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DNA Release from Polyaziridine Polyplexes Aided by Biomacromolecules: Effect of pH

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ABSTRACT. The pH dependence of heparin and dextran sulfate aided release of DNA from its polyplexes with polyaziridine (PA) has been investigated using Quartz crystal microbalance with dissipation monitoring (QCM-D). The acoustic behavior of ternary systems indicated that the DNA decondensation was favored at physiological pH. When pH < 7.4, PA was significantly positively charged and ternary complexation occurred. When pH exceeded 7.4, very small proportion of DNA was released upon heparin addition due to marked reduction in cationic nature of PA. An increase in thickness at the interface due to adsorbed material at pH 9.0 showed the retention of heparin on the surface despite of negligible release of DNA. In terms of PA binding, heparin proved to be a better competitor than dextran sulfate.

Keywords: Transfection; heparin; dextran sulfate; QCM-D; acoustic sensing.

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Introduction

Cationic polymers and lipids have been widely harnessed as a non-viral gene delivery vectors (Dave et al., 2020; Uddin, 2022). The complexes of DNA with lipids and polymers are called lipoplexes and polyplexes, respectively. The latter are more effective in preventing degradation by serum components and cargo sorting by endosomes (Wan Leung et al., 2022). Spherical nucleic acids themselves deliver functional proteins to cells via transfection (Ebrahimi, Samanta, Kusmierz, & Mirkin, 2022).

Among polymers, polyaziridines (PA) have frequently been employed for gene therapy (Dhas et al., 2021; Wang et al., 2020). Linear PA have protonable nitrogen at every third atom of the polymer backbone (Sun, Tang, Uludağ, & Cuervo, 2011). In media having labile H^+, they form polycations, capable of binding with anionic phosphate groups in DNA (Amin, Rahimizadeh, Eshghi, Dehshahri, & Ramezani, 2013). The complex formation is chiefly driven by entropy change occurring upon counter-ion release from the double dielectric layer, though van der Waal forces and hydrogen bonding interactions could also contribute towards stability of polyplexes (Perevyazko et al., 2012). The efficacy of transfer of DNA condensates with PA is also dependent on rigidity of cell adhesion substrates (Kong et al., 2005). The quantification of PA-DNA interactions has also been attempted (Kou, Zhang, Zhang, 2016).

After transfer, DNA decondensation is a vital step in DNA transfection (Chong, Yeap, & Ho, 2021). This can be affected by addition of (bio)macromolecules that can compete with DNA for binding with polycations. Polyanionic heparin and dextran sulfate are ideal candidates owing to their affinity for DNA (Wang et al., 2021). pH of medium can augment or inhibit the DNA release from polyplexes by the competitor binder (Abebe et al., 2015; Coelho, Botelho, Paris, Marques, & Silva, 2021)

Efforts have been made to monitor the release of DNA from its polyplexes (Clamme, Azoulay, & Mély, 2003). Papadakis and coworkers have reported the acoustic detection of DNA in biological samples (Papadakis, Palladino, Chronaki, Tsortos, & Gizeli, 2017). Ultrasound triggered release (Liao et al., 2017) and acoustophoretic transfection (Vasileiou, Foresti, Bayram, Poulikakos, & Ferrari, 2016) have also been attempted. Quartz Crystal Microbalance (QCM) has been utilized for in-depth analysis of surface-bound (bio)molecules (Adamczyk, Sadowska, & Żeliszewska, 2020; Milioni, Tsortos, Velez, & Gizeli, 2017). In the present study, we have employed QCM-D for investigating pH triggered release of DNA from DNA-PA polyplexes that is aided by heparin and dextran sulfate. The change in frequency and dissipation relates to mass and elastic/viscoelastic properties of the deposited material (Easley et al., 2021). The release of DNA at solid-liquid interface occurs differently than that occurring in the bulk in solution and may serve as a better mimic of the complicated biological systems (Hill, Wildman, & Mata, 2022).

