2017

Studying the Drug Delivery Kinetics of Nanosponges Using a MIP-Based Thermal Sensing Platform

Ariane Perez-Gavilan
Dublin Institute of Technology, ariane.perezgavilan@dit.ie

Christopher Pawley

Kaelin Foley
Dublin Institute of Technology

Gabrielle Tuijthof
Dublin Institute of Technology

Erik Steen Redeker

See next page for additional authors

Recommended Citation

Pawley, Christopher J., et al. "Studying the Drug Delivery Kinetics of a Nanoporous Matrix Using a MIP-Based Thermal Sensing Platform." *Polymers* 9.11 (2017): 560. doi:10.3390/polym9110560

Authors
Ariane Perez-Gavilan, Christopher Pawley, Kaelin Foley, Gabrielle Tuijthof, Erik Steen Redeker, Hanne Dillen, Kasper Eersels, Bart Van Grinsven, and Thomas J. Cleij

polymers

MDPI

Article

Studying the drug delivery kinetics of nanosponges using a MIP-based thermal sensing platform

Christopher J. Pawley [1*], Ariane Perez-Gavilan [2], Kaelin S. Foley [1,2], Gabrielle Tuijthof [3], Erik Steen Redeker [1], Hanne Diliën [1], Kasper Eersels [1], Bart van Grinsven [1] and Thomas J. Cleij [1]

[1] Maastricht University, Maastricht Science Programme, P.O. Box 616, 6200 MD Maastricht, the Netherlands.
[2] Dublin Institute of Technology, School of Chemical and Pharmaceutical Sciences, 143-149 Rathmines Road, Dublin 8, Ireland.
[3] Zuyd University of Applied Science, Faculty of Beta Sciences and Technology, Smart devices unit, Nieuw Eyckholt 300, 6419 DJ Heerlen, the Netherlands.
* Correspondence: c.pawley@maastrichtuniversity.nl; Tel.: +31-(0)43-388-5186

Academic Editor: name
Received: date; Accepted: date; Published: date

Abstract: The implementation of Molecularly Imprinted Polymers (MIPs) into sensing systems has been demonstrated abundantly over the past decades. In this article, a novel application for a MIP-based thermal sensing platform is introduced by using the sensor to characterize the drug release kinetics of a nano-sized silver-organic framework. This so-called Ag-nanosponge was loaded with acetylsalicylic acid (aspirin) which was used as a model drug compound in this study. The drug elution properties were studied by placing the nanosponge in phosphate buffered saline solution for two days and measuring the drug concentration at regular time intervals. To this extent, an acrylamide-based MIP was synthesized that was able to detect aspirin in a specific and selective manner. Rebinding of the template to the MIP was analyzed using a thermal sensor platform. The results illustrate that addition of aspirin into the sensing chamber leads to a concentration-dependent increase in the phase shift of a thermal wave that propagates through the MIP-coated sensor chip. After constructing a dose-response curve, this system was used to study the drug release kinetics of the nanosponge, clearly demonstrating that the metalorganic framework releases the drug steadily over the course of the first hour after which the concentration reaches a plateau. These findings were further confirmed by UV-VIS spectroscopy, illustrating a similar time-dependent release in the same concentration range, which demonstrates that the MIP-based platform can be used as a low-cost, straightforward tool to assess the efficacy of drug delivery systems in a lab environment.

Keywords: Molecularly imprinted polymers, thermal detection, nanosponge, drug delivery.

1. Introduction

Molecular imprinting technology originally focused on the development of imprinted particles that could be packed into columns for affinity separation, exploiting the affinity and selectivity to extract a molecule of interest from complex matrices [1-3]. The concept was soon extended by using molecularly imprinted polymers (MIPs) as antibody or enzyme mimics [4, 5]. One of the most interesting applications for MIPs is their incorporation into biomimetic sensing devices as they mimic the affinity of a natural receptor for its target, but are superior in terms of their chemical, thermal and long term stability [6-8] and can be made through a straightforward and relatively low-cost synthesis process [9]. Although MIPs have been combined with optical [10, 11], electrochemical [12-15], microgravimetric [16, 17], and thermal transducers [18-22] in devices with great potential for *e.g.* diagnostic applications, the translation of these lab-based devices into commercially available sensors is still challenging due to difficulties with reproducibility, integrated sampling and automated signal

processing [23]. Therefore, this paper illustrates a new potential application for MIP-based sensing systems, *i.e.* the study of the elution kinetics of drug delivery systems.

