Letter

Biodegradation resistant multilayers coated with gold nanoparticles. Towards tailor-made artificial extracellular matrix

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Biodegradation resistant multilayers coated with gold nanoparticles. Towards tailor-made artificial extracellular matrix

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Abstract: Polymer multicomponent coatings such as multilayers mimic extracellular matrix (ECM) that attracts significant attention for their use as functional supports for advanced cell culture and tissue engineering. Herein, biodegradation and molecular transport in hyaluronan/polylysine multilayers coated with gold nanoparticles was described. Nanoparticle

coating acts as semipermeable barrier that governs molecular transport into/from the multilayers and makes them biodegradation resistant. Model protein lysozyme (mimics of ECM soluble signals) diffuses in the multilayers as fast and slow diffusing populations existing in an equilibrium. Such composite system may have high potential to be exploited as degradation-resistant drug delivery platforms suitable for cell-based applications.

The extracellular matrix (ECM) provides not only a structural support for cellular growth, but also a biochemical milieu, which together define cell fate.[1] Artificial ECM is designed to mimic real ECM and to achieve cellular functions in laboratory designed materials similar as in nature. Artificial ECM consists of degradable supports made of natural or (semi)synthetic polymers which: i) recapitulate physical-chemical properties of real ECM, ii) host bioactive signaling molecules (e.g. hormones, cytokines, growth factors) to be presented to cells, and iii) possess tuned biodegradability.[2-3] The issues above are challenging for the fabrication of artificial ECM but are essential for successful applications in tissue engineering, diagnostics, and animal-free studies.

Introduced in the early nineties,[4] layer-by-layer deposition proofed to be extremely useful in the field of bio-functional coatings. It is powerful technique for the fabrication of polymer-based multilayer films with dimensions from nano to micro, adjusted composition, and tunable cellular response.[5-11] Control over cellular functions such as adhesion, differentiation, and proliferation,[12-15] along with reservoir properties for a variety of biomolecules such as proteins, growth factors, genes, and drugs[7, 16-20] makes these films an attractive platform for cell-based applications.

Despite the evident biological potential to employ the multilayers to recapitulate ECM,[3] the options to tune multilayer biodegradability are mostly limited to multilayer chemical

crosslinking[13, 21-22] that can significantly change multilayer properties. There is also a lack in understanding the mechanism of biomolecule-multilayer interaction that obviously defines biomolecule presentation/release from multilayers to cells and, as a result, cellular response.[23-24]

In this work a new approach for the design of artificial ECM with controlled biodegradation based on the coating of multilayers with gold nanoparticles (GNPs) is present. The multilayers were assembled from glycosaminoglycan hyaluronic acid (HA) and polyaminoacid poly-L-lysine (PLL). Molecular transport through the GNP-coated multilayers is investigated. The model protein lysozyme (Lys) has been loaded into multilayers as a mimic of ECM soluble signaling molecule. Protein mobility has been assessed by Fluorescence Recovery after Photobleaching (FRAP) to reveal protein availability to cellular receptors. The schematics in Figure 1A-C illustrates the multilayer biodegradation and the exchange of Lys in solution and in multilayers.