Material and methods

Materials

The calf thymus DNA (as water soluble, sodium salt) and Polyaziridine (MW = 25 KDa) were obtained from Sigma-Aldrich. Heparin was provided by Biodee (China). All products were used as received. PA (10 mmol L^{-1}, based on monomeric molecular weight), DNA (100 µg mL^{-1}), heparin (1 mg mL^{-1}) and different concentrations of dextran sulfate were prepared in 1000 mM phosphate buffer saline (PBS) buffer at pH 5.6, 7.4 and 9.0 with 100 mmol L^{-1} NaCl. These pH values were selected as DNA is stable in this range (Wang, Lim, & Son, 2014). Ultrapure water from Milli-Q Advantage A10 System of Millipore (France) was used in preparations.

Methods

QCM-D frequency and dissipation data were obtained using quartz crystal microbalance with dissipation monitoring (Q-Sense D300) using method reported elsewhere (Feiler, Davies, & Vincent, 2011; Lee et al., 2012). The QCM-D sensor consists of disk-shaped AT-cut quartz crystal with a fundamental resonant frequency of 5 MHz and crystal constant (*C*) of 17.7 ng cm^{-2} Hz. The quartz crystal was mounted in fluid cell with one side exposed to solution. The frequency values were reproducible within ±2 Hz. The active site of the quartz crystal was in continuous contact with aqueous solutions of PA, DNA, dextran sulfate, heparin, and buffer. Δf and ΔD values from the fundamental that were noisy were excluded and the third overtone was used for analysis (Easley et al., 2021; Feiler et al., 2011). Prior to use, the gold coated resonator was cleansed using piranha solution composed of one part of H_2O_2 and three parts of H_2SO_4, rinsed with ultrapure water and dried using stream of nitrogen gas (Bibi & Siddiq, 2012). A measurement of layer-by-layer deposition process was initiated by switching the liquid exposed to the resonator from PBS buffer to a PA (1 mg mL^{-1}). PA was allowed to adsorb until a stable baseline was obtained. Later, it was rinsed with PBS buffer to confirm a uniform positive charge coating. After rinsing with PBS buffer, DNA, heparin, or dextran sulfate were introduced in QCM chamber, alternatively. During the experiments, QCM-D cell was rinsed with PBS during each deposition and the process was considered complete when dissipation and frequency change was negligible. All experiments were conducted at 20 ± 0.02 ºC. QSense Q-Tools and Origin Pro 8.0 software was used for data analysis.

Results and discussion

PA-DNA complex formation at interface can be attained through a simple electrostatically driven layer by layer adsorption process. Initially, PA was adsorbed at the interface. The onset occurred at point *A* and *a* in the frequency and dissipation plots, respectively (Figure 1). At pH 5.6, the adsorption of PA to the substrate surface was indicated by decline in frequency (from ***A*** to ***B***) showing an increase in mass deposited on the surface of the crystal. The frequency decline is about 67 Hz. The dissipation relates to rigidity of the polymer film on the surface. When a film is rigid, dissipation is negligibly changed. A concurrent increase in dissipation (from ***a*** to ***b***) observed in our case reflects the existence of loosely bound macromolecules at the solid-liquid interface. The dissipation is altered by a magnitude of ~ 2.0 $x10^{-6}$, based on which it can be inferred that the PA film is not rigid (Medina, Farinha, Emwas, Tabatabai, & Leiknes, 2020).

After rinsing with PBS buffer and allowing the system ample of time for stabilization during which no mass change occurred (see segment ***BC***), the PA-modified surface was exposed to DNA. A sharp decrease in frequency was observed, indicated by segment ***CD*** in Figure1. During this process, a change of 181.9 Hz is seen in Δf. This can be correlated to mass deposition based on Sauerbrey model (Cougnon, Gautier, Pilard, Casse, & Chénais, 2008) or Voigh viscoelastic model (Medina et al., 2020). The results reveal an increase in thickness at the surface due to greater assembly of macromolecules, obviously due to complex formation between PA and DNA. There is displacement of water of hydration, and in addition to existence of electrostatic forces, the complex formation reflects a trade-off of entropy change, hydrogen bonding and van der Waals interactions (Neu, Fischer, & Kissel, 2005; Thomas, Tajmir-Riahi, & Thomas, 2016). The simultaneous elevation in dissipation upon DNA addition of the magnitude 10.6 x 10^{-6} (***cd*** segment in the plot) is seen. At this stage, the film is highly flexible. At pH < 7, the phosphate groups of DNA are not fully ionized and van der Waals interactions with PA become significantly important (Mazumdar, 2001). Still a more intimate contact between quarternized nitrogen atoms in PA and phosphate groups in DNA is possible