Nanosponges are virus-sized particles that can be used as smart drug delivery systems due to their biodegradable polymer framework [24]. Cross-linking of the polymer leads to the formation of spherical nanoparticles with a large amount of binding cavities that could be used to store drugs for steady release [25]. The solubility of drugs that poorly dissolve in water can be regulated by carefully tuning the composition of the polymer and balancing the ratio of lipophilic and hydrophilic segments [26]. Over the past ten years, nano-sized metal-organic frameworks have emerged as an interesting class of nanosponges due to their non-toxic nature and unusually large loadings of a wide variety of drugs [27]. In addition, they can be made without using organic solvents and have shown to release drugs in a gradual and sustaining manner which can be tuned by modifying the organic linkers within their binding cavities [28]. Due to their size they can reach tightly controlled areas of the body, allowing to selectively target a desired area.

Drug elution kinetics from smart drug delivery systems are traditionally studied by measuring the concentration of the drug in the medium surrounding the drug carrier. The drug concentration is usually determined by classic laboratory devices/techniques including radioactive assays [28], fluorescent resonance energy transfer [29], liquid chromatography [30] and UV-visible spectroscopy [31]. Although these methods are usually very sensitive and allow for a very accurate determination of the drug concentration and hence the elution profile, most of them require expensive readout devices, involve target labeling, or suffer from interference, limiting their performance in complex media. Therefore selective, label-free techniques have been studied based on *e.g.* electrochemical [32] and microgravimetrical [33] readout methods. These platforms allow for a straightforward and relatively fast analysis of the eluted medium but still require ultrasensitive, expensive readout technology, conductive electrodes and involved data processing and interpretation.

Therefore, we introduce an elegant MIP-based platform for the study of drug elution from a metal-organic framework in this article. Silver metalorganic frameworks were synthesized crosslinking $AgNO_3$ with ethylenediamine in a sodium hydroxide solution leading to a sponge-like structure containing a wide distribution of nanocavities. These nanosponges were loaded with the model drug, acetylsalicylic acid (aspirin), one of the drugs most abundantly used for the treatment of pain, fever, or inflammation that has also demonstrated to possess antithrombotic effects which positively affects patients at risk for heart failure [34]. The drug elution under physiological conditions was studied in the lab by applying aspirin-imprinted MIPs to aluminum electrodes and studying the propagation of a thermal wave through the chip in function of an increasing concentration of acetylsalicylic acid, a technique that was used successfully for the detection of dopamine in banana juice in previous work [21]. Next, the constructed dose-response curve from this experiment was used to determine the aspirin concentration in PBS solution containing drug eluted from the nanosponge. The results demonstrated that the nanosponge releases the drugs in a burst-like manner, releasing the drug in a constant fashion during the first hour, after which the concentration in the media surrounding the sponge remains stable. These results were validated using UV-Visible spectroscopy, which shows a similar behavior and a similar concentration range, illustrating the potential of the platform for drug elution studies.

2. Materials and Methods

2.1. Chemicals

Acrylamide (AA), azobisisobutyronitrile (AIBN), polyvinylchloride (PVC) and silver nitrate (AgNO3) were obtained from Sigma-Aldrich. Methanol (absolute) and acetonitrile were bought at Biosolve. Acetone (pure), sodium hydroxide, sulfuric acid, ethanol, salicylic acid and phosphate buffered saline (PBS) tablets were acquired from VWR chemicals. Ethylenediamine (EDA), hydrazine

hydrate and ethylene glycol dimethacrylate (EGDM) were procured from Merck Schuchardt OHG. Polydimethysiloxane (PDMS) stamps were made with the Sylgard 184 elastomer kit from Mavom NV. Aluminum chips were purchased at Brico NV and cut to the desired dimensions.

