FINAL REPORT

Name: Beatrix Péter

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Title: Biophysics of living cells and cell adhesive coatings exposed to novel compounds

(Újszerű hatóanyagokkal kezelt adhezív bevonatok és élő sejtek biofizikája)

I. Introduction and the major research question

Various plant tissues can be applied for medical applications because of their accumulation of special bioactive metabolites with diverse molecular structures. Natural products with their broad chemical diversity and bioactivity spectrum are sought after by the pharmaceutical industry, and they continue to provide new structures with promising effects and offer templates for the development of scaffolds of novel drug candidates.

My aim was to answer the question during the project period: Do natural compounds affect the adhesion matrix of living cells? If yes, how this affects the behavior of living cells? These are very relevant and important questions to clearly see the effects of natural compounds on living cells and tissues and to develop new drugs based on natural compounds. Therapeutics search for natural substances that are beneficial to human health, exerting also anti-inflammatory and anticancer (antiproliferative) effects. We propose that they are mediated by influencing cellular adhesion and migration via various signaling pathways and by directly inactivating key cell adhesion surface receptor sites. In general, the so-called classical labeling techniques are used to test their effect on cellular adhesion and migration. However, labels or dyes may disturb the samples. Furthermore, natural compounds usually have small molecular weights where labeling can be problematic or even impossible, especially if their binding pocket is small or embedded. Label-free biosensors are emerging tools to investigate the mode of action of small molecules as well. They eliminate all of the disadvantages of classical techniques.

In my measurements, I applied epigallocatechin-gallate (green tea polyphenol which has got proven anticancer activity), aryltetralin lignans isolated from inland plants (for example *A. sylvestris*) with yet unknown effects, and positively and negatively charged gold nanoparticles.

II. Summary of the results

II.1. Review the role of natural compounds in cellular adhesion and movement

As a starting point, I looked around in the literature in this topic. As a result, my review article was published open access with the title „Natural compounds as target biomolecules in cellular adhesion and migration: From biomolecular stimulation to label-free discovery and bioactivity-based isolation". In this review, we concluded by highlighting the possibilities of screening natural compounds faster and more easily by applying new label-free methods, which also enable a far greater degree of quantification than the conventional methods used hitherto. We have systematically classified recent studies regarding the effects of natural compounds on cellular adhesion and movement, characterizing the active substances according to their organismal origin (plants, animals or fungi). Finally, we also summarized the results of recent studies and experiments on SARS-CoV-2 treatments by natural extracts affect mainly the adhesion and entry of the virus (*Péter et al. Biomedicines, 2021*) [1].

II.2. The effect of green tea polyphenol on the cell adhesivity of fibronectin coatings

The main compound of green tea, epigallocatechin-gallate (EGCG) is probably the most studied polyphenol for decades. A lot of studies showed its beneficial effects on human health, for instance, its anticancer, anti-inflammatory, and anti-metastatic activities. These processes are in connection with cellular adhesion. Some experiments with cancer cell lines proved that this active substance effectively decreases adhesion to different extracellular matrix proteins like laminin, fibronectin, and collagen. These results highlight the potential anticancer effect of EGCG. As a continuation of my previous work (*Peter et al. Green tea polyphenol tailors cell adhesivity of RGD displaying surfaces: multicomponent models monitored optically, Scientific Reports, 2017*), I focused on studying the indirect effects of EGCG on living cells through an extracellular matrix component, fibronectin. Fibronectin is a high-molecular-weight (~500-~600 kDa) glycoprotein that binds to integrin receptors of the cells, thus, it plays a major role in cell adhesion, growth, migration, and differentiation.