(Cherng, 2009). When N/P ratio exceeds 10, the positively charged polyplexes are formed and the excess PA causes DNA condensation (Jorge, Dias, Pereira, & Pais, 2010; Mocci, Laaksonen, Engelbrecht, Vasiliu, & Perepelytsya, 2022). The Δf and ΔD curves soon level-off because strongly protonated state need shorter time for complex formation (see segments ***DE*** and ***de*** in Figure 1). After this, the surface was rinsed with PBS buffer and no obvious change has been observed. At this stage, heparin was introduced to initiate DNA decondensation (Clamme et al., 2003). Heparin is most negatively charged of cellular biomolecules with high affinity for cations (Paiva et al., 2021). A change in third over tone frequency Δf (i.e., 67.27 Hz) as well as dissipation ΔD (i.e., 8.6 x 10^{-6}) occurred (see segments ***EF*** and ***ef*** in Figure 1), clearly indicating increased thickness at the resonator surface due to mass accumulation but absence of rigidity in the deposited material. It was therefore inferred that heparin was involved in ternary complexation. In the region ***FG***, the Δf values were slightly regained (~ 18 Hz), showing some reduction in mass most probably due to slight decondensation of DNA. The dissipation values remained unaltered in the corresponding region ***fg*** unraveling no change in flexibility of polymeric layers at the solid-liquid interface. Based on these it can be concluded that heparin was unable to compete with DNA in binding with PA and therefore the polyplexes were not dissociated significantly. Higher heparin concentrations (3 to 5 mg mL^{-1}) yielded similar results (not shown).

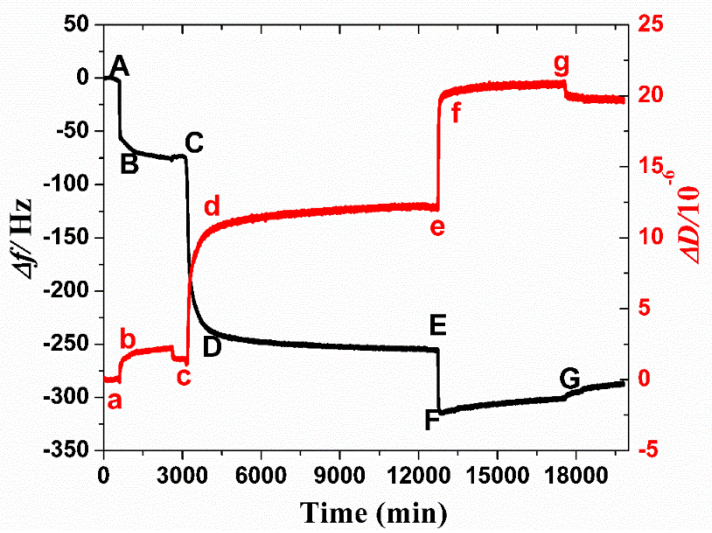

Figure 1. Third overtone frequency (black) of SiO_2 coated quartz crystal during the adsorption of PA (point A), DNA (point C) and heparin (point E) at pH 5.6. The corresponding change in dissipation is indicated by plot in red, and similar points by lower case letters (a, c, and e).

In Figure 2, the effects produced by dextran sulfate are presented. The regions from ***A*** to ***E*** for frequency change (and from ***a*** to ***e*** for change in dissipation) represent deposition of PA followed by DNA at the resonator surface and trends appear quite similar to those obtained earlier. Here also, a decrease in Δf (i.e., 90.5 Hz) and a simultaneous increase in ΔD (i.e., 11.9 x 10^{-6}) were observed after introduction of dextran sulfate at point ***E*** showing the increase in mass deposited but absence of compactness/rigidity. Along the segment ***FG*** (or ***fg***), the frequency or dissipation was altered negligibly.

The dextran sulfate can attach to bound PA and form cross-links between various polyplexes. Besides, it may engage into complexation with excess PA molecules, which are only weakly associated with PA-DNA polyplexes, but does not directly engage in complexation with DNA (Cherng, 2009).

Based on patterns shown in Figures 1 and 2, we conclude that at pH 5.6, the effectiveness of biomacromolecules, heparin and dextran sulfate to set DNA free from its polyplexes is extremely low (López-Cornejo et al., 2022; Moret et al., 2001).