2.2. Synthesis of the metalorganic framework (silver nanosponge)

Nanosponges were synthesized by mixing aqueous $AgNO_3$ (5 mL, 0.4M, 2 mmol) with aqueous NaOH (150 mL, 15 M, 2.25 mol). Crosslinking was initiated by the addition of aqueous EDA (1.5 mL, 99% w/v, 0.25 mmol) and aqueous hydrazine hydrate (0.2 mL, 80% w/v, 5.0 mmol) to the solution. The mixture was then purged with N_2 and refluxed at 80 °C for 90 minutes. After cooling the reaction flask to room temperature, silver nanosponges were isolated by vacuum filtration and air dried before being freeze dried for 6 hours.

2.3. Molecular imprinting procedure

Pre-polymerization mixtures composed of aspirin (0.090 g, 0.5 mmol), 3 mmol AA (0.213 g) and 15 mmol EGDM were dissolved in 5 mL acetonitrile. Polymerization was initiated by adding 0.15 mmol AIBN (0.025 g) to the pre-polymerization mixture. After sonication, the mixture was deoxygenated by purging it for 5 minutes with N_2 and heated to 60 °C during 24 hours while shielding the mixture from light to prevent the template from degrading. The resulting monolith was mechanically ground using a Fritsch Planetary Micro Mill Pulverisette 7 premium line (700 rpm, 5 minutes, 10 mm balls) and sieved using a using a Fritsch Analysette 3 for 4 hours with a 20 μm mesh. The resulting powder was extracted during 96 hours at 105 °C, using a Soxhlet apparatus filled with with a mixture of methanol and ethanoic acid (7:3 v/v). Finally, the MIP particles were dried for three hours in an oven at 50 °C. Non-imprinted polymers (NIPs), serving as a reference, were synthesized in the same manner without the presence of a template.

2.4. Chip preparation

Polished aluminium plates were cut to obtain chips with the desired dimensions (10 × 10 mm2). To immobilize MIP particles onto the surface of the measurement chip, a 100 nm PVC adhesive layer (0.35 wt% PVC dissolved in tetrahydrofuran) was applied onto the chip by spin coating at 3000 rpm for 60 seconds with an acceleration of 1100 rpm/s. MIP and NIP particles were stamped into this layer using a PDMS substrate that was covered with a monolayer of polymer particles. The PVC layer was heated for 2 hours at a temperature of 100 °C way above its glass transition temperature (80 °C) allowing beads to sink into the polymer layer. The samples were cooled down prior to thermal measurements and any unbound particles were washed off with distilled water.

2.5. Selectivity test and dose-response curve

The thermal detection platform is described thoroughly in previous work [18-22]. Functionalized chips (MIP or NIP) were pressed mechanically with their backside onto a copper block serving as a heat provider. The temperature of the copper underneath the sample, T_1, was monitored by a K-type thermocouple (TC Direct). This information was fed into a temperature control unit that stringently controls T_1 by modifying the voltage over the power resistor (Farnell) that heats the copper, using a software-based (Labview, National Instruments) proportional-integral-derivative (PID) controller (P= 10, I= 8, D= 0). The functionalized side of the chip faced a polyether ether ketone (PEEK) flow cell which was sealed with an O-ring to avoid leakage, defining a contact area of 28 mm^2 and an inner volume of 110 μL. The flow cell is connected to a tubing system, allowing the administration of liquids in a controlled and automated fashion by means of a syringe pump. The temperature of the liquid inside the flow cell, T_2, is measured by a second thermocouple, placed 1 mm above the chip. For each rebinding measurement the signal was stabilized in PBS at pH 7.4 which was used as to mimic physiological conditions.

2.6. Loading of nanosponges with aspirin

Aspirin was absorbed into the nanosponges by solvent evaporation. To this extent 1.85 mmol (0.100 g) of the nanosponge was incubated with a 1.85 mmol aspirin in 6.2 mL of ethanol. The mixture was shaken for 48 hours at 750 rpm. The solvent was removed under vacuum (30 °C, 300 mbar, 90 rpm) and dried at 65 °C for 3 hours.

