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ARTICLE

Synthetic sialylglycopolymer receptor for virus detection using cantilever-based sensors

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We describe the rapid, label-free detection of Influenza A viruses using a cantilever transducer modified with a synthetic sialylglycopolymer receptor layer. Surface stresses induced by viruses binding to the receptor layer were used as the analytical signal. The synthetic sialylglycopolymer receptor layer can be used in nanoscale strain-gauge cantilever transducers for highly sensitive virus detection. Strain-gage transducers using such sensor layers exhibit long lifetimes, high sensitivities, and possible regeneration. Nanomechanical cantilever systems using optical detectors were used for the surface stress measurements. We demonstrated the positive, label-free detection of Influenza A at concentrations below 10^6 viruses per ml. In contrast to hemagglutination assays, cantilever sensors are label free, in situ, and rapid (less than 30 min), and they require minimal or nearly no sample preparation.

Introduction

The flu is a worldwide problem for people infected with the influenza viruses. The three major types of influenza are influenza A, B, and C. Influenza A commonly circulates among birds and mammals, often causing death, whereas influenza B mainly occurs in humans. While uncommon, influenza C can also cause illness [1]. Early detection of influenza is the most effective way to protect from the flu. The most convenient method of influenza virus detection in clinical practice – hemagglutination assay has sensitivity in the range of 10^6 pfu/ml [2]. This assay takes ~1–2 hours to complete and results can differ widely based on the technical expertise of the operator. It relies on the fact that hemagglutinin, a surface protein of influenza viruses, agglutinates red blood cells and requires fresh erythrocyte suspension. Herein, we propose using a synthetic sialylglycopolymer receptor layer on a cantilever transducer as a highly sensitive virus detector. The hemagglutinin molecule is responsible for viral attachment to cell-surface sialylglycolipids or sialylglycoproteins [3]. Due to erythrocyte surface membrane has sialyl residues red blood cells can react with Influenza viruses and cause hemagglutination. Nowadays the hemagglutination assay is a common method for virus detection was invented in 1941–42 by American virologist George Hirst [3]. Suzuki et al. [4] reported that the hemagglutinins of the influenza A, B, and C viruses exhibited strict and different specificities for the sialyloligosaccharide sequences that serve as their receptor

determinants on erythrocytes. Sialyloligosaccharides bearing terminal Sia(α2-3)Gal- and Sia(α2-6)Gal-moieties exhibit different molecular conformations, which exhibit various affinities to different Influenza virions. Human and avian influenza viruses are particularly selective. Generally, human viruses preferentially binding to Sia(α2-6)Gal-terminated receptors while avian viruses prefer Sia(α2-3)Gal-containing ones [5]. The third saccharide could also affect the receptor affinity for different influenza viruses [6]. However, all virus species from different hosts could bind different sialylglycan receptors to a varying degree; therefore, determining the exact specificity of our receptor is impossible. We could only determine the different affinities for different virus and host species, which has no clear relation to the surface glycoprotein compositions (hemagglutinin and neuraminidase) as shown in [7]. Nevertheless, this trait also provides a universal receptor for several types of viruses via the combination of different receptors into one polymer matrix. We propose using oligosaccharides specific to Influenza A viruses as the receptor sequence. We propose using synthetic sialyloligosaccharide sequences immobilized in a polyacrilamide matrix to extend the lifetime beyond the commonly used biological antibody natural receptors. Using a polymer matrix as the carrier for biologically active carbohydrate ligands is preferable due to its inertness and stability [8, 9, and 10]. The oligosaccharide stability does not affect the overall receptor stability [11, 12].

Studies using microcantilevers for molecular sensing were first reported 20 years ago at IBM and the University of Basel [13]. Currently, numerous publications describe potentially using cantilever transducers for highly sensitive chemical [14, 15], gravimetric [16] and biological sensing [17, 18 and 19].

The cantilever sensor sensitivity depends on the surface stress arising from the sensor layer binding to specific target molecules. To increase sensor response, receptor molecules

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can be incorporated into the matrix layer. In such a case, the surface stress increases due to the improved interactions of complexes with not only each other but also the matrix molecules. We propose using polyacrylamide as a matrix.