Figure 3 shows the acoustic monitoring of condensation and decondensation of DNA at physiological pH (7.4). When pH > 7.0, the extent of PA protonation is reduced, this has a direct bearing on its complexation with DNA. At pH 7.4, only 20% of the total nitrogen atoms in PA are protonated. Therefore, in addition to electrostatic interaction, an indirect binding between DNA and PA mediated through water molecule/s is developed (Cherng, 2009). There is a decline in Δf after addition of DNA at point ***C***. The ***CD*** segment represents a frequency difference of 189.3 Hz, showing the significant deposition of DNA on PA modified interface. However, beyond point ***D*** the Δf values gradually regained become less negative by the factor of 62.5 Hz, showing that DNA is retained only partly at the interface. After introduction of heparin at point ***E***, a further increase in Δf (~ 57 Hz) is seen (segment ***EF***) that clearly indicates DNA displacement by heparin.

There is concurrent decrease in dissipation beyond point ***e*** and ΔD change of magnitude 2.89 x 10^{-6} is observed. This indicates some reduction in flexibility after DNA departure and complexation between heparin and PA, which is likely considering the fact that heparin has greater anionic character compared to DNA at physiological pH (Paiva et al., 2021). Unexpectedly, dextran sulfate did not produce similar effects, even when its concentration was raised to 20 mg mL^{-1}. Therefore, we divorced from dextran sulfate and continued further investigations with heparin only.

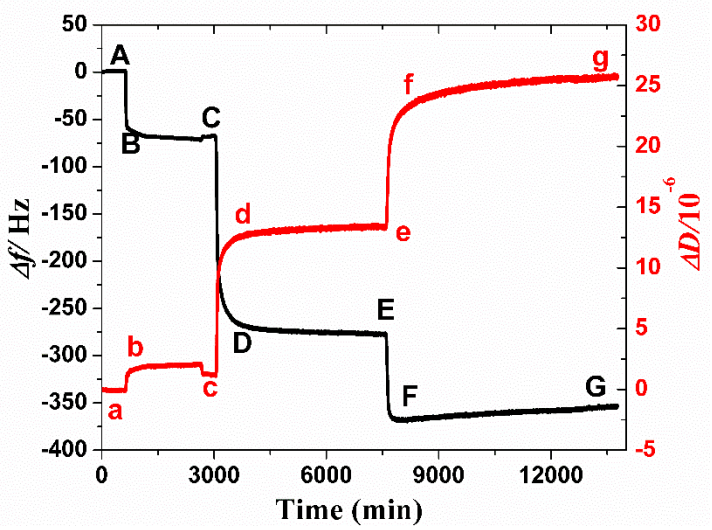

Figure 2. Third overtone frequency (black) of SiO_2 coated quartz crystal during the adsorption of PA (point A), DNA (point C) and dextran sulfate (point E) at pH 5.6. The corresponding change in dissipation is indicated by plot in red, and similar points by lower case letters (a, c, and e).

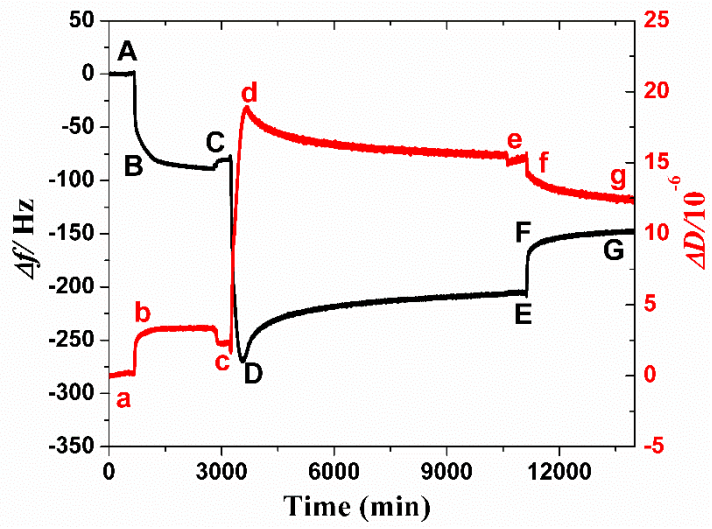

Figure 3. Third overtone frequency (black) of SiO_2 coated quartz crystal during the adsorption of PA (point), DNA (point C) and heparin (point E) at pH 7.4. The corresponding change in dissipation is indicated by plot in red, and similar points by lower case letters (a, c, and e).