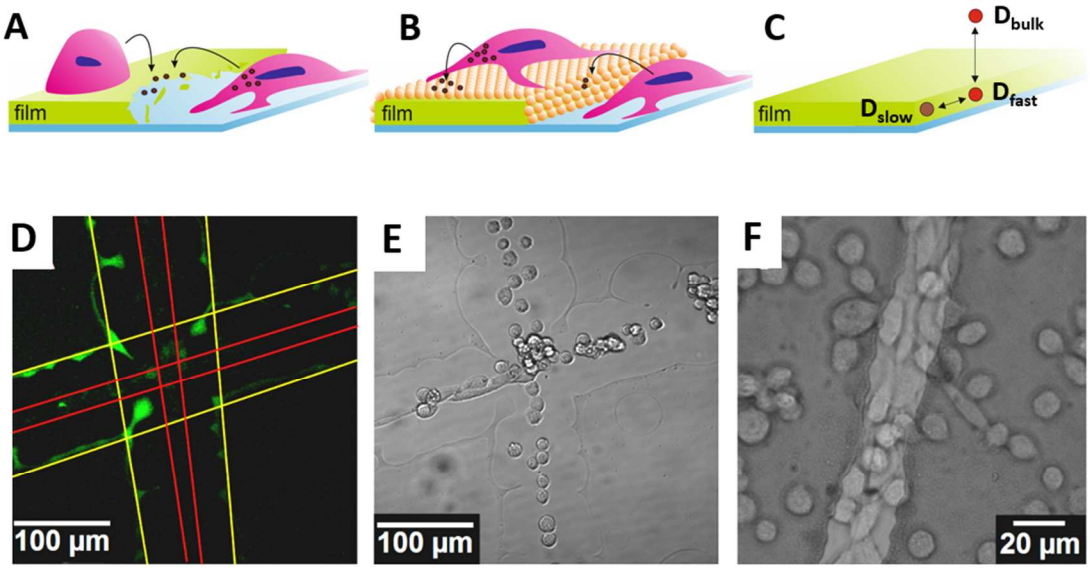

Figure 1. Schematics of cellular interaction with (A) bare and (B) GNP-coated $(HA/PLL)_{24}$ multilayers. The coating protects the multilayer from degradation by the cells adhering on it. (C) illustrates exchange of Lys (red cycles) between bulk and the multilayers where fast and slow diffusive populations are present. (D) CLSM fluorescence and (E, F) transmission images of

L929 fibroblasts cells cultured for three days on $(HA/PLL)_{24}$ and on (F) GNP-coated $(HA/PLL)_{24}$ multilayers ($\gamma = 3$). Red and yellow lines in (D) represent scratch boundaries before and after cell cultivation, respectively.

Firstly, cellular interaction with the $(HA/PLL)_{24}$ multilayers has been assessed. The multilayers have been scratched by a needle to form a visible scratched area of about 20 μm (Figure S1). This followed by seeding of L929 fibroblast cells which grow preferentially within the scratch (Figure 1D-E) due to soft nature of bare multilayers.[21, 25] The scratch width has significantly widened up to 100 μm demonstrating the enzymatic degradation potential of cells. The multilayers incubated without cells are stable, whereas those incubated with cells underwent considerable degradation, most probably due to hydrolytic enzymes secreted by cells (Figure 1, S1). Reduction of fluorescence in PLL-FITC containing multilayers is most likely caused by biodegradation (Figure S1).

Figure 1F shows a transmission image of the GNP-coated multilayers after 3 days of cell culture. The amount of GNPs corresponds to three theoretical monolayers of compactly packed spheres ($\gamma = 3$). Improved cellular adhesion (Figure 1F and Figure S2) compared to uncoated multilayers correlates well with our previous results showing that better adhesion of cells was attributed to multilayer stiffening induced through the presence of nanoparticles on the multilayer surface.[26-27] Hence, the coated multilayers become not only cell-adhesive but also resistant to biodegradation, as indicated by a constant width of the scratch during and after cellular growth (Figure 1F). One can assume that the coating acts as a barrier and prevents diffusion of cell-expressed enzymes into the multilayers and, as a result, suppresses multilayer biodegradation.

To prove this assumption, transport of the molecules of different sizes and charges into the GNP-capped multilayers has been studied. A small dye 5(6)-carboxyfluorescein (376 Da, CF), large positively charged PLL-FITC (15-30 kDa), and dextrans-FITC of various molecular masses (10-500 kDa) have been employed as probes. The level of GNP coverage (GNPs covers only the multilayer surface) has been varied by adjusting the number of theoretical GNP monolayers (γ = 1.5 or 3).[26] The diffusion of CF into the film occurs almost instantaneously, regardless of γ (Figure S3A). However, the situation is essentially different for larger PLL-FITC. While PLL-FITC saturates the bare films within 20-30 min, its diffusion is slowed down for the coated films (γ = 1.5) and totally blocked if γ = 3 (Figure 2A and Figure S3B). The fact that the diffusion of PLL-FITC is not significantly reduced for γ = 1.5 compared to that at γ = 1.5 may be related to defects in the GNP coating. The coating is not ideally homogeneous as proven by microscopy analysis. GNPs form clusters of several hundred nanometers and significant reinforcement of mechanical properties of the GNP coated $(HA/PLL)_{24}$ multilayers takes place at γ more than 1.5 when the clusters are well interconnected.[26]

One can assume that the GNP coating provides a kind of size-exclusion semipermeable barrier that does not possess diffusion limitations for small molecules. Diffusion of macromolecules is significantly reduced and depends on γ. However, molecular transport into the coated multilayers can not only be driven by the size of diffusing molecules but also by the interactions between the probe and multilayers. In the case of charged molecules (CF, PLL) electrostatics may play a significant role because of a large number of both positive and negative uncompensated charges in multilayers.[23] Negative charge on citric acid stabilized nanoparticles may thus also play a role.