The effects of EGCG-treated fibronectin coatings on HeLa (cancer) cell adhesion were investigated by using a multi-component model system, like in our previous study. We used a label-free, high-resolution technique (Epic BT, resonant waveguide grating biosensor with original fibronectin-coated Epic BT biosensor plates). The EGCG was dissolved in the assay buffer and diluted to different concentrations (0.05-500 μg/ml), and we used the oxidized form of EGCG and bare biosensor surface (without fibronectin coating) as well. Our results showed that EGCG and its oxidized form bind to fibronectin in a concentration-dependent manner and can form multilayers as well. Furthermore, both polyphenol forms inhibited cellular adhesion; however, the effect was more pronounced in the case of the oxidized form. The results were compared to the measurements performed on bare biosensor surfaces without fibronectin, and the roles of oxidation were investigated. It is suggested that the polyphenols can interact and

block important cell adhesion protein motifs and affect the rigidity of the layers as well. As a result, at 500 μg/ml EGCG, approximately 2 layers, while in the case of 500 μg/ml oxidized EGCG, 5 layers were formed on the fibronectin coating. On the bare surface, 2 and 3 layers were adsorbed, respectively. We proposed that the polyphenol molecules bound less between the fibronectin chains, and thus form fewer (approximately half) multilayers than in the case of synthetic copolymer coatings /PLL-*g*-PEG (poly(l-lysine)-graft-poly(ethylene glycol)) and PP:PPR (RGD (Arg-Gly-Asp) containing form of PLL-*g*-PEG)/. We suggest that at high concentrations the formed multilayers can effectively block RGD or PHSRN (or both) cell adhesion motifs, decreasing cell adhesion and spreading on the polyphenol-exposed protein films. Moreover, a novel molecular scale active mechanism involving the disulfide bridges of fibronectin was proposed to explain the recorded kinetic signals and highlight that these proteins can be active participants in the molecular scale transformations affecting adhesion. This modeling provided a plausible interpretation of the redox transformation mediated by fibronectin-linked thiol/disulfide residues.

The introduced method is capable of illuminating the most important features of EGCG-adhesion matrix interactions, highlighting the importance of ligand oxidation during cellular interactions. Our results have been published open access with the title of „Epigallocatechin-gallate tailors the cell adhesivity of fibronectin coatings in oxidation and concentration-dependent manner" (*Peter et al. Materials Advances, 2022*) [2].

II.3. The effect of other natural compounds on the cell adhesivity of fibronectin coatings

Preliminary experiments on the effects of other natural compounds (from fungi and plants) on various cell lines have been also investigated by using Epic BT biosensor. The method was the same as used in the II.2 chapter described above (fibronectin-coated biosensor plates, with or without washing out the molecules from the coating). The measurements have been performed on both cancer (HeLa) and healthy preosteoblast (MC3T3-E1) cell lines. We measured in total 18 (9+9 in two terms) active substances. In the first term, we found 3 of the 9 compounds which inhibited cancer cell adhesion and do not have any effects on healthy cells. MTT cell viability tests were performed at the Eötvös Lóránd University. During the second term, another 9 compounds (aryltetralin lignans) isolated from *A. sylvestris* were tested as well in the same cell lines and circumstances. The tested new active substances have an inhibitory effect on cell adhesion on the fibronectin surface. The compounds were tested on polystyrene surface (material of Petri dishes) by Holomonitor M4 holographic transmission microscopy as well to investigate how they affect cell migation, movement, and adhesion on a single cell level. We found that the active substances have an inhibitory effect on cell adhesion on the fibronectin, but they apparently have no significant effect on the cells that have already adhered to the polystyrene surface. The active substance molecules may bind to fibronectin, thus occupying its binding sites, however, further investigations are still necessary for proving this theory. Our aim with this study is to find and select from a lot of substances those compounds which are harmful for cancer cells but do not affect the healthy cells by a fast and relatively easy method. These selected compounds can be the cure for several illnesses in the near future after further experiments. The manuscript in this topic is under preparation, we may publish this huge work in 2023.

II.4. Binding affinity of compounds to human serum serum albumin protein

QCM measurements were originally planned in order to shed light on the effects of the compounds on the rigidity of the extracellular matrix and cells. During the project time, however, we decided that it would be more interesting and – perhaps more useful from the point of view of the drug industry and development – to map the interactions between the active substance and the transport protein found in the blood (serum albumin). The reliable, high-throughput, and sensitive characterization of the binding kinetics of low molecular weight compounds to key proteins is a challenging task. Often, the compounds are not available in large quantities, or a pharmaceutical pre-screening is needed when sample consumption and rapidity of the measurements is a critical issue.