Another factor affecting cantilever sensor operation is nonspecific binding, which may either yield false signals or decrease the true signal value. To prevent nonspecific binding, all adsorption centres on the cantilever surface except the specific receptor molecules should be blocked. A blocking function could use matrix molecules or another substance. Therefore the sensor layer should consist of a matrix layer with integrated receptor molecules to specifically bind the target molecules. Herein, we propose a new synthetic sensor layer based on oligosaccharide sequences linked to a polymer matrix. This receptor layer is considered for strain-gauge transducer sensing of the Influenza virus. Such strain-gauge sensors for virus detection have a potential for miniaturization and implementation for home appliance devices for environment monitoring.

Materials and methods

Viruses

Influenza A virus A/Duck/Moscow/4182/2008 (M.P. Chumakov Institute of Poliomyelitis and Viral Encephalitis RAS, Moscow) with the H5N3 surface glycoprotein composition was used as our investigation target. The virus stock was propagated in the allantoic cavity of 10-day-old embryonated specific pathogen-free chicken eggs. Allantoic liquid is a biological media containing a complicated protein mixture with total concentration of approximately 2 mg/ml [20], which provides a good background for determining the sensor selectivity. Virus-free allantoic liquid from non-infected embryos was used to prepare samples with different virus concentrations. The initial virus concentration was ~10^8 virions/ml. Hemmaglutination assay and TEM were used to check virus activity and concentration of a sample right before the experiment. Several sample solutions with concentrations of 10^8, 10^7, 10^6 and 0 virions/ml were prepared by diluting the initial virus solution with allantoic liquid.

Receptor

Synthetic sialylglycoconjugates based on a polymer matrix (3'SL-PAA) were used as the virus receptor in a cantilever sensor (Figure 1). The structure of such a polymer-based receptor is similar to sialylglycoprotein fetuin containing three N-linked and three O-linked sialylated glycan side chains that biospecifically bind hemagglutinin proteins on the influenza viruses. High molecular weight polyacrylamide conjugates
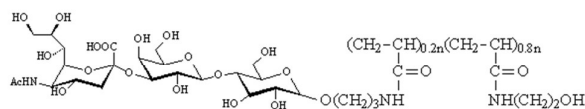
containing Neu5Ac2-3Gal1-4Glc (3'SL) were synthesized by coupling statistically spaced oligosaccharides to poly(N-oxisuccinimidyl acrylate) as previously described [21, 22, 23 and 24]. The average molecular mass for the poly(N-2-hydroxyethylacrylamide) carrier was approximately 1500 kDa based on gel-permeation chromatography. The sialosaccharide content in the polymer was 0.76 nmol/mg of polymer.

Receptor immobilization. Preparing the sensor and reference cantilevers.

3'SL-PAA is a water-soluble polymer that can be easily immobilized on an aminated surface via the sialic acids carboxylic groups. Gold coated Arrow™ TL1Au (NanoWorld, Switzerland) cantilevers were used in all experiments. Two cantilevers were required for each experiment: one sensing cantilever and another as reference. The surface was cleaned using piranha solution (H_2SO_4 x H_2O_2) before each experiment. The piranha solution was prepared immediately before the cleaning procedure by adding concentrated H_2SO_4 to 37% H_2O_2. The cantilevers were incubated in a piranha solution for 10 min, followed by washing with Milli-Q water and absolute ethanol several times. The receptor layer was immobilized on the silicon side of the cantilever. The silicon surface was modified with 3-aminopropyltrimethoxysilane (APTMS). The stability of immobilized APTMS on silicon or glass surfaces is why industry uses this reagent to provide superior bonding between inorganic substrates and organic polymers for a broad range of applications [25]. Both cantilevers were incubated 1 h in a 10% APTMS solution in ethanol. The cantilevers were washed with absolute ethanol and Milli-Q water several times after modifying. The sensor cantilever was immersed in a 10^{-3} M 3'SL-PAA water solution for 16 h. The amide bond formation is slow, which is why we incubated the cantilever overnight in the 3'SL-PAA water solution. Some receptors should be deactivated after the adsorption; furthermore, numerous active receptors in the polymer structure do not interact with the surface. The sensor cantilever was washed with Milli-Q water after the 3'SL-PAA immobilization and immersed in an aqueous BSA solution (1 mg/ml) for 16 h. The reference cantilever was incubated in an aqueous BSA solution (1 mg/ml) for 16 h. BSA was used to prevent non-specific adsorption to the sensor cantilever (a non-modified gold surface) and create a reference cantilever with similar mechanical properties to the sensing one. After this modification, both cantilevers were washed several times with Milli-Q water.