Therefore, uncharged molecules - dextrans - of various molecular masses (10, 70, and 500 kDa) have been employed to evaluate the impact of molecular mass to permeability. Figure 2B

shows that the cut-off of the uncoated films is between 70 and 500 kDa. Diffusion of 500 kDa dextran is fully hindered and 10 and 70 kDa dextrans can rather easily penetrate into the film, reaching saturation levels after 30 min of incubation (Figure 2B). A similar time is necessary to saturate the film with charged PLL (Figure 2A). This allows one to assume that the mesh size of the polymer network of the film plays the pivotal role in diffusion of macromolecules. In the case of the GNP-coated multilayers (γ = 3) the dextrans do not diffuse into the films at all (Figure 2C). Systematic analysis of molecular diffusion through the GNP coating may allow adjusting the diffusion rate of a molecule of chose. Altogether, the coating can effectively suppress the degradation of the multilayers by preventing the diffusion of rather large enzymes such as hyaluronidase (Mw ~ 54 kDa) and proteases (Mw are usually few tens of kDa). At the same time, transport of smaller protein-based biomolecules such as growth factors into/from the multilayers could still be possible and can also be adjusted by the γ.

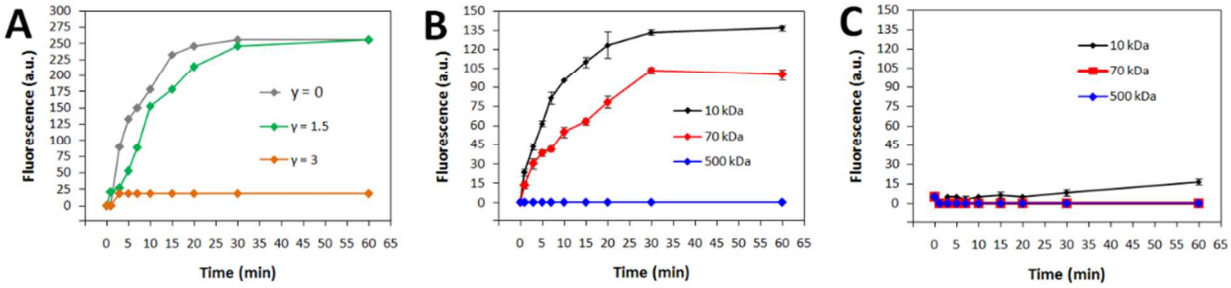

Figure 2. (A) Relative fluorescence of bare and GNP-coated $(HA/PLL)_{24}$ multilayers in the presence of PLL-FITC; the coating at γ=1.5 or γ=3. (B) Relative fluorescence of the bare and (C) coated multilayers (γ = 3) in the presence of 10, 70, and 500 kDa dextran-FITC.

The semipermeable nature and potential to adjust molecular transport into/from the multilayers make the coating attractive for the design of biomaterials with controlled degradability and controlled presentation/release of the loaded bio-molecules. The $(HA/PLL)_{24}$multilayers can host

bio-macromolecules such as proteins which are rather mobile in the multilayers. Diffusion coefficient (*D*) of proteins papain, lysozyme, and catalase were found to be about 2-4 $\mu m^2/s$[23] that shows the multilayers as good mimics of ECM in terms of mobility of proteins/growth factors. The mechanism of protein loading/release into/from multilayers is still not clear showing complex character of protein-multilayer interaction because of no systematic effect of protein charge and size.[23]

