

Supporting information

Protein-repellent and Antimicrobial Nanoparticle Coatings from Hyaluronic Acid and a Lysine- Derived Biocompatible Surfactant

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Dynamic light scattering (DLS)

The HA-MKM mean particle hydrodynamic diameters were determined by DLS using a Zetasizer Nano ZS device (Malvern Instruments Ltd, United Kingdom, wavelength: 633 nm, scattering angle: 173°). The measurements were conducted at 25° C. Each sample was measured in three successive runs and in each run the sample was scanned ten times. Mean particle diameters were approximated as the effective (z -average) diameters from the bimodal size distribution.¹ The measurements were repeated five times.

Quartz crystal microbalance with dissipation (QCM-D)

A QCM-D instrument (model E4) from Q-Sense, Gothenburg, Sweden was used. The instrument simultaneously measures changes in the resonance frequency (Δf) and energy dissipation (ΔD) when the mass of an oscillating piezoelectric crystal changes upon increase/decrease in the mass of the crystal surface due to the added/deduced mass. Dissipation refers to the frictional losses that lead to damping of the oscillation depending on the viscoelastic properties of the material. For a rigid adsorbed layer that is fully coupled to the oscillation of the crystal, Δf_n is given by the Sauerbrey equation ² (1)

$$\Delta m = C \frac{\Delta f_n}{n} \quad (1)$$

where Δf_n is the observed frequency shift, C is the Sauerbrey constant ($-0.177 \text{ mg Hz}^{-1} \text{ m}^{-2}$ for a 5 MHz crystal), n is the overtone number ($n = 1, 3, 5, \text{ etc.}$), and Δm is the change in (wet) mass of the crystal due to the adsorbed layer. The mass of a soft (i.e. viscoelastic) film is not fully coupled to the oscillation and the Sauerbrey relation is not valid since energy is dissipated in the film during the oscillation. The damping (or dissipation) (D) is defined as

$$D = \frac{E_{diss}}{2\pi E_{stor}} \quad (2)$$

where E_{diss} is the energy dissipated and E_{stor} is the total energy stored in the oscillator during

one oscillation cycle. The condition for using the Sauerbrey equation is that the adsorbed film is rigid, homogeneous and evenly distributed on the sensors. Assuming that the adsorbed HA-MKM NPs is homogeneous and tightly bound to the surface, equation 1 was used to calculate the dry mass of the three layers of HA-MKM coated on PDMS thin film under condition II. For that purpose, the third overtone resonance frequency of the crystal before and after HA-MKM (II) deposition following the drying process was measured for at least 10 minutes. All calculations were carried out using the software package QTools 3.0.12 (Q-Sense).

For creating PEI (branched, average $M_w \sim 25,000$, Sigma-Aldrich, Austria) layer on PDMS surface, the PDMS coated sensors were mounted in the QCM-D flow cell and equilibrated with water followed by PBS buffer (pH 7.4) until a constant frequency signal was established. The PEI solution (0.5%, w/v, dissolved in PBS buffer) was pumped over the PDMS surface for 90 min followed by rinsing with PBS buffer until a constant signal was established. Following this step, the PEI coated PDMS films were equilibrated with the PBS solution for 5 min. Then, the protein solutions (BSA, Fib and Lyz, 10 mg mL^{-1} , dissolved in PBS) were pumped through the flow cells for 60 min followed by rinsing with PBS buffer for 30 min at a flow rate of 0.1 mL min^{-1} . All experiments were performed at $21 \pm 0.1 \text{ }^\circ\text{C}$. Each adsorption experiment has been performed in three parallels.

X-ray photoelectron spectroscopy (XPS)

The X-ray photoelectron spectroscopy (XPS) spectra of the uncoated and HA-MKM coated PDMS surfaces were recorded with a monochromatic K-Alpha spectrometer equipped with an Al X-ray source (1486.6 eV) operating with a base pressure in the range of 10^{-8} to 10^{-10} mbar. Survey scans were recorded with a pass energy of 100 eV and a step size of 1.0 eV. All spectra were normalized to the Au $4f_{7/2}$ peak. The average chemical composition was calculated from wide scan spectra and all analyses were performed at room temperature.

Profilometry

The layer thickness of the uncoated and coated PDMS films was determined by profilometry using a DEKTAK 150 Stylus Profiler from Veeco (Plainview, NY, USA). The scan length was set to 1000 μm over the time duration of 3 seconds. The diamond stylus had a radius of 12.5 μm and the force was 3 mg with a resolution of 0.333 $\mu\text{m}/\text{sample}$ and a measurement range of 6.5 μm . The profile was set to hills and valleys. Prior to the surface scanning, the coating was scratched to remove the films in order to determine the thickness of the coating using a step-height profile. The thickness was determined at 3 independent positions.