Set-up for surface stress measurements

The BioScan nanomechanical cantilever system (Biosensor Academy, Russia) measures the cantilever deflection induced by lateral intermolecular forces in the layer on one side of the cantilever. This system provides measurements with two cantilevers simultaneously. The reference cantilevers allows to consider the contribution of false signal caused by temperature fluctuations, non-specific adsorption and other environment factors. So the valid signal is a difference between sensor and reference cantilever deflection. The deflection value depends on the cantilever parameters and

Figure 1. Structure of polyacrylamide modified with Neu5Acα 2-3Gal β 1-4Glcβ (3'SL-PAA).

cannot be universal. Therefore, the independent characteristics of the lateral intermolecular forces must be used. Such characteristics include the surface stress, which relates to the cantilever deflection via Stoney's formula (1):

$$\Delta\sigma = \frac{E \cdot t^2}{3 \cdot (1-\vartheta) \cdot L^2} \Delta z \qquad (1)$$

where E and v are Young's modulus and Poisson's ratio of cantilever material, respectively, t and L are the cantilever thickness and length, respectively, and Δz is the cantilever free end deflection. Arrow™ TL1Au cantilevers are made from silicon (for silicon E=180 GPa and v=0.22 was used), and have 500±5 microns length and 1 micron thickness (0.5 - 2.5 µm range).

Herein, all BioScan nanomechanical cantilever system results are presented as a difference signal between sensor and reference cantilevers in terms of the surface stress.

Experiments using the BioScan cantilever system were performed with a continuous flow. A special setup was constructed to provide measurements during a flow (figure 2). A syringe pump (New Era Pump Systems, Inc., USA) with a 10-ml syringe was connected to the 6th port of a two position 6-way valve (Upchurch, USA), the BioScan cantilever system liquid chamber was connected to the 5th port, a 300 µl loop was connected to the 1st and 4th ports, the sample was injected through the 2nd port, and wastes exited the 3rd port (figure 2).

A syringe pump flow rate of one millilitre per hour was used for all virus detection experiments. A flow system containing the syringe, valve, loop and tubes was filled 10 times with PBS diluted from a 10X stock (800 mg NaCl, 20 mg KCl, 144 mg $Na_2HPO_4 \cdot 2H_2O$, 24 mg KH_2PO_4, 8 mL of distilled water).

The initial loop volume for PBS was 300 µl. The sample solution replaced PBS during injection when the valve was in position 1 (Figure 2). Switching the valve to position 2 caused PBS from the syringe pump to push the sample solution into the
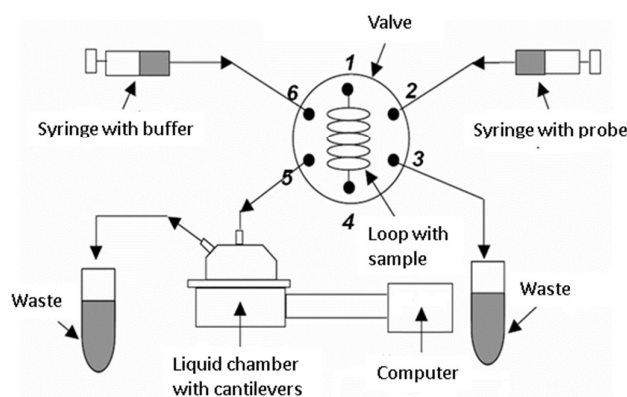

Figure 2. Setup scheme for the flow experiments. The sample injection loop is connected to ports 1 and 4 in a two position six-way valve. A syringe with a syringe pump is connected to port 6. The cantilever chamber is connected to port 5. Samples are injected into the loop through port 2. Port 3 is for wastes. The six way valve has two positions. Position 1 fills the loop with the sample solution. Position 2 moves the sample solution in the loop to the cantilever chamber.

measurement chamber. The time required for the sample to enter the measurement chamber was approximately 7 min. The target molecule concentration increased gradually, while sample solution displaced PBS from the chamber. The flow provided continuous influx of target molecules in a region near cantilever surface maintaining a constant concentration, which decreased the virus adsorption time, caused by diffusion; therefore, the surface stress began changing immediately after the sample solution reached the cantilever chamber.