For this purpose, we used the high-resolution GCI (grating coupled interferometry) label-free optical biosensor technique instead. Investigation of the interaction between small molecules and proteins has always played a major role in the field of drug research and biochemistry. Binding affinity and the strength of the binding interaction between protein and small molecule ligands are tipically described by the equilibrium dissociation constant which represents the ratio of free and complexed protein amounts. The dissociation constants of protein and small-molecule agents were determined by GCI, which is, among other applications, a well-established method in the binding analysis of small-molecule drugs at low concentrations. GCI biosensor based on the phenomena of evanescent field; thus alternation in the immediate proximity of the sensing surface can be detected, and the multi-channel arrangement facilitates parallel measurements. This novel biosensor combines optical waveguide and interferometric techniques that allow the monitoring of molecular interactions with excessively high sensitivity in a real-time manner. This work aimed to study the interaction between drugs with known interaction parameters first (norfloxacin, diazepam, warfarin) and human serum albumin (HSA) protein in order to develop an appropriate measurement procedure and map the interaction between a drug under development with unknown parameters and the protein.
After developing the optimal measuring method by well-known drugs, we continued the experiments with the mentioned compounds from *A. sylvestris* with small molecular weight and unknown binding parameters to HSA.

In our work the binding of two aryltetralin lignans – promising as antitumor agents, and isolated by us in excellent quality –, deoxypodophyllotoxin (DPT), and angeloyl podophyllotoxin (APT) to the transfer protein human serum albumin (HSA) was in-depth analyzed for the first time. During measurements, we used polycarboxylate-based hydrogel layer with immobilized HSA on top of it. With this engineered model surface, we could measure the binding parameters of two aryltetralin lignans, deoxypodophyllotoxin (DPT), and angeloyl podophyllotoxin (APT) to HSA. Exploiting the multi-channel referencing ability, the unique surface sensitivity and the throughput of this sensing method, the experiments revealed the specific biomolecular interactions. Traditional label-free kinetic measurements were also compared with a novel way of affinity kinetics; namely by employing repeated analyte pulses of increasing duration (RAPID). In the case of DPT, the RAPID measurements resulted in the two estimated K_d (dissociation constant) values, K_{d1} = 1±0.03 μM and K_{d2} = 1.8±0.09 μM. Traditional kinetic measurements resulted in K_{d1} = 8.7±0.09 μM and K_{d2} = 5.4±0.02 μM. In the case of APT, the repeated analyte pulses of increasing duration measurements resulted in K_d =

8±1.8 μM, while K_d = 6.5±0.02 μM by traditional kinetic measurements. From these results, we can conclude that the values received by the two methods are comparable and reliable, but the latter sensing method offers faster results from less sample material. We found that this new method is reliable and optimal for novel active substance testing and screening, with a significantly lower sample consumption. This can be critical when screening of large amounts of ligands and precise kinetic affinity data is needed. Our manuscript has been submitted to the International Journal of Biological Macromolecules and is under revision [3].

II.5. Penetration of gold nanoparticles into glycocalyx-digested cells

Functionalized nanoparticles (NPs) can be applied as cell-targeting drug carriers in biomedical applications. The penetration of NPs into cells has significant implications for medical treatments. The glycocalyx is a multifunctional cell surface sugar layer composed mainly of glycoproteins, glycolipids, and proteoglycans. Glycocalyx components are supposed to take part in the regulation of receptor functions. It can also facilitate or inhibit the cellular uptake of growth factors, viruses, and functionalized NPs. The type and efficiency of uptake proved to depend on the NP size and coat, the intercellular medium composition, the cell type, and the cell line as well. Removal of some components of the glycocalyx by enzymatic treatment can cause changes in its 3D structure (pore size) and the shape of the cell membrane and can also affect the arrangement and activity of the receptors. The digestion of this sugar-rich coating of the cells can be caused by the treatment of chondroitinase ABC (ChrABC), heparinase III, and neuraminidase enzymes, among others.