For the reasons given above, the interaction of the model protein lysozyme with the multilayers has further been examined. Lys-FITC has been loaded into the multilayers, washed out with Tris buffer and further with cell culture medium with systematic assessment of the *D* of Lys and protein fluorescence in the multilayers. *D* of Lys in the multilayers has been evaluated by FRAP according to the procedure described elsewhere, which is based on the analytical solution of Fick's law of diffusion.[23, 28-29] The protein-loaded multilayers have been bleached within a predefined area (marked with a yellow rectangle in Figure 3A) and the averaged 2D fluorescence profile has been taken perpendicular to the bleached line. During the time course the bleached and non-bleached Lys-FITC molecules exchange in the multilayers, resulting in the widening of the fluorescence profile (profile width *w*) and reduction of the depth of the profile (*A*) (Figure 3B and 3C). The intensity of fluorescence $I(x,t)$ across the bleached region has been approximated by the Gaussian function and is described by Equation 1:

$$I(x,t) = I_0 - A(t) \cdot \exp\left(-\frac{x^2}{2w^2}\right) \qquad \text{(Equation 1)}$$

where I_0 is the intensity of fluorescence before bleaching; $A(t)$ is the depth of dip after time t following the bleaching; x is the spatial distance from the minimum of the dip and w describes the half width of the Gaussian curve at the inflexion point.

High level of consistency of the experimental data fitted by the Equation 1 allows D to be determined reliably from the slope of linear regression of w^2 plotted versus t (Figure 3D). The mean D of the protein loaded into the film has been found to be 1.2 ± 0.1 $\mu m^2/s$ (in cell culture medium). Further two protein populations have been revealed in the multilayers (for details see Experimental part). The time evolution of the dip depth A was meant to test if a complete fluorescence recovery takes place, which would be indicative for a single protein diffusing population. Extrapolation of the initial kinetics of the changes of A indicates that A approaches a constant value (Figure 3E) which represents the fraction of protein molecules (about 10 % of all protein) that diffuse much slower compared to the main protein population in the multilayers. Thus, one can identify two populations of the protein molecules in the multilayers: Fast diffusing (major fraction, 90%, D_{fast}) and slow diffusing proteins (minor fraction, 10%, D_{slow}). Both D_{fast} and D_{slow} contribute to the mean D measured with $D_{fast} >> D_{slow}$. The slow diffusing fraction is approximated as immobile; diffusion slower than 0.01 $\mu m^2/s$ is undistinguishable from immobile behaviour because of spatiotemporal resolution of the experiment. Due to the small content of slow diffusing protein with D_{slow}, its contribution can be considered as minor, thus assuming that $D_{fast} \approx 1.0$ $\mu m^2/s$. This value indicates rather fast protein diffusion and differs only by two orders of magnitude to protein diffusion in solution (D_{bulk}) which is approximately 100 $\mu m^2/s$, according to Stokes-Einstein equation and reported literature data.[23] Figure 1C depicts exchange of Lys molecules between bulk and the film where fast and slow diffusive populations are present.

To understand if equilibrium between the diffusing populations is established, both the protein content in the film reflected by Lys-FITC fluorescence intensity and the content of immobile protein fraction have been considered during the protein loading and after sequential washing out

with Tris-buffer and further with cell culture medium (Figure 3F). It is of note that cell culture medium does not affect Lys-FITC fluorescence (data not shown) meaning that the change in fluorescence indicates loss of protein washed out of the multilayers. After washing with buffer and finally with cell culture medium, the amount of protein in the multilayer film has been significantly reduced down to 11 ± 2 % compared to the initial protein content. At the same time, the content of the slow diffusing fraction is not enhanced and did not exceed 10% of the total protein (Figure 3F). This means that equilibrium is established between slow and fast protein fractions in the films within the time scale of tens of minutes (washing time).