Figure 4 shows the change in frequency or dissipation because of PA deposition (segment ***AB, ab***), its complexation with DNA (segment ***CD, cd***), and DNA decondensation by heparin (segment ***EF***, ***ef***) at pH 9.0. At this pH, electrostatic interaction between DNA and PA is weak, so loosely bounded complexes are formed. Apparently, about 13% of total nitrogen atoms in PA are protonated at this stage (Sun et al., 2011) and interactions are possible through intervening water molecules. Only small fraction of DNA segments is in close contact with PA. When heparin was introduced (Figure 4, point ***E***), a small decrease in Δf by a magnitude of 13 Hz occurred (segment ***EF***). This was followed by regain of frequency and in the latter stages the frequency became less negative. An overall increase in frequency was about 25 Hz. The concurrent increase in ΔD was 4.91 x 10^{-6} (segment ***ef***). However, in the following stage a continuous decline in ΔD took place, leading to overall shift of 1.18 x 10^{-6}. The deposited layer is considered rigid only when $\Delta D < 1 \times 10^{-6}$ (Lee et al., 2012). Hence, the values of dissipation obtained in our case reflects only reduction in flexibility. The results indicated that heparin was retained on the surface, but only negligible amounts of DNA were released.

Overall, heparin proved to be a better agent for DNA release compared to dextran sulfate all pH values, This is probably due to repulsive interactions between DNA and highly anionic dextran sulfate.

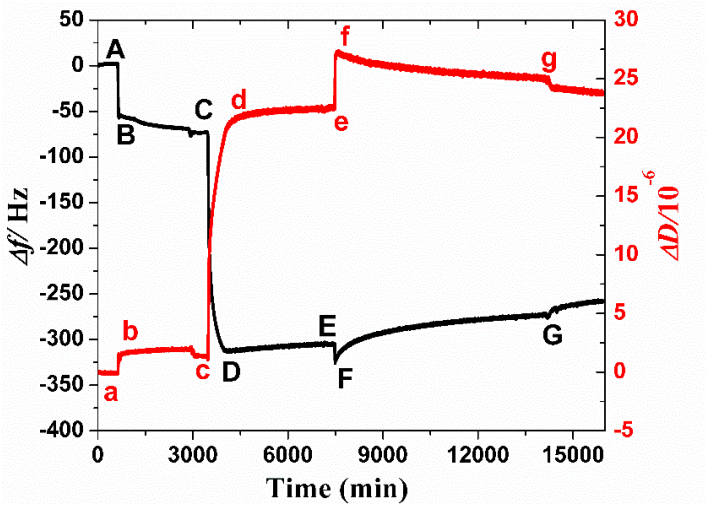

Figure 4. Third overtone frequency (black) of SiO_2 coated quartz crystal during the adsorption of PA (point), DNA (point C) and heparin (point E) at pH 9.0. The corresponding change in dissipation is indicated by plot in red, and similar points by lower case letters (a, c, and e).

Conclusion

The acoustic sensing results showed that both the formation of DNA polyplexes with PA and its dissociation by heparin occurred in pH dependent manner. Ternary PA/DNA/heparin complexes were evident under acidic conditions. Under basic conditions (i.e., pH 9.0) heparin was able to cause DNA release is small quantity. The significant DNA release occurred at physiological pH of 7.4. Contrary to our expectations, dextran sulfate proved to be inefficient in dissociating polyplexes. Based on these findings, biocompatible systems can be developed for more efficient transfection of genetic materials (Chong et al., 2021; Ebrahimi et al., 2022).