In our work, we continued the measurements with positive gold nanoparticles (previous study: *Peter et al. Interaction of positively charged gold nanoparticles with cancer cells monitored by an in situ label-free optical biosensor and transmission electron microscopy. ACS AMI, 2018*) as well together with enzyme-treated HeLa cells. The enzyme chondroitinase ABC digests the glycocalyx of the cells. With these measurements, we would like to study the effect of the digestion of glycocalyx on the penetration of gold nanoparticles with different diameter sizes, because the exact role of the cellular glycocalyx in NP uptake is still uncovered.

We in situ monitored the cellular uptake of gold NPs─functionalized with positively charged alkaline thiol (TMA)─into adhered cancer cells with or without preliminary glycocalyx digestion. Proteoglycan (PG) components of the glycocalyx were treated with the chondroitinase ABC enzyme. It acts on chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, and slowly on hyaluronate. The uptake measurements of HeLa cells were performed by applying a high-throughput label-free optical biosensor based on resonant waveguide gratings (RWG). The positively charged gold NPs were used with different sizes [d = 2.6, 4.2, and 7.0 nm, small (S), medium (M), and large (L), respectively]. Negatively charged citrate-capped tannic acid (CTA, d = 5.5 nm) NPs were also used in control experiments. Real-time biosensor data confirmed the cellular uptake of the functionalized NPs, which was visually proved by transmission electron microscopy. It was found that the enzymatic digestion facilitated the entry of the positively charged S- and M-sized NPs, being more pronounced for the M-sized. Other enzymes digesting different components of the glycocalyx were also employed, and the results were compared. Glycosaminoglycan digesting heparinase III treatment also increased, while glycoprotein and glycolipid modifying neuraminidase decreased the NP uptake by HeLa cells.

This suggests that the sialic acid residues increase, while heparan sulfate decreases the uptake of positively charged NPs. Our results raise the hypothesis that cellular uptake of 2–4 nm positively charged NPs is facilitated by glycoprotein and glycolipid components of the glycocalyx but inhibited by PGs. Our article has been published open access with a title of „Glycocalyx components detune the cellular uptake of gold nanoparticles in a size- and charge-dependent manner" in the (*Peter et al. ACS Applied Bio Materials, 2022*) [4]. Our work has been selected for the cover page of the actual issue as well.

II.6. Preliminary studies with fluidic force microscopy (FluidFM) and computer-controlled micropipette (CCMP) in a single cell level

My aim was to investigate the effect of compounds on the level of single cells as well. However, these methods are novel and the measuring protocol has to be set and invented to be capable of examining the impact of active substances. For these purposes, basic researches were performed by using fluidic force microscopy (FluidFM) and computer-controlled micropipette.

We published open access an article with the title „Single-cell adhesion force kinetics of cell populations from combined label-free optical biosensor and robotic fluidic force microscopy" (*Sztilkovics et al. Scientific Reports, 2020*) [5]. In this study, a high spatial and temporal resolution resonant waveguide grating-based label-free optical biosensor was combined with robotic fluidic force microscopy to monitor the adhesion of living cancer cells. In contrast to traditional fluidic force microscopy methods with a manipulation range in the order of 300–400 micrometers, the robotic device employed here can address single cells over mm-cm scale areas. This feature significantly increased measurement throughput, and opened the way to combine the technology with the employed microplate-based, large area biosensor. After calibrating the biosensor signals with the direct force measuring technology on 30 individual cells, the kinetic evaluation of the adhesion force and energy of large cell populations was performed for the first time. We concluded that the distribution of the single-cell adhesion force and energy can be fitted by log-normal functions as cells are spreading on the surface and revealed the dynamic changes in these distributions. The present methodology opens the way for the quantitative assessment of the kinetics of single-cell adhesion force and energy with unprecedented throughput and time resolution, in a completely non-invasive manner. This study was in the top 100 downloaded articles in 2020 in the Scientific Reports. However, in this work we did not apply any active substances; these findings are essential for further investigations with FluidFM with single cells and natural compounds.