2.7. Drug elution analysis

Loaded nanosponges (0.1 g) were incubated in 300 mL PBS (pH 7.4 at 37 °C) while gently stirringat 100 rpm to mimic physiological conditions. Over the course of two days, 3 mL samples of the PBS were taken at regular time intervals and the aspirin concentration was analyzed by both TWTA and UV-visible spectroscopy to create an elution profile.

3. Results and Discussion

3.1. Surface characterization of Ag nanosponges

Upon synthesis the Ag nanosponges were analyzed using scanning electron microscopy (SEM). This analysis reveals that the metalorganic framework indeeds has a nanosponge structure containing a large set of nano-sized cavities, providing a large a surface area that can be loaded with drug molecules.

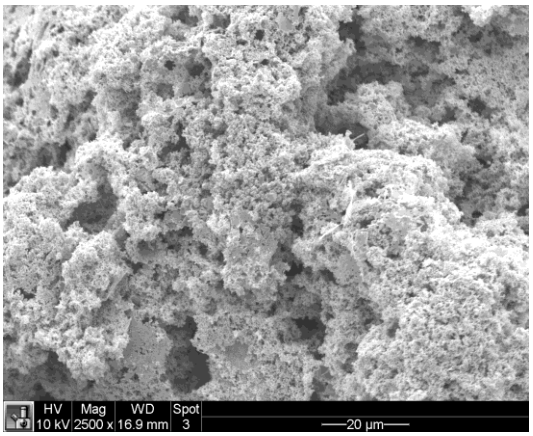

Figure 1. Surface analysis of an Ag-nanosponge using a scanning electron microscope at magnification 2500x.

3.2. Quantification of aspirin in PBS

To assess whether it was possible to accurately determine the concentration of drug eluted from the nanosponge, a dose-response curve was constructed by exposing a MIP-coated chip to an increasing concentration of aspirin in PBS. The thermal analysis clearly indicates that exposing the MIP to aspirin in increasing concentrations results in a decrease of the liquid temperature inside the flow chamber (Figure 2a) and a increase in the phase shift observed in the transmitted wave (Figure 2b). The results in figure 2b were used to construct a thermal bode plot which shows a the phase shift for each transmitted frequency in function of the cummulative concentration of aspirin present in the flow cell (Figure 3c). Although the phase shift at every concentration is most pronounced at the highest input frequency, the resolution appears to be optimal at 0.03 Hz. These findings are in line with previously obtained results with dopamine MIPs in a similar setup [21]. The time-dependent TWTA data at 0.03 Hz are used to construct a dose-response curve (figure 3d), which will be used to assess the concentration of aspirin that has eluted from the Ag-nanosponge.