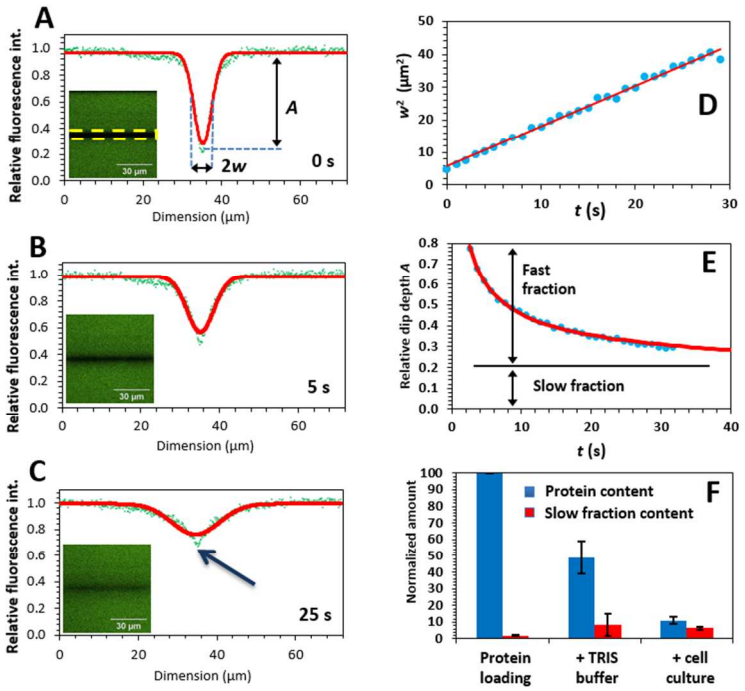

Figure 3. (A-C) - The recovery profiles at various time points (0, 5, and 25 s) after bleaching of $(HA/PLL)_{24}$ multilayers loaded with Lys-FITC (fluorescence images are as insets, the bleached area is marked by the dashed yellow rectangle). The experimental data are in green and the fitting curve in red; the fitting is performed using the Eq. 1. Gaussian width of the profiles (w) and the relative dip depth (A) have been determined as shown in (A). The blue arrow in (C) points to a dip in the recovery curve that indicates a protein fraction diffusing much slower than

the majority of protein molecules in the film. (D) - w^2 versus recovery time t after bleaching, (E) - time dependence of relative dip depth A after bleaching plotted versus recovery time t, (F) - normalized fluorescence of multilayers loaded with Lys-FITC (in blue) and content of slow diffusing protein fraction (in red) after the protein loading followed by washing with TRIS and further with cell culture medium.

The presence of two protein populations in the film with significantly different mobility may be attributed either to i) the presence of two types of protein binding sites in the multilayers or ii) the formation of protein aggregates which are significantly larger than single protein molecules and thus diffuse much slower. The increase of the slow diffusing fraction with time during protein loading into the film[30] suggests that the slow diffusing fraction exists due to inter-protein interactions in the multilayers (protein aggregation). The presence of different protein binding sites would result in preferential protein binding to the site with stronger affinity and as a result reduction of the amount of slow diffusing proteins during the protein loading. The findings of this study stimulate further investigation, which we plan in the future in order to better understand the interactions between proteins and multilayers and tune multilayer biodegradation rate.

In conclusion, the coating of biopolymer-based $(HA/PLL)_{24}$ multilayers with GNPs results in the formation of a size-exclusion semipermeable membrane on the multilayer surface with a cut-off tuned by GNP density. The GNP coating significantly improves cellular adhesion and protect the multilayers from biodegradation Analysis of protein (Lys) diffusion in the multilayers has revealed that the loaded protein molecules are present in two populations with high ($D \approx 1.0$ $\mu m^2/s$) and low ($D_{slow} < 0.01$ $\mu m^2/s$) mobility. The populations are in equilibrium with each other and the fast diffusing population dominates, representing about 90% of the loaded protein. The

slow diffusing population is likely a result of protein aggregation. High mobility of the proteins in the multilayers shows high promise to use the multilayers as hosts to present the loaded bioactive molecules (e.g. growth factors, cytokines, hormones) to cell receptors. Finally, this study demonstrates that GNP-coated $(HA/PLL)_{24}$ multilayers can potentially be exploited as degradation-resistant drug delivery platforms suitable for cell culture and tissue engineering.

Supporting Information The supporting information includes experimental section, multilayers stability tests, cellular adhesion to and kinetics of loading of CF and PLL-FITC into bare and GNP-coated multilayers. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡Vladimir Z. Prokopović and Anna S. Vikulina contributed equally to this work.

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REFERENCES

1. Lutolf, M. P.; Hubbell, J. A. Synthetic Biomaterials as Instructive Extracellular Microenvironments for Morphogenesis in Tissue Engineering. Nat Biotech 2005, 23 (1), 47-55.