Atomic Force Microscopy

Silicon surface was used as a substrate in AFM experiments. Receptor immobilization for silicon surface was made using the same procedures as for the cantilevers, but without immersing in BSA solution at the final stage. Multimode atomic force microscope Nanoscope-3a (Digital Instruments, USA) was used in our studies. For AFM scanning in solution we have used silicon nitride cantilevers NP-S1 (Veeco, USA) (both in contact and in tapping modes). Also we have used silicon cantilevers fpN11 (Lukin Research Institute of Physical Problems, Russia) and CSG10S (NT-MDT, Russia) for AFM measurements in air in contact and tapping modes correspondingly. Scratching experiments in contact mode were made by applying maximal force while acquiring relatively small (typically 500 nm) AFM image. After this, normal force (near disengagement on the AFM force curve) was restored. Scratching experiments in tapping mode were made by adjusting small setpoint values (about 20 % of the initial setpoint) while acquiring relatively small (typically 500 nm) AFM image. After this, normal setpoint value was restored. AFM images typically consisted 512×512 pixels obtained with the frequency 2.1 Hz (for tapping mode images) and 5 Hz (for contact mode images). Image processing was performed using FemtoScan Online software (Advanced Technologies Center, Russia).

Transmission electron microscopy (TEM)

Dimensions of virions where characterized using transmission electron microscopy LEO 912 AB (Zeiss, Germany).

Results and Discussions

3'SL-PAA polymer immobilized on a cantilever surface was chosen as the virus detection sensor layer. The main advantages of such a layer are the long lifetime from its synthetic origin [21, 22, 23 and 24] and regeneration during continuous nanomechanical cantilever sensor measurements using surface stress changes as the analytical signal. Sialyloligosaccharides are not widely used as biosensor receptors; however, there is industrial interest in using such compounds for industrial-scale sensor production [26]. The main problem with using sialyloligosaccharide receptor layers on cantilevers is the low level surface stress generated during biospecific binding. Herein, we propose using sialyloligosaccharides linked to a polymer base as the receptor layer for virus detection. Polyacrylamide conjugates bearing Neu5Ac α 2-3Gal β 1-4Glcβ can covalently attach to a silicon cantilever sensor modified with APTMS.
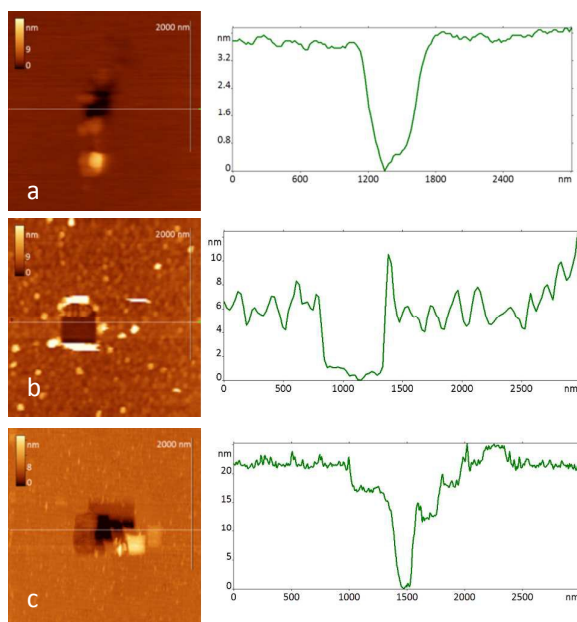
Figure 3. AFM height images of (a) APTMS modified silicon surface in air (contact mode), sialyloligosaccharide receptor layer on APTMS modified silicon surface (b) in air (contact mode) and (c) in water (tapping mode) after scratching experiments. Section analysis along the white line on the corresponding AFM image is shown on the right.

The formation of the receptor layer has been studied on the model silicon surface using AFM. Figure 3 demonstrates obtained in air AFM images after scratching of (a) APTMS modified silicon surface and (b-c) sialyloligosaccharide receptor layer on APTMS modified silicon surface. APTMS modified silicon surface is rather smooth (mean squared roughness, MSR, less than 0.5 nm) with depth about 3-4 nm (Figure 3a). In contrast, the receptor layer demonstrates coarse topography (with MSR exceeding 3 nm). The measurements of the total width of the receptor layer imaged in air gives 5-6 nm (not taking into account coarse topography, Figure 3b, which would add several nanometers more). At the same time, the apparent width of the receptor layer obtained from AFM images in solution is significantly bigger, about 20 nm (Figure 3c). This is probably connected with swelling of the polymer matrix in aqueous solution. Dimensions of virions where characterized with TEM (Figure 4). Diameters of particles were in average about 100 nm.