After this work, we started to inject gold nanoparticles into the cytosol of HeLa cells by FluidFM and make TEM images of the injected cells. This work is a very rudimentary stage, but we intend to publish our results in the near future.

In another study, we applied a computer-controlled micropipette (CCMP) to measure the dissociation constant (K_d) of integrin-RGD-binding. Surface coatings with varying RGD densities were prepared, and the detachment of single cells from these surfaces was measured by applying a local flow-inducing hydrodynamic lifting force on the targeted cells in discrete steps. The average behavior of the populations was then fit according to the chemical law of mass action. To verify the resulting value of $K_d^{2d} = (4503 \pm 1673)\ 1/\mu m^2$, a resonant waveguide

grating-based biosensor was used, characterizing and fitting the adhesion kinetics of the cell populations. Both methods yielded a K_d within the same range. Furthermore, an analysis of subpopulations was presented, confirming the ability of CCMP to characterize cell adhesion both on single-cell and whole population levels. This work was published open access with the title of „Dissociation constant of integrin-RGD binding in live cells from an automated micropipette and label-free optical data" (*Gerecsei et al. Biosensors, 2021*) [6]. Our work has been selected for the cover page of the actual issue this time as well. The introduced methodologies offer convenient and automated routes to quantify the adhesivity of living cells before their further processing, for example treating them with natural compounds or nanoparticles.

II.7. Supervision of students

During the project period, I was a supervisor of 3 students from the Budapest University of Technology and Economics: Kinga Tóth ((BME-VBK (BSc) and BME-VIK (MSc)), Barbara Majoros (BME-VIK (MSc)), and Viktor Kovács (BME-VBK (BSc)).

In the autumn semester of 2020, Kinga Tóth participated in the TDK conference (BME-VBK) with the work titled „Interaction of small molecules and protein investigated by high-resolution optical biosensor technique". My student received a diploma of merit for her work. After that, she received her BSc diploma in January 2021. Her BSc thesis aimed to study the interaction between drugs with known interaction parameters (norfloxacin, diazepam, warfarin) and human serum albumin (HSA) protein in order to develop an appropriate measurement procedure and map the interaction between a drug under development with unknown parameters (called TB1602) and the protein with grating coupled interferometry (*Kinga Tóth: Biosensors for high precision measurement of molecular interactions. Bsc thesis, 2020*). In the autumn semester of 2022, she participated in the TDK conference (BME-VIK) again with the work titled „Revealing the effect of natural compounds on cell adhesion". The topic of the presentation was described earlier in II.3. chapter. My student received 1st place for her work, and she intends to participate in the OTDK conference in 2023 spring. She writes her MSc thesis on this topic as well.

Barbara Majoros participated in the GCI measurements (described in the II.4. chapter). She writes her MSc thesis with the title of „Examination of pharmaceuticals by applying label-free biosensors".

In 2021 autumn, Viktor Kovács participated in the TDK conference (BME-VBK) with the work titled „Impact of glycocalyx digestion on cell adhesion and nanoparticle uptake of cancer cells" (described in the II.5. chapter). In this work, the effect of digestion of glycocalyx on the penetration of nanoparticles with different diameter sizes by RWG biosensor (cell population level) and Epic Cardio biosensor (single cell level) as well. My student received the diploma of merit for his work.

II.8. Conference presentations

Due to the Covid-19 pandemic starting in March 2020 in Hungary (worldwide a little bit earlier), I was unable to travel and present my results at any conferences for a long time. Because of this reason, the rest of the original budget for conferences was shared for other purposes (for example buying chemicals and measurement tools and paying for the ones who participated in the project).

I could participate in an international conference only in August 2022 when the pandemic situation calmed down. I presented my work with the title of „Label-free discovery of natural compounds as target biomolecules in cellular adhesion and migration" (poster) [7]. My student, Barbara Majoros also showed her work with the title of „Grating Coupled Interferometry (GCI) for kinetic interaction analysis of small molecules and their target proteins" [8] at the Regional Biophysics Conference (Pécs, Hungary, August 22-26, 2022).