References

Abebe, D. G., Kandil, R., Kraus, T., Elsayed, M., Merkel, O. M., & Fujiwara, T. (2015). Three-layered biodegradable micelles prepared by two-step self-assembly of PLA-PEI-PLA and PLA-PEG-PLA triblock copolymers as efficient gene delivery system. *Macromolecular Bioscience*, *15* (5), 698–711. DOI: https://doi.org/10.1002/mabi.201400488

Adamczyk, Z., Sadowska, M., & Żeliszewska, P. (2020). Applicability of QCM-D for Quantitative Measurements of Nano- And Microparticle Deposition Kinetics: Theoretical Modeling and Experiments. *Analytical Chemistry*, *92*(22), 15087-15095. DOI: https://doi.org/10.1021/acs.analchem.0c03115

Amin, Z. R., Rahimizadeh, M., Eshghi, H., Dehshahri, A., & Ramezani, M. (2013). The effect of cationic charge density change on transfection efficiency of Polyethylenimine. *Iranian Journal of Basic Medical Sciences*, *16* (2), 150–156.

Bibi, I., & Siddiq, M. (2012). Conformational transition of poly (vinylbenzyltrimethylammonium chloride) (PVBTMAC) brush in the presence of hofmeister anions. *Journal of Polymer Research*, *19* (9). DOI: https://doi.org/10.1007/s10965-012-9961-y

Cherng J. Y. (2009). Investigation of DNA spectral conformational changes and polymer buffering capacity in relation to transfection efficiency of DNA/polymer complexes. *Journal of pharmacy & pharmaceutical sciences: a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques*, *12* (3), 346–356. DOI: https://doi.org/10.18433/j3859k.

Chong, Z. X., Yeap, S. K., & Ho, W. Y. (2021). Transfection types, methods and strategies: A technical review. *PeerJ*, *9*, 1–37. DOI: https://doi.org/10.7717/peerj.11165

Clamme, J. P., Azoulay, J., & Mély, Y. (2003). Monitoring of the formation and dissociation of polyethylenimine/DNA complexes by two photon fluorescence correlation spectroscopy. *Biophysical Journal*, *84*(3), 1960–1968. DOI: https://doi.org/10.1016/S0006-3495(03)75004-8

Coelho, F., Botelho, C., Paris, J. L., Marques, E. F., & Silva, B. F. B. (2021). Influence of the media ionic strength on the formation and in vitro biological performance of polycation-DNA complexes. *Journal of Molecular Liquids*, *344*, 117930. DOI: https://doi.org/10.1016/j.molliq.2021.117930

Cougnon, C., Gautier, C., Pilard, J. F., Casse, N., & Chénais, B. (2008). Redox and ion-exchange properties in surface-tethered DNA-conducting polymers. *Biosensors and Bioelectronics*, *23*(7), 1171–1174. DOI: https://doi.org/10.1016/j.bios.2007.10.016

Dave, K. M., Han, L., Jackson, M. A., Kadlecik, L., Duvall, C. L., & S Manickam, D. (2020). DNA Polyplexes of a Phosphorylcholine-Based Zwitterionic Polymer for Gene Delivery. *Pharmaceutical Research*, *37, 176*. DOI: https://doi.org/10.1007/s11095-020-02899-5

Dhas, Nn., Mehta, T., Sharma, S., Garkal, A., Yadav, D., Hariharan, K., … Kalyankar, P. (2021). Intranasal gene therapy for the treatment of neurological disorders. In C. V. Pardeshi & E. B. Souto (Eds.), *Direct Nose-to-Brain Drug Delivery: Mechanism, Technological Advances, Applications and Regulatory Updates* (p. 351–387). Academic Press, Elsevier Inc. DOI: https://doi.org/https://doi.org/10.1016/B978-0-12-822522-6.00017-5

Easley, A. D., Ma, T., Eneh, C. I., Yun, J., Thakur, R. M., & Lutkenhaus, J. L. (2021). A practical guide to quartz crystal microbalance with dissipation monitoring of thin polymer films. *Journal of Polymer Science*, *June*, 1–18. DOI: https://doi.org/10.1002/pol.20210324

Ebrahimi, S. B., Samanta, D., Kusmierz, C. D., & Mirkin, C. A. (2022). Protein transfection via spherical nucleic acids. *Nature Protocols,* 17, 327-357. DOI: https://doi.org/10.1038/s41596-021-00642-x.