Such a receptor layer yields a variable surface stress for different concentrations due to the absorption of virions into the receptor layer based on the model described by Wentzel et al. [27]. The sensitivity and regeneration capabilities of the 3'SL-PAA receptor layer were investigated using a BioScan nanomechanical cantilever system, which can simultaneously measure the analytical signal from the sensor and reference cantilevers. A commercially available BioScan nanomechanical cantilever systems that uses standard AFM cantilevers was chosen to show the proof of concept for implementing 3'SL-PAA in strain-gauge sensing elements.
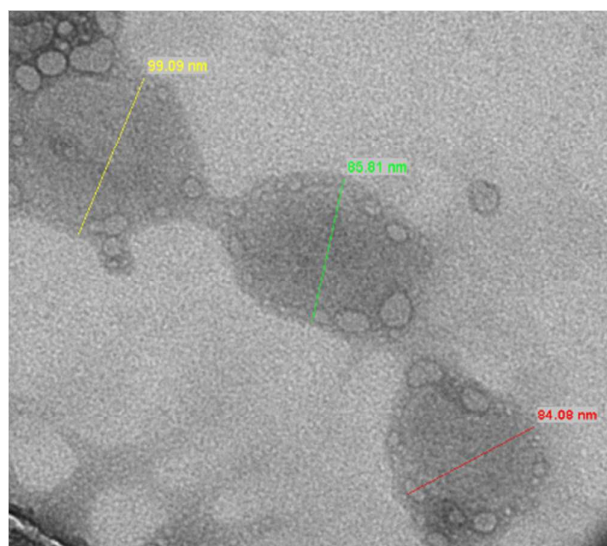

Figure 4. TEM image of Influenza A virus A/Duck/Moscow/4182/2008

Interactions between the target substance and receptor layer leads to attractive or repulsive lateral forces that deflect the cantilever in one direction or another [28]. In our case, the surface stress is positive for attractive intermolecular lateral forces in the receptor layer and negative for repulsive forces. Virion adsorption to the 3'SL-PAA receptor layer generate positive surface stresses (Figure 5). There are shown three independent measurements for different cantilevers for same concentration and surface functionalization on figure 5. Relative deviation of cantilever deflection caused by virus adsorption is less than 20% of average signal amplitude.

Sialyloligosaccharides specifically bind with hemagglutinin proteins on the virion capsid. Virions have about hundred hemagglutinins interacting with 3'SL-PAA macromolecules that appear as an attractive lateral intermolecular force in the receptor layer. Water being a good soluble agent for such polymers yields larger Gaussian ball polymer globule radii in
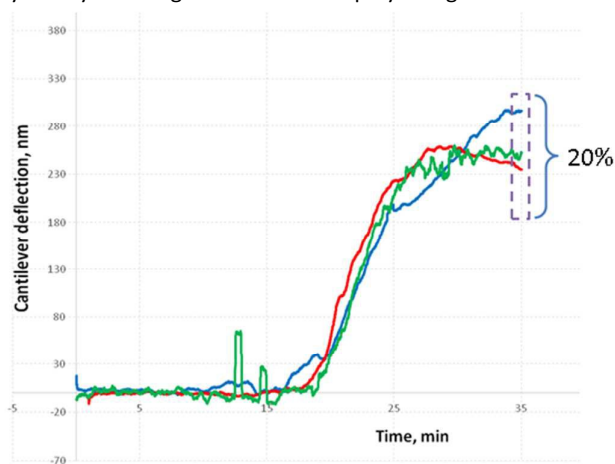

