.8	25.4
Q-factor	158	168	162	161	157.6
Free air Amplitude (m/√Hz)	7.8×10^{-15}	9.4×10^{-15}	9.2×10^{-15}	8.6×10^{-15}	8.8×10^{-15}
Spring constant (N/m)	2.8	1.8	2	2.35	2.3
Set Point (nm)	27	26	29	22	22

Base film variations

A 20 nm-thick plasma-processed MePPOx base film functionalised with 68 nm Au NPs was selected as an antibacterial nanostructured surface due to the reasonable control of plasma time for adjusting polymer film thickness and for the ease of altering the ratio of NP size to the thickness of the overcoating film. Other plasma-derived polymers and Au NP sizes were analysed for their overall roughness in both tapping and contact mode topography over a 5 x 5 μm scan area. A force curve test was performed to compare adhesion and friction forces using a SiN conical cantilever tip. All analysis was performed with four NT-MDT NSG30 cantilevers with a typical Sader spring constant in the 30-40 N/m range and a torsional spring constant in the 6E-9 N/m range, as shown in Table S2(a) below. Au NP of three sizes, 16, 32, and 68 nm diameter, was placed on three plasma polymer coatings, with and without a 20-nanometre-thick MePPOx overcoating. These polymers are shown in Table S2(b), with variations in the NP size, whether they have a ~20 nm overcoating of the same base film material, and the cantilever used, as presented in Table S1 (a).

Table S2. (A) NTMDT NSG30 cantilever calibration values. (B) The range of samples tested and compared. Sample numbers are labelled by the polymer base film, MePPOx (M), Allylamine (AA), Acrylic Acid (AC), the Au NP diameter of either 16, 32, or 68 nm, and if the sample has a 20nm overcoating of the

same polymer as the base film, with no overcoating (N), and with an overcoating (Y). Four NT-MDT NSG30 cantilevers were used, labelled C1–C4.

A

Cantilever Number	Resonant Frequency (kHz)	Normal Sader SC (N/m)	Torsional SC (E-9 N/m)	Q-factor
C1	281.7	32	6.45	512
C2	281.6	32.8	6.6	531
C3	273.9	30.2	6.1	507
C4	281	32.4	6.6	531

B

Sample Number	Base Material	Overcoating (Y/N)	Cantilever Number
M16N	MePPOx	N	C1, C2
M16Y	MePPOx	Y	C1, C2
M38N	MePPOx	N	C1, C2
M38Y	MePPOx	Y	C1, C2
M68N	MePPOx	N	C1, C2
M68Y	MePPOx	Y	C1, C2
AA16N	Allylamine	N	C3, C4
AA38N	Allylamine	N	C3, C4
AA68N	Allylamine	N	C3, C4
AC16N	Acrylic Acid	N	C3, C4
AC38N	Acrylic Acid	N	C3, C4

Roughness values were compared not only between tapping and contact topography modes but also for samples with and without plasma overcoating applied at a thickness of 20 ± 5 nm, Table S2–S7. Most of the sample's topography was scanned using AFM, and each contained a small amount of dirt, dust, plasma polymer remnants, etc. that adhered to the substrate at some point during processing. Data processing software has tools that allow for the removal of these anomalies that change roughness values. The *-marked sample values highlight the roughness values of the surface filtered by Gwyddion software to remove surface anomalies. A significant contrast in roughness values occured between the two topography modes, with and without overcoating, and in the software-filtered values. This was expected owing to differences in tip–substrate topography forces, the change in Au NP interaction with the tip with and without an applied overcoating and removing topographical features through software processing.

Table S3. (A) Roughness values of MePPOx with **16 nm** Au NP measured and compared in tapping and contact topography mode, and (B) Force curve values of the force load (the force of the cantilever tip's contact with the substrate), the adhesion force of the cantilever tip as it retracts from the surface, and the friction force as measured through LFM data. The average friction force for the M16Y* sample shown in (b) was not able to be calculated due to computer failure.

A

Roughness value	Sample M16N		Sample M16Y		Sample M16Y*	
	Tapping	Contact	Tapping	Contact	Tapping	Contact
RMS (nm)	3.1	2.7	24.3	2	5.8	1.3
Ra (nm)	2.2	2.3	10.8	1.5	4.7	1
Peak height (nm)	96	18	518	53	59	7

B

Force Curve Values	M16Y	M16Y*
Force load (nN)	0.44	2.116
Adhesion force (nN)	0.37	1.536
Av friction force (nN)	0.33	-

Table S4. (A) Roughness values of MePPOx with 32 nm Au NP measured and compared in tapping and contact topography modes, and (B) force curve values of the force load, the adhesion force of the cantilever tip as it retracts from the surface, and friction force as measured through LFM data.