III. Summary

During the project term (2019-2022) I accomplished the major research aims with extracellular matrix components and small molecule compounds (natural compounds and nanoparticles) by applying label-free techniques:

- In our review article we concluded by highlighting the possibilities to screen natural compounds faster and more easily by applying new label-free methods, which also enable a far greater degree of quantification than the conventional methods [1].
- It is suggested that the polyphenols can interact and block important cell adhesion protein motifs and affect the rigidity of the layers as well. In our work with EGCG, we proposed that the polyphenol molecules bound less between the fibronectin chains than in the case of synthetic copolymers. We provided a plausible interpretation of the redox transformation mediated by fibronectin-linked thiol/disulfide residues as well [2].
- Other natural compounds have been measured with the same methodology with RWG biosensor on the same extracellular matrix component.
- Natural compounds deoxypodophyllotoxin (DPT), and angeloyl podophyllotoxin (APT) to the transfer protein human serum albumin (HSA) was in-depth analyzed for the first time by applying the GCI method [3].
- The effect of the digestion of glycocalyx on the penetration of gold nanoparticles with different diameter sizes and charges was analyzed [4].
- Preliminary studies proved that FluidFM and computer-controlled micropipette techniques offer convenient and automated routes to quantify the adhesivity of living cells before their further processing, for example treating with natural compounds or nanoparticles [5,6].
- From our results, 3 first-author publications have been published [1-3], 1 is under publication [4], 1 manuscript is still in the writing phase, and there were 2 conference presentations as well [7-8].

- In these topics, 1 BSc thesis and 3 TDK conference presentations were performed under my supervision, and 2 MSc theses are under preparation.
- During this period, I was a co-author of some other studies as well [9-17].

As a possible continuation I would like to highlight the screening platforms developed in the present project, which could be adapted to other types of biomolecules as well (both novel ligands and important medical targets in adhesion and/or signalization). Targeting and characterizing glycocalyx-small molecule interactions, and the more extensive application of FluidFM in this field could be alo potential future directions.

IV. Publication list

Most relevant, highlighted publications in the project topic:

[1] Beatrix Péter, Imre Boldizsár, Gábor M. Kovács, Anna Erdei, Zsuzsa Bajtay, Alexandra Vörös, Jeremy J. Ramsden, Ildikó Szabó, Szilvia, Robert Horvath. Natural compounds as target biomolecules in cellular adhesion and migration: from biomolecular stimulation to label-free discovery and bioactivity-based isolation. Biomedicines, 2021.

[2] Beatrix Peter, Nicolett Kanyo, Inna Szekacs, Antal Csampai, Szilvia Bosze, Robert Horvath. Epigallocatechin-gallate tailors the cell adhesivity of fibronectin coatings in oxidation and concentration-dependent manner. Materials Advances, 2022.

[3] Beatrix Péter, Barbara Majoros, Sándor Kurunczi, Andrea Violetta Ács, Inna Szekacs, Szilvia Bősze, Gábor M. Kovács, Imre Boldizsár, Robert Horvath. Label-free biosensing of lignans for therapeutics using engineered model surfaces. Submitted to International Journal of Biological Macromolecules.

[4] Beatrix Peter, Nicolett Kanyo, Kinga Dora Kovacs, Viktor Kovács, Inna Szekacs, Béla Pécz, Kinga Molnár, Hideyuki Nakanishi, Istvan Lagzi, Robert Horvath. Glycocalyx components detune the cellular uptake of gold nanoparticles in a size- and charge-dependent manner. ACS Applied Bio Materials, 2022.

[5] Milan Sztilkovics, Tamas Gerecsei, Beatrix Peter, Andras Saftics, Sandor Kurunczi, Inna Szekacs, Balint Szabo, Robert Horvath. Single-cell adhesion force kinetics of cell populations from combined label-free optical biosensor and robotic fluidic force microscopy. Scientific Reports, 2020.

[6] Tamás Gerecsei, Péter Chrenkó, Nicolett Kanyo, Beatrix Péter, Attila Bonyár, Inna Székács, Balint Szabo, Robert Horvath. Dissociation constant of integrin-RGD binding in live cells from automated micropipette and label-free optical data, Biosensors, 2021.