Atomic force microscopy (AFM)

The surface morphology of the samples was characterized by atomic force microscopy (AFM) in tapping mode with an Agilent 5500 AFM multimode scanning probe microscope (Agilent, Santa Barbara, CA). The images were acquired after drying the films in a stream of dry nitrogen. The images were scanned using silicon cantilevers (ATEC-NC-20, Nanosensors, Germany) with a resonance frequency of 210-490 kHz and a force constant of 12-110 N m^{-1} . All measurements were performed at room temperature. All images were processed using Gwyddion software package.³

Confocal laser scanning microscopy (CLSM)

The uncoated and HA-MKM coated PDMS surfaces were stained with a FITC solution ($c = 10 \mu\text{g mL}^{-1}$, dissolved in MilliQ-water, pH 6.8). The stained samples were left to react for 30 min in a dark place under exclusion of light. Afterwards, the samples were rinsed with MilliQ-water and blow dried with N_2 gas. A confocal laser scanning microscope (Leica TCS SP5 II laser scanning confocal microscope equipped with a LAS AF imaging software, Leica Microsystems, Germany) was used to observe the surface morphology of the stained samples.

The FITC dye was excited at 490 nm, and the emission at 520 nm was recorded. The image size was 512×512 pixels, and the images were scanned at a scan speed of 290 frames s⁻¹.

pH-potentiometric titrations

pH-potentiometric titrations were performed for the HA-MKM complex. A glass titration cell was filled with HA-MKM dispersion and titrated in a forward (from acidic to alkaline) and backward (from alkaline to acidic) manner in the pH region between 2.5 and 11 using 0.1 M hydrochloric acid and 0.1 M potassium hydroxide. The ionic strength of the solutions was set to 0.1 M using potassium chloride. The titrants were added to the system in a dynamic mode using a double burette Mettler Toledo T70 automatic titration unit. The pH value was measured using a Mettler Toledo InLab Routine combined glass electrode. The equilibrium criteria for a stable pH reading was set to $dE/dt = 0.1 \text{ mV}/30 \text{ s}$. The minimum time for the reading was thus set to 30 s, while the maximum time was set to 300 s. (ii)

Determination of the amount of charged functional groups is described elsewhere⁴⁻⁵. Only a brief description is presented in this paper. In the titration system, as described above, the ionic species present are H⁺, OH⁻, their counter ions K⁺ and Cl⁻ as well as the species of interest, denoted as A_k^n , where n is the charge number and k is the enumerator. The total charge Q , due to the presence of A_k^n is calculated using the electro-neutrality condition according to Eq. 1:

$$Q(\text{pH}) = FV_t \sum_k n [A_k^n] = FV_t ([\text{Cl}^-] - [\text{K}^+] + [\text{OH}^-] - [\text{H}^+]) \quad (1)$$

where square brackets denote the ion concentrations in mol/dm³, V_t the total volume and F the Faraday's constant. The potassium and chloride ion concentrations, $[\text{K}^+]$ and $[\text{Cl}^-]$, respectively, are known from the titrant additions, while the hydrogen and hydroxyl ion concentrations, $[\text{H}^+]$ and $[\text{OH}^-]$, respectively, are measured with a pH metre. In a blank titration without the species of interest only H⁺, OH⁻, K⁺, and Cl⁻ ions are present, thus $Q = 0$

for any given pH. This allows replacing the $[\text{OH}^-]-[\text{H}^+]$ term in Eq. 1 by the difference $[\text{K}^+]_{\text{blank}}-[\text{Cl}^-]_{\text{blank}}$ and results in (Eq. 2):

$$Q_{\text{AC}}(\text{pH}) = FV_t ([\text{Cl}^-] - [\text{K}^+] + [\text{K}^+]_{\text{blank}} - [\text{Cl}^-]_{\text{blank}}) \quad (2)$$

The latter approach is recommended because it permits eliminating the error due to the presence of dissolved carbon dioxide in the titration system. The titrant volume was normalised to the mass of the titrated samples and expressed as charges per mass (in mmol/g) versus pH curve. The $Q(\text{pH})$ curves are referred to as charging isotherms.