A

Roughness value	Sample M32N		Sample M32N*		Sample M32Y		Sample M32Y*	
	Tapping	Contact	Tapping	Contact	Tapping	Contact	Tapping	Contact
RMS (nm)	31.3	6.8	16.8	3.3	28.3	5.3	3	5.3
Ra (nm)	19.4	5	12	2.8	23.5	4.2	15.5	2.6
Peak height (nm)	559	73	366	27	322	64	238	14

B

Force curve values	M32N	M32Y
Force load (nN)	0.18	0.33
Adhesion force (nN)	0.27	0.37
Av friction force (nN)	0.24	0.22

Table S5. (A) Roughness values of MePPOx with **68 nm** Au NP measured and compared in tapping and contact topography modes, and (B) force curve values of the force load, the adhesion force of the cantilever tip as it retracts from the surface, and friction force as measured through LFM data.

A

Roughness value	Sample M68N		Sample M68N*		Sample M68Y		Sample M68Y*	
	Tapping	Contact	Tapping	Contact	Tapping	Contact	Tapping	Contact
RMS (nm)	63.3	2	12.7	0.73	27.5	3.1	12.3	1
Ra (nm)	26.2	1.3	9.1	0.56	16.3	2.1	8.6	0.8
Peak height (nm)	1006	70	147	7	522	72	256	6

B

Force Curve Values	M68N	M68Y
Force load (nN)	0.65	0.59
Adhesion force (nN)	0.58	0.68
Av friction force (nN)	0.5	0.62

Table S6. Roughness values of MePPOx without AuNP measured and compared in tapping and contact topography modes. Additionally, force curve values of the force load, the adhesion force of the cantilever tip as it retracts from the surface, and friction force as measured through LFM data are shown.

Roughness value	Tapping	Contact	Force curve values	
RMS (nm)	4.78	4.33	**Force load (nN)**	0.96
Ra (nm)	3.4	3.51	**Adhesion force (nN)**	0.48
Peak height (nm)	153	85		

Table S7. Roughness values of Allylamine with (A) 16 nm Au NP, (B) 32 nm Au NP, (C) 68 nm Au NP measured and compared in tapping and contact topography modes, and (D) force curve values of the force load, the adhesion force of the cantilever tip as it retracts from the surface, and friction force as measured through LFM data.

A

	Sample AA16N		Sample AA16N*	
Roughness value	**Tapping**	**Contact**	**Tapping**	**Contact**
RMS (nm)	15.3	3.5	8.7	1.3
Ra (nm)	12.9	2	7.3	1.1
Peak height (nm)	92	76	58	8

B

	Sample AA32N		Sample AA32N*	
Roughness value	**Tapping**	**Contact**	**Tapping**	**Contact**
RMS (nm)	6.8	2.3	2.9	1.4
Ra (nm)	2.5	1.7	1.8	1.2
Peak height (nm)	339	48	97	8

C

	Sample AA68N		Sample AA68N*	
Roughness value	**Tapping**	**Contact**	**Tapping**	**Contact**
RMS (nm)	31	2	27.3	1
Ra (nm)	25.3	1.3	22.7	0.7
Peak height (nm)	292	52	225	8

D

Force curve values	**AA16N**	**AA32N**	**AA68N**
Force load (nN)	0.16	0.2	0.13
Adhesion force (nN)	0.2	0.27	0.07
Av friction force (nN)	0.28	0.1	0.5

Table S8. (A) Roughness values of Acrylic Acid with 16 and 32 nm Au NP measured and compared in tapping and contact topography mode, and (B) force curve values of the force load, the adhesion force of the cantilever tip as it retracts from the surface, and friction force as measured through LFM data.

A

	Sample AC16N		Sample AC16N*		Sample AC3NY		Sample AC32N*	
Roughness value	**Tapping**	**Contact**	**Tapping**	**Contact**	**Tapping**	**Contact**	**Tapping**	**Contact**
RMS (nm)	5.7	2.1	5.7	1.3	18	23	17.6	11.2
Ra (nm)	4	1.58	4	1	14.7	16	14.7	9.5
Peak height (nm)	198	45	41	8	255	203	132	76

B

Force curve values	**AC16N**	**AC32N**
Force load (nN)	0.13	0.12
Adhesion force (nN)	0.4	0.37
Av friction force (nN)	0.32	0.4