Feiler, A. A., Davies, P. T., & Vincent, B. (2011). Adsorption of anionic gold nanoparticles by a layer of cationic microgel particles deposited on a gold-coated, quartz surface: Studied by quartz crystal microbalance and atomic force microscopy. *Soft Matter*, *7*(14), 6660–6670. DOI: https://doi.org/10.1039/c0sm01319f

Hill, J., Wildman, R., & Mata, A. (2022). Exploiting the fundamentals of biological organization for the advancement of biofabrication. *Current Opinion in Biotechnology*, *74*, 42–54. DOI: https://doi.org/10.1016/j.copbio.2021.10.016.

Jorge, A. F., Dias, R. S., Pereira, J. C., & Pais, A. A. C. C. (2010). DNA condensation by pH-responsive polycations. *Biomacromolecules*, *11*(9), 2399–2406. DOI: https://doi.org/10.1021/bm100565r

Kong, H. J., Liu, J., Riddle, K., Matsumoto, T., Leach, K., & Mooney, D. J. (2005). Non-viral gene delivery regulated by stiffness of cell adhesion substrates. *Nature Materials*, *4*(6), 460–464. DOI: https://doi.org/10.1038/nmat1392

Kou, X., Zhang, W., & Zhang, W. (2016). Quantifying the Interactions between PEI and Double-Stranded DNA: Toward the Understanding of the Role of PEI in Gene Delivery. *ACS Applied Materials and Interfaces*, *8*(32), 21055–21062. DOI: https://doi.org/10.1021/acsami.6b06399

Lee, H. S., Yee, M. Q., Eckmann, Y. Y., Hickok, N. J., Eckmann, D. M., & Composto, R. J. (2012). Reversible swelling of chitosan and quaternary ammonium modified chitosan brush layers: Effects of pH and counter anion size and functionality. *Journal of Materials Chemistry*, *22*(37), 19605–19616. DOI: https://doi.org/10.1039/c2jm34316a

Liao, W. H., Hsiao, M. Y., Lo, C. W., Yang, H. S., Sun, M. K., Lin, F. H., … Chen, W. S. (2017). Intracellular triggered release of DNA-quaternary ammonium polyplex by ultrasound. *Ultrasonics Sonochemistry*, *36*, 70–77. DOI: https://doi.org/10.1016/j.ultsonch.2016.11.002

López-Cornejo, P., Lebrón, J. A., Ostos, F. J., López-López, M., Moyá, M. L., Bernal, E., & Martín, C. (2022). Metallosurfactants as Non-viral Vectors in Transfection. In S. K. Mehta & R. Kaur (Eds.), *Metallosurfactants: From Fundamentals to Catalytic and Biomedical Applications.* (p. 133–149). Germany: Wiley-VCH.

Mazumdar, S. (2001). Interactions of Surfactants with Biomolecules and Mimics. In H. S. Nalwa (Ed.), *Handbook of Surfaces and Interfaces of Materials* (Vol. 3, p. 73–128). Academic Press. DOI: https://doi.org/10.1016/B978-0-12-513910-6.50060-8

Medina, S. C., Farinha, A. S. F., Emwas, A. H., Tabatabai, A., & Leiknes, T. O. (2020). A fundamental study of adsorption kinetics of surfactants onto metal oxides using quartz crystal microbalance with dissipation (QCM-D). *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *586*. DOI: https://doi.org/10.1016/j.colsurfa.2019.124237

Milioni, D., Tsortos, A., Velez, M., & Gizeli, E. (2017). Extracting the Shape and Size of Biomolecules Attached to a Surface as Suspended Discrete Nanoparticles. *Analytical Chemistry*, *89*(7), 4198–4203. DOI: https://doi.org/10.1021/acs.analchem.7b00206

Mocci, F., Laaksonen, A., Engelbrecht, L., Vasiliu, T., & Perepelytsya, S. (2022). DNA-Polyamine Interactions: Insight from Molecular Dynamics Simulations on the Sequence-Specific Binding of Spermidine. In L. Bulavin & N. Lebovka (Eds.), *Soft Matter Systems for Biomedical Applications. Springer Proceedings in Physics* (Vol. 266, Issue July, pp. 1–23). Springer, Cham. DOI: https://doi.org/https://doi.org/10.1007/978-3-030-80924-9_6

Moret, I., Esteban Peris, J., Guillem, V. M., Benet, M., Revert, F., Dasí, F., Crespo, A., & Alio, S. F. (2001). Stability of PEI-DNA and DOTAP-DNA complexes: Effect of alkaline pH, heparin and serum. *Journal of Controlled Release*, *76*(1), 169–181. DOI: https://doi.org/10.1016/S0168-3659(01)00415-1