Figure 5. Three independent experimental results for various cantilevers deflection from time (green, blue and red curves) – measuring of the sorption kinetics of viruses (10^7 particles in ml) on cantilever surface.
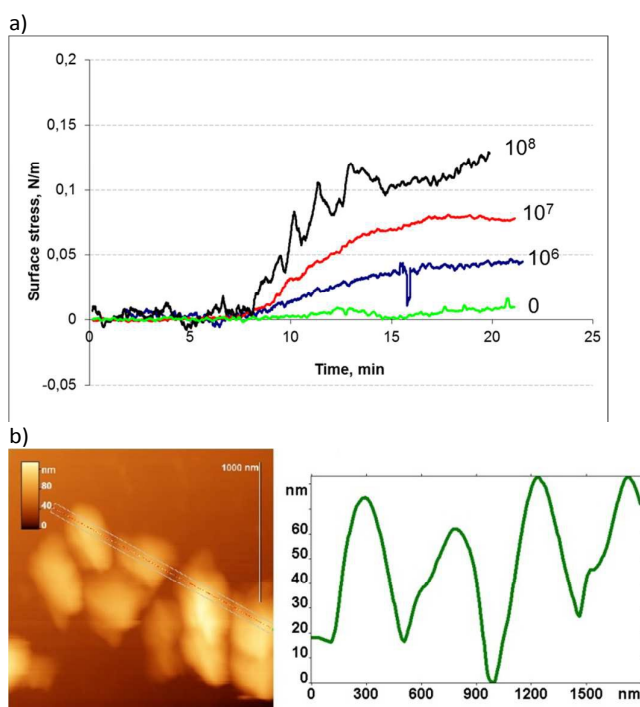
a)

b)

Figure 6. Duck virus detection using 3'SL-PAA receptor molecules. (a) Time dependence of the differential surface stress for different concentrations: black line – 10^8 viruses/ml, red line – 10^7 viruses/ml, blue line - 10^6 viruses/ml, green line – 0 viruses/ml. (b) Obtained in liquid tapping mode AFM image of the model receptor layer on silicon surface after its exposition to the duck virus suspension. Section analysis along the white line on the corresponding AFM image is shown.

solution. 3'SL-PAA being a water soluble polymer may provide some free space inside the layer. Decreasing of density of polymer layer was also confirmed by AFM data. Polymer adsorbed to the silicon surface is sufficiently viscous to absorb virions into the layer [27]. In this case, we assume that the virions act as a crosslinking centres that binds different receptor fragments linked to the polymer chains. Evidently, increasing the virions concentration in solution increases the virus particle absorption into the sensor layer. Each virion absorbed into the 3'SL-PAA layer becomes a cross-linker because of interchain interactions between sialyloligosaccharides on the polymer and hemagglutinin proteins on the capsid. Increase the virion number also increases both the sensor cross-linking density and the resultant positive surface stress.

We obtained meaningful differences between signals for concentrations of 0, 10^6, 10^7, and 10^8 virions/ml (Figure 6a). Steady-state deflections of surface stress as a function of virus concentration are presented at figure 7. Allantoic liquid was used as the zero probe. Allantoic liquid containing different proteins changed the receptor layer stress less than solutions containing the virus particles. We concluded the cantilever sensor using 3'SL-PAA receptors was highly specific to virions
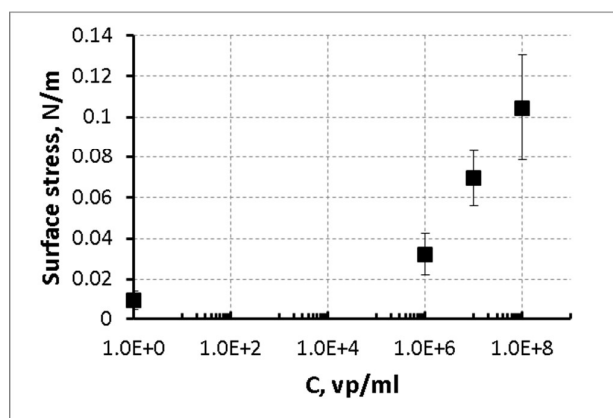

Figure 7. Concentration dependence of the surface stress for virus detection. Every data point on this plot represents an average of cantilever deflections obtained in multiple experiments done with different cantilevers, whereas the range of deflections obtained from these experiments is shown as the error bar

over other proteins. Binding of virus particles to the receptor layer was confirmed by their AFM visualization on model surface in water solution (Figure 6b). Viral particles can be distinguished by their height of ~100 nm, which substantially exceeds the roughness of the receptor surface.

Several standard methods for dissociating specifically bound substrates were considered for the regeneration procedure. NaOH (1 M and 0.01 M) and NaCl (10% in water) did not provide satisfactory results. Thermal treatment (incubation at 80-100 °C water) provided no regeneration as well.