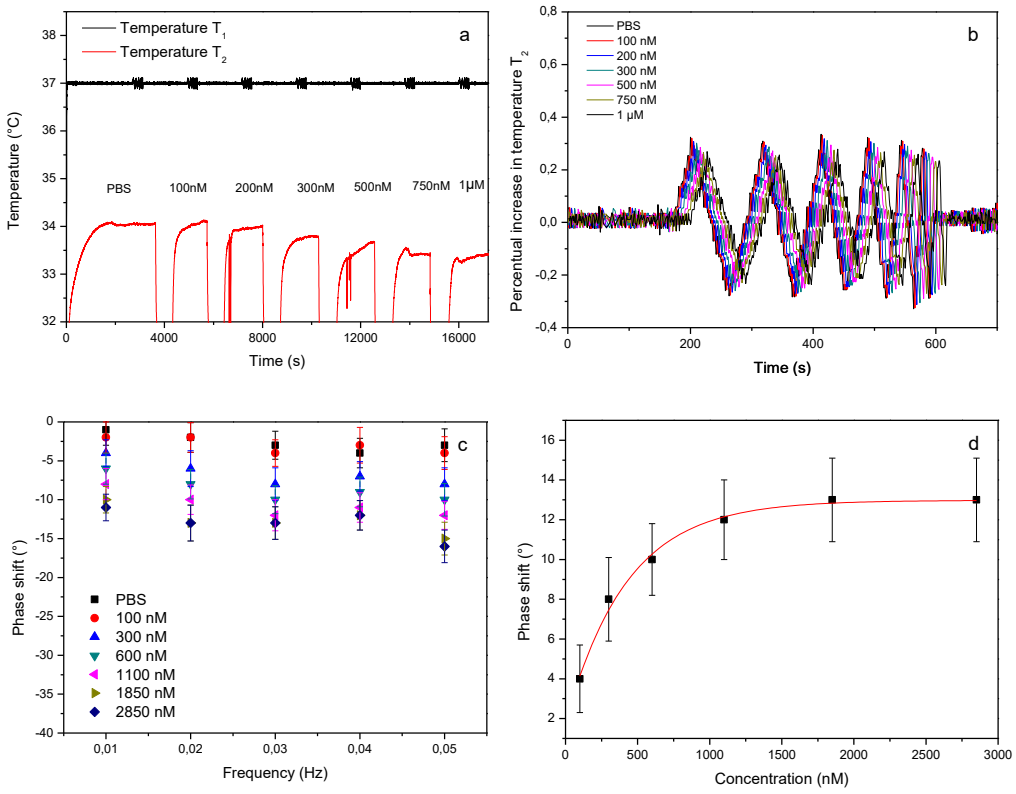

Figure 2. Aspirin rebinding analysis. The time-dependent temperature data (a) and thermal wave transport analysis spectrum (b) are shown in response to adding an increasing concentration of aspirin. The phase shift at an optimal resolution at frequency 0,03 Hz (c) and a dose-response curve (d) are constructed from these data and are plotted in function of the cummulative concetration present in the flow chamber.

3.3. Selectivity test

In order to assess whether the aspirin recognition was selective and specific, the experiment summarized in the previous section was repeated for a NIP-coated electrode. In addition, both MIP and NIP were exposed to an increasing concentration of acetaminophen (paracetamol). The resulting dose-response curves and the corresponding fits are shown in Figure 3.

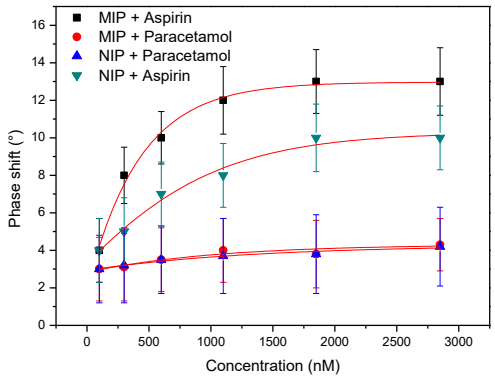

Figure 3. MIP selectivity test. The data show that exposing both the aspirin MIP and the NIP do not respond to an increasing concentration of the analogue molecule. However, the imprinting factor is limited as the difference between MIP and NIP is small.

The results in figure 3, illustrate that although the MIP is suprisingly selective in discriminating between paracetamol and aspirin, the imprinting factor is small. This in line with previous findings with similar AA-based MIPs and can be explained by the fact that at a neutral pH, not all binding

sites and functional groups on the target will be protonated (pK_a of aspirin is 3.49) [35]. The fact that paracetamol only slightly increases the phase shift is suprising as the non-specific binding to the MIP was expected to be similar. Although previous work has indicated that the MIP would be more specific and therefore, selective at acidic pHs, the authors decided to continue measuring at a pH of 7.4 to simulate drug elution. The results in Figure 2 indicate that the dose-response curve is highly usable and as the release pattern will be studied in PBS no interference from other molecules is to be expected. However, if the concept would be extend to complex matrices in the future the MIP synthesis route should be revised.

3.4. Thermal analysis of drug elution

The elution of aspirin from the nanosponges was studied by incubating them in PBS and retrieving a sample from the surrounding medium after 1, 10, 30, 120 and 360 minutes and after 48 hours. The elutions were diluted 5000 times with PBS to fit the linear range of the sensor. MIP-coated electrodes were exposed to these diluted elutions and their response was summarized in a temperature bode plot (figure 4a). The resulting phase shifts at 0.03 Hz were used to construct an elution profile that was compared to the previously obtained dose response curve (figure 4b) to determine the aspirin concentration in each of the eluted samples.