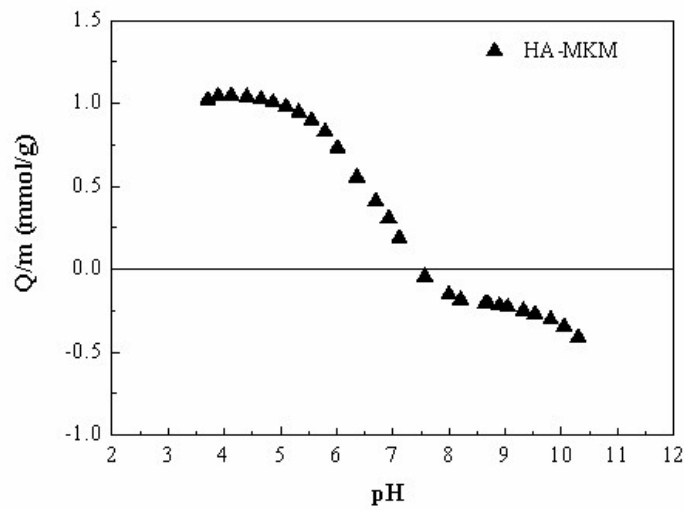


Fig. S1. pH potentiometric neutralization curve (Q/m) as a function of pH for HA-MKM NPs dispersion

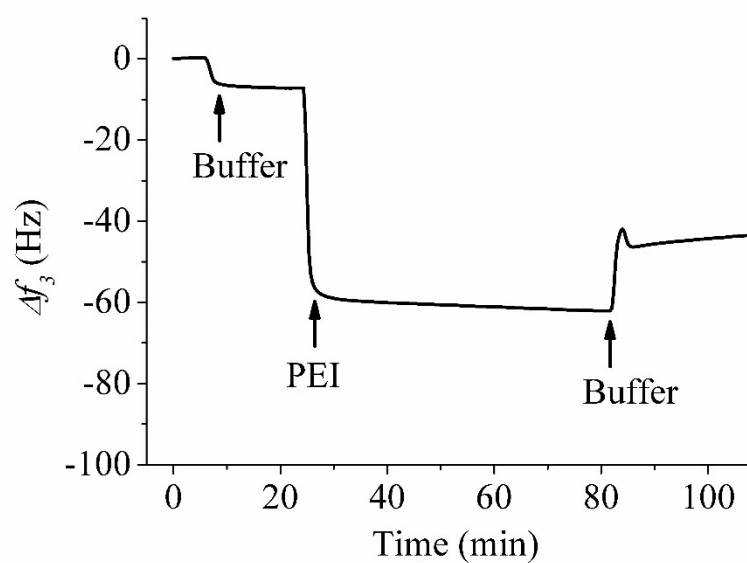


Figure S2. QCM-D frequency changes for the adsorption of PEI on PDMS thin films.

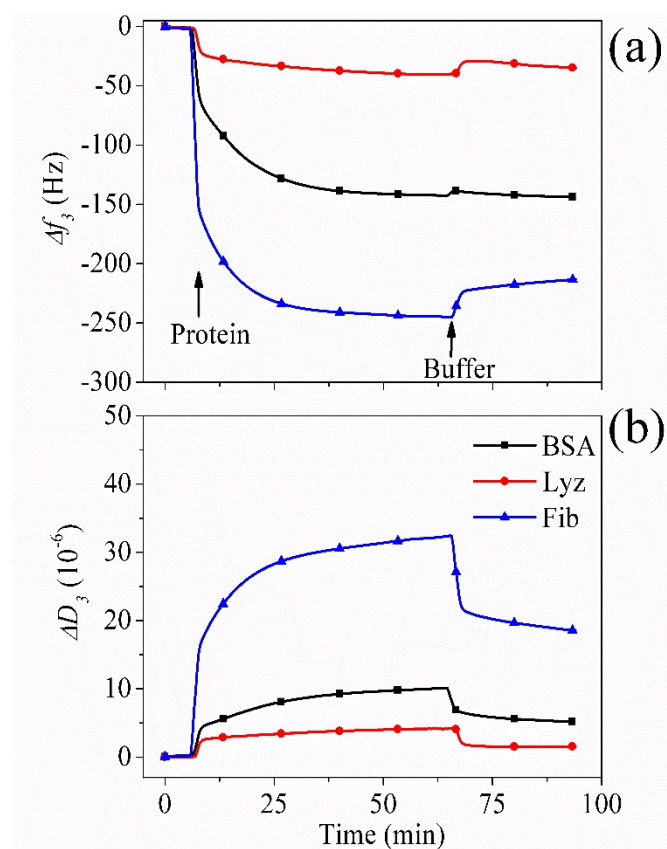


Figure S3. QCM-D frequency (a) and dissipation (b) changes for the adsorption of proteins on PEI coated PDMS thin films.

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