Neu, M., Fischer, D., & Kissel, T. (2005). Recent advances in rational gene transfer vector design based on poly(ethylene imine) and its derivatives. *The Journal of Gene Medicine*, *7*(8), 992–1009. DOI: https://doi.org/10.1002/jgm.773

Paiva, R. E. F., Peterson, E. J., Malina, J., Zoepfl, M., Hampton, J. D., Johnson, W. E., Graminha, A., Ourahmane, A., McVoy, M. A., Brabec, V., Berners-Price, S. J., & Farrell, N. P. (2021). On the Biology of Werner's Complex. *Angewandte Chemie*, *133*(31), 17260–17267. DOI: https://doi.org/10.1002/ange.202105019

Papadakis, G., Palladino, P., Chronaki, D., Tsortos, A., & Gizeli, E. (2017). Sample-to-answer acoustic detection of DNA in complex samples. *Chemical Communications*, *53*(57), 8058–8061. DOI: https://doi.org/10.1039/C6CC10175E

Perevyazko, I. Y., Bauer, M., Pavlov, G. M., Hoeppener, S., Schubert, S., Fischer, D., & Schubert, U. S. (2012). Polyelectrolyte complexes of DNA and linear PEI: Formation, composition and properties. *Langmuir*, *28*(46), 16167–16176. DOI: https://doi.org/10.1021/la303094b

Sun, C., Tang, T., Uludağ, H., & Cuervo, J. E. (2011). Molecular dynamics simulations of DNA/PEI complexes: Effect of PEI branching and protonation state. *Biophysical Journal*, *100*(11), 2754–2763. https://doi.org/10.1016/j.bpj.2011.04.045

Thomas, T. J., Tajmir-Riahi, H. A., & Thomas, T. (2016). Polyamine–DNA interactions and development of gene delivery vehicles. *Amino Acids*, *48*(10), 2423–2431. DOI: https://doi.org/10.1007/s00726-016-2246-8

Uddin, N. M. (2022). *Succinylated Polyethylenimine Gene Delivery Agents for Enhanced Transfection Efficacy, PhD Dissertation* [University of Kentucky, USA]. Retrieved from https://uknowledge.uky.edu/chemistry_etds/153

Vasileiou, T., Foresti, D., Bayram, A., Poulikakos, D., & Ferrari, A. (2016). Toward Contactless Biology: Acoustophoretic DNA Transfection. *Scientific Reports*, *6*(1). DOI: https://doi.org/10.1038/srep20023

Wan Leung, S., Cheng, P.-C., Chou, C.-M., Lin, C., Kuo, Y.-C., Amy Lee, Y.-L., Liu, C.-Y., Mi, F.-L., & Cheng, C.-H. (2022). A novel low - molecular - weight chitosan/gamma - polyglutamic acid polyplexes for nucleic acid delivery into zebrafish larvae. *International Journal of Biological Macromolecules*, *194*, 384–39. DOI: https://doi.org/https://doi.org/10.1016/j.ijbiomac.2021.11.080

Wang, C., Bao, X., Ding, X., Ding, Y., Abbad, S., Wang, Y., Li, M., Su, Y., Wang, W., & Zhou, J. (2020). A multifunctional self-dissociative polyethyleneimine derivative coating polymer for enhancing the gene transfection efficiency of DNA/polyethyleneimine polyplexes in vitro and in vivo Cheng. *Polymer Chemistry*, *11*(3), 752. DOI: https://doi.org/10.1039/d0py90010a

Wang, G., Chen, S., Qiu, N., Wu, B., Zhu, D., Zhou, Z., Piao, Y., Tang, J., & Shen, Y. (2021). Virus-mimetic DNA-ejecting polyplexes for efficient intracellular cancer gene delivery. *Nano Today*, *39*, 101215. DOI: https://doi.org/10.1016/j.nantod.2021.101215

Wang, X., Lim, H. J., & Son, A. (2014). Characterization of denaturation and renaturation of DNA for DNA hybridization. *Environmental Analysis Health and Toxicology*, *29*, e2014007. DOI: https://doi.org/10.5620/eht.2014.29.e2014007