Urea (carbamide) exhibited good regeneration results. The experiments were performed as described above. Sample solutions with 10^7 virions/ml were used for the regeneration experiments to test any lost receptor layer sensitivity. Figure 8 shows the data obtained for the cantilever sensor before (blue
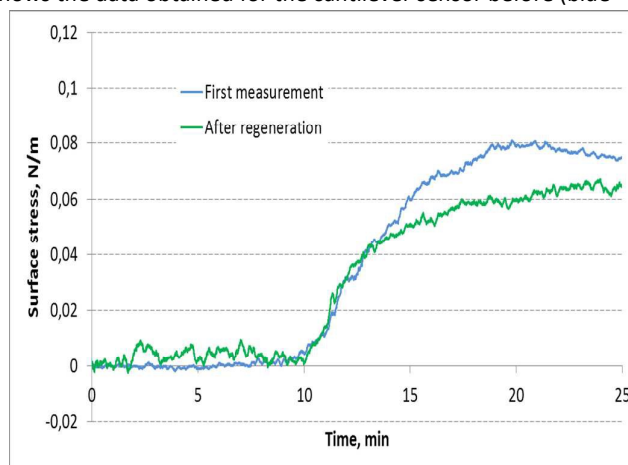

Figure 8. Regeneration of 3'SL-PAA receptor molecules for virus detection. Blue graph - injection a 10^7 virus/ml solution before regeneration. Green graph – second injection of the 10^7 virions/ml solution after regenerating with 10% urea water solution

line) and after (green line) regeneration. This regeneration involved incubating the sensor and reference cantilevers in a 10% urea water solution flow at 1 ml per h for 18 min. Due to the greater Van der Waals attraction of the protein for urea compared with water aqueous urea tends to destabilize folded protein structures [29]. Denaturation of capsid hemagglutinin proteins induces destruction of specific binding between 3'SL-PAA and viruses.

The signal intensity after regeneration was over 80% the initial target-receptor system measurements. We have repeated experiments with regeneration on different concentration for 10^7 virions/ml concentration (more than 3 times). Relative deviation of each measurement was less than 20 %. The baseline exhibited larger fluctuations after regeneration than before because the urea water solution must be replaced with PBS. When the urea solution was fully replaced in the probe, the noise level decreased. The fluctuation amplitude in the plateaus was equal for experiments before and after regeneration.

Conclusions

We demonstrated the possible usage of 3'SL-PAA as a receptor layer in strain-gauge sensors. The interaction between the virus particles and 3'SL-PAA layer induced attractive lateral intermolecular forces in layers covalently attached to the silicon side of a cantilever. We have obtained meaningful differences in signals at concentrations of 0, 10^6, 10^7, and 10^8 virions/ml and demonstrated the dependence of the surface stress on the virion concentration. 3'SL-PAA layer could be regenerated. The flow regeneration of the sensor by incubating it and the reference cantilevers in 10% urea water solution was above 80%.

Acknowledgements

References

1 Gopinath, S.C.B.; Tang T.-H.; Chen Y.; Citartan M.; Tominaga, J.; Lakshmipriya T. *Biosensors and Bioelectronics.* 2014, **61**, 357–369.

2 Killian, M.L., *Hemagglutination Assay for the Avian Influenza Virus. Avian Influenza Virus 436. Humana Press.* 2008, 47–52

3 Hirst, G.K. *Journal of Experimental Medicine*. 1942, **75**, 49–64

4 Suzuki, Y., Nakao, T., Ito, T., Watanabe, N., Toda, Y., Xu,G., Suzuki, T., Kobayashi, T., Kimura, Y., Yamada, A., Sugawara, K., Nishimura, H., Kitame, F., Nakamura, K., Deya, E., Kiso, M., & Hasegawa, A. *Virology.* 1992, **189**, 121-131

5 Yoshihiro Kawaoka. *Influenza Virology: Current Topics Caister Academic Press, United Kingdom*, 2006, 372.

6 Gambaryan, A.; Yamnikova, S.; Lvov, D.; Tuzikov, A.; Chinarev, A.; Pazynina, G.; Websterd, R.; Matrosovich M.; Bovin, N. *Virology*. 2005, **334**, 276-283.

7 Gambaryan, A.S.; Tuzikov, A.B.; Piskarev, V.E.; Yamnikova, S.S.; Lvov, D.K.; Robertson, J.S.; Bovin, N.V.; Matrosovich, M.N. *Virology*. 1997, **232 (2)**, 345-350.