[7] Beatrix Péter, Inna Székács, Szilvia Bősze, Imre Boldizsár, Gábor M. Kovács, Robert Horvath. Label-free discovery of natural compounds as target biomolecules in cellular adhesion and migration. Regional Biophysics Conference, Pécs, Hungary. August 22-26, 2022 (poster presentation).

[8] Barbara Majoros, Beatrix Péter, Imre Boldizsár, Szilvia Bősze, Inna Szekacs, Sándor Kurunczi, Robert Horvath. Grating Coupled Interferometry (GCI) for kinetic interaction analysis of small molecules and their target proteins. Regional Biophysics Conference, Pécs, Hungary. August 22-26, 2022 (poster presentation).

Additional publications forming the basis of possible continuation:

[9] Nicolett Kanyo, Kinga Dora Kovacs, Andras Saftics, Inna Szekacs, Beatrix Peter, Ana R. Santa-Maria, Fruzsina R. Walter, András Dér, Mária A. Deli, Robert Horvath. Glycocalyx regulates the strength and kinetics of cancer cell adhesion revealed by biophysical models based on high resolution label-free optical data. Scientific Reports, 2020.

[10] Andras Saftics, Sándor Kurunczi, Beatrix Peter, Inna Szekacs, Jeremy J. Ramsden, Robert Horvath. Data evaluation for surface-sensitive label-free methods to obtain real-time kinetic and structural information of thin films: A practical review with related software packages. Advances in Colloid and Interface Science, 2021.

[11] Rita Ungai-Salánki, Benjamin Csippa, Tamás Gerecsei, Beatrix Péter, Robert Horvath, Bálint Szabó. Nanonewton scale adhesion force measurements on biotinylated microbeads with a robotic micropipette. Journal of Colloid and Interface Science, 2021.

[12] Kristof Kliment, Inna Szekacs, Beatrix Peter, Anna Erdei, Istvan Kurucz, Robert Horvath. Label-free real-time monitoring of the BCR-triggered activation of primary human B cells modulated by the simultaneous engagement of inhibitory receptors. Biosensors and Bioelectronics, 2021.

[13] Eniko Farkas, Robert Tarr, Tamás Gerecsei, Andras Saftics, Kinga Dóra Kovács, Balazs Stercz, Judit Domokos, Beatrix Peter, Sandor Kurunczi, Inna Szekacs, Attila Bonyár, Anita Bányai, Péter Fürjes, Szilvia Ruszkai-Szaniszló, Máté Varga, Barnabás Szabó, Eszter Ostorházi, Dóra Szabó, Robert Horvath. Development and in-depth characterization of bacteria repellent and bacteria adhesive antibody-coated surfaces using optical waveguide biosensing. Biosensors, 2022.

[14] Nicolett Kanyo, Kinga Dóra Kovács, Sándor Viktor Kovács, Bálint Béres, Beatrix Péter, Inna Székács, Robert Horvath. Single-cell adhesivity distribution of glycocalyx digested cancer cells from high spatial resolution label-free biosensor measurement. Matrix Biology Plus, 2022.

[15] Beatrix Péter, Eniko Farkas, Sandor Kurunczi, Zoltán Szittner, Szilvia Bősze, Jeremy J. Ramsden, Inna Szekacs, Robert Horvath. Review of label-free monitoring of bacteria: From challenging practical applications to basic research perspectives. Biosensors, 2022.

[16] Tamás Gerecsei, Beatrix Peter, Rita Ungai-Salánki, Sándor Kurunczi, Inna Szekács, Bálint Szabó, Robert Horvath. Prospects of fluidic force microscopy and related biosensors for medical applications (book chapter). In Nanobioanalytical Approaches to Medical Diagnostics. Woodhead Publishing Series in Biomaterials, Elsevier, 2022.

[17] Zoltán Szittner, Beatrix Péter, Sándor Kurunczi, Inna Székács, Robert Horvath. Functional blood cell analysis by label-free biosensors and single-cell technologies. Advances in Colloid and Interface Science, 2022.