2. Kim, B.-S.; Park, I.-K.; Hoshiba, T.; Jiang, H.-L.; Choi, Y.-J.; Akaike, T.; Cho, C.-S. Design of Artificial Extracellular Matrices for Tissue Engineering. Prog. Polym. Sci. 2011, 36 (2), 238-268.

3. Guillame-Gentil, O.; Semenov, O.; Roca, A. S.; Groth, T.; Zahn, R.; Voros, J.; Zenobi-Wong, M. Engineering the Extracellular Environment: Strategies for Building 2D and 3D Cellular Structures. Adv. Mater. 2010, 22 (48), 5443-5462.

4. Decher, G.; Hong, J.-D. Buildup of Ultrathin Multilayer Films by a Self-Assembly Process: I. Consecutive Adsorption of Anionic and Cationic Bipolar Amphiphiles. Makromol. Chem. 1991, 46, 321.

5. Gribova, V.; Auzely-Velty, R.; Picart, C. Polyelectrolyte Multilayer Assemblies on Materials Surfaces: From Cell Adhesion to Tissue Engineering. Chem. Mat. 2011, 24 (5), 854-869.

6. Lavalle, P.; Voegel, J. C.; Vautier, D.; Senger, B.; Schaaf, P.; Ball, V. Dynamic Aspects of Films Prepared by a Sequential Deposition of Species: Perspectives for Smart and Responsive Materials. Adv. Mater. 2011, 23 (10), 1191-1221.

7. Pavlukhina, S.; Sukhishvili, S. Polymer Assemblies for Controlled Delivery of Bioactive Molecules from Surfaces. Adv. Drug Deliv. Rev. 2011, 63 (9), 822-836.

8. de Villiers, M. M.; Otto, D. P.; Strydom, S. J.; Lvov, Y. M. Introduction to Nanocoatings Produced by Layer-by-layer (LbL) Self-assembly. Adv. Drug Deliv. Rev. 2011, 63 (9), 701-715.

9. Ariga, K.; Lvov, Y. M.; Kawakami, K.; Ji, Q. M.; Hill, J. P. Layer-by-Layer Self-assembled Shells for Drug Delivery. Adv. Drug Deliv. Rev. 2011**,** 63 (9), 762-771.

10. Ariga, K.; Yamauchi, Y.; Rydzek, G.; Ji, Q. M.; Yonamine, Y.; Wu, K. C. W.; Hill, J. P. Layer-by-layer Nanoarchitectonics: Invention, Innovation, and Evolution. Chem. Lett. 2014**,** 43 (1), 36-68.

11. Gentile, P.; Carmagnola, I.; Nardo, T.; Chiono, V. Layer-by-layer Assembly for Biomedical Applications in the Last Decade. Nanotechnology 2015**,** 26 (42).

12. Richert, L.; Lavalle, P.; Payan, E.; Shu, X. Z.; Prestwich, G. D.; Stoltz, J.-F.; Schaaf, P.; Voegel, J.-C.; Picart, C. Layer by Layer Buildup of Polysaccharide Films: Physical Chemistry and Cellular Adhesion Aspects. Langmuir 2004**,** 20, 448-458.

13. Boudou, T.; Crouzier, T.; Ren, K.; Blin, G.; Picart, C. Multiple Functionalities of Polyelectrolyte Multilayer Films: New Biomedical Applications. Adv. Mater. 2010**,** 22 (4), 441-467.

14. Crouzier, T.; Fourel, L.; Boudou, T.; Albiges-Rizo, C.; Picart, C. Presentation of BMP-2 from a Soft Biopolymeric Film Unveils its Activity on Cell Adhesion and Migration. Adv. Mater. 2011**,** 23 (12), H111-H118.

15. Muzzio, N. E.; Pasquale, M. A.; Gregurec, D.; Diamanti, E.; Kosutic, M.; Azzaroni, O.; Moya, S. E. Polyelectrolytes Multilayers to Modulate Cell Adhesion: A Study of the Influence of Film Composition and Polyelectrolyte Interdigitation on the Adhesion of the A549 Cell Line. Macromol. Biosci. 2016**,** 16 (4), 482-495.