8 Bovin, N. V. Polyacrylamide-based glycoconjugates as tools in glycobiology. *Glycoconj. J.*, 1998, **15 (5)**, 431-446.

9 Říhová, B.; Rathi, R.C.; Kopečková, P.; Kopeček, J. *Int. J. Pharmaceutics.* 1992, **87**, 105-116.

10 Dean, B.; Oguchi, H.; Cai, S.; Otsuji, E.; Tashiro, K.; Hakomori, S.; Toyokuni, T. *Carbohydr Res.* 1993, **245**, 175-92.

11 Slimestada, R.; Vågenb, I.M. *Journal of Chromatography A.* 2006, **1118 (2)**, 281–284.

12 Shallenberger, R.S.; Mattick, L.R. 1983**, 12 (3)**, 159–165.

13 Gimzewski, J.K.; Gerber, C.; Meyer, E.; Schlittler, R.R. Observation of a chemical-reaction using a micromechanical sensor. Chem. Phys. Lett. 1994, 217, 589 – 594.

14 Gorelkin, P.V.; Mukhin, D.S.; Majouga, A.G.; Romashkina, R.B.; Beloglazkina, E.K.; Yaminsky, I.V.; Zyk, N.V. Mendeleev commun. 2010, **20**, 329 – 331.

15 Beloglazkina E.K., Majouga A.G., Zyk N.V., Rakhimov R.D., Yaminsky I.V., Gorelkin P.V., Kiselev G.A., Kutateladze A.G. Thin solid films, 2007, **515 (11)**, 4649–4661.

16 Erofeev, A.S; Yaminsky, I.V. Determination of the Mass of Polymer Films Using an AFM Cantilever. Moscow University Physics Bulletin, 2011, 66 (3), 283–286.

17 Fritz, J.; Cantilever biosensors. Analyst, 2008,**133**, 855-863

18 Gorelkin, P.; Kiselev, G.; Mukhin, D.; Kim, T.; Kim, S.; Lee, S.; Yaminskii, I. *Polymer Science Series A.* 2010, **52 (10)**, 1023-1033.

19 Kolesov, D.V.; Kiselev, G.A.; Moiseev, M.A.; Kudrinskiy, A.A.; Yaminskiy, I.V. J. *Phys.: Conf. Ser.* 2012, **345**, 012042

20 Moors, A. *Ann. Soc. Belg. Med. Trop.* 1972; **52(1)**, 9-17

21 Tuzikov, A.; Gambaryan, A.; Juneja, L.; Bovin, N. *J. Carbohydr. Chem.* 2000, **19**, 1191–1200

22 Pazynina, G.; Tuzikov, A.; Chinarev, A.; Obukhova, P.; Bovin, N. *Tetrahedr. Lett.* 2002, **43**, 8011–8013.

23 Mochalova, L.; Gambaryan, A.; Romanova, J.; Tuzikov, A.; Chinarev, A.; Katinger, D.; Katinger, H.; Egorov, A.; Bovin, N. *Virology*. 2003, **313**, 473–480.

24 Shilova, N.V.; Galanina, O.E.; Pochechueva, T.V.; Chinarev, A.A.; Kadykov, V.A.; Tuzikov, A.B.; Bovin, N.V. *Glycoconj. J.* 2005, **22**, 43–51.

25 Wang, H.; Huang, Y.; Tan, Z.; Hu, X. *Analytica Chimica Acta*. 2004, **526**, 13–17

26 Ishihara Kei; Wada Yasuhiro; Toujima Eisuke Cantilever Sensor, Sensor System and Method of Detecting Analyte in Specimen Liquid WO2005JP19498 20051024

27 Wenzel, M.J.; Josse, F.; Heinrich, S.M.; Yaz, E.; Datskos, P.G. *J. Appl. Phys.* 2008, **103**, 064913-1–064913-11.

28 Godin, M.; Tabard-Cossa, V.; Miyahara, Y.; Monga, T.; Williams, P.J.; Beaulieu, L.Y.; Bruce Lennox, R.; Grutter, P. *Nanotechnology*. 2010, **21(7)**, 75501.

29 Rossky P. *Proc. Natl. Acad. Sci.* 2008, **105(44)**, 16825–16826.