16. Prokopović, V. Z.; Duschl, C.; Volodkin, D. V. Hyaluronic Acid/Poly-l-Lysine Multilayers as Reservoirs for Storage and Release of Small Charged Molecules. Macromol. Biosci. 2014**,** 15 (10), 1357-1363.

17. Volodkin, D.; Skirtach, A.; Mohwald, H., LbL Films as Reservoirs for Bioactive Molecules. In *Bioactive Surfaces*, Borner, H. G.; Lutz, J. F., Eds. Springer-Verlag Berlin: Berlin, **2011**, pp 135-161.

18. Jewell, C. M.; Lynn, D. M. Multilayered Polyelectrolyte Assemblies as Platforms for the Delivery of DNA and other Nucleic Acid-based Therapeutics. Adv. Drug Deliv. Rev. 2008**,** 60 (9), 979-999.

19. Hirano, Y.; Mooney, D. J. Peptide and Protein Presenting Materials for Tissue Engineering. Adv. Mater. 2004**,** 16 (1), 17-25.

20. Chuang, H. F.; Smith, R. C.; Hammond, P. T. Polyelectrolyte Multilayers for Tunable Release of Antibiotics. Biomacromolecules 2008**,** 9 (6), 1660-1668.

21. Richert, L.; Boulmedais, F.; Lavalle, P.; Mutterer, J.; Ferreux, E.; Decher, G.; Schaaf, P.; Voegel, J.-C.; Picart, C. Improvement of Stability and Cell Adhesion Properties of Polyelectrolyte Multilayer Films by Chemical Cross-Linking. Biomacromolecules 2003**,** 5 (2), 284-294.

22. Schneider, A.; Vodouhê, C.; Richert, L.; Francius, G.; Le Guen, E.; Schaaf, P.; Voegel, J.-C.; Frisch, B.; Picart, C. Multifunctional Polyelectrolyte Multilayer Films: Combining Mechanical Resistance, Biodegradability, and Bioactivity. Biomacromolecules 2007**,** 8, 139-145.

23. Uhlig, K.; Madaboosi, N.; Schmidt, S.; Jager, M. S.; Rose, J.; Duschl, C.; Volodkin, D. V. 3d localization and Diffusion of Proteins in Polyelectrolyte Multilayers. Soft Matter 2012**,** 8 (47), 11786-11789.

24. Vogt, C.; Ball, V.; Mutterer, J.; Schaaf, P.; Voegel, J. C.; Senger, B.; Lavalle, P. Mobility of Proteins in Highly Hydrated Polyelectrolyte Multilayer Films. J. Phys. Chem. B 2012**,** 116 (17), 5269-5278.

25. Üzüm, C.; Hellweg, J.; Madaboosi, N.; Volodkin, D. V.; von Klitzing, R. Growth Behaviour and Mechanical Properties of PLL/HA Multilayer Films Studied by AFM. Beilstein J. Nanotechnol. 2012**,** 3, 778-788.

26. Schmidt, S.; Madaboosi, N.; Uhlig, K.; Köhler, D.; Skirtach, A.; Duschl, C.; Möhwald, H.; Volodkin, D. V. Control of Cell Adhesion by Mechanical Reinforcement of Soft Polyelectrolyte Films with Nanoparticles. Langmuir 2012**,** 28 (18), 7249-7257.

27. Prokopović, V. Z.; Duschl, C.; Volodkin, D. V. Hyaluronic Acid/Poly-L-lysine Multilayers Coated with Gold Nanoparticles: Cellular Response and Permeability Study. Polymer. Adv. Tech. 2014**,** (25), 1342-1348.

28. Sustr, D.; Duschl, C.; Volodkin, D. A FRAP-based Evaluation of Protein Diffusion in Polyelectrolyte Multilayers. Eur. Polym. J. 2015**,** 68, 665-670.

29. Seiffert, S.; Oppermann, W. Systematic Evaluation of FRAP Experiments Performed in a Confocal Laser Scanning Microscope. J. Microsc.-Oxf. 2005**,** 220, 20-30.

30. Velk, N.; Uhlig, K.; Duschl, C.; Volodkin, D. Mobility of Lysozyme in Poly(L-lysine)/Hyaluronic Acid Multilayer Films. Colloids Surfaces B: Biointerfaces 2016**,** 147, 343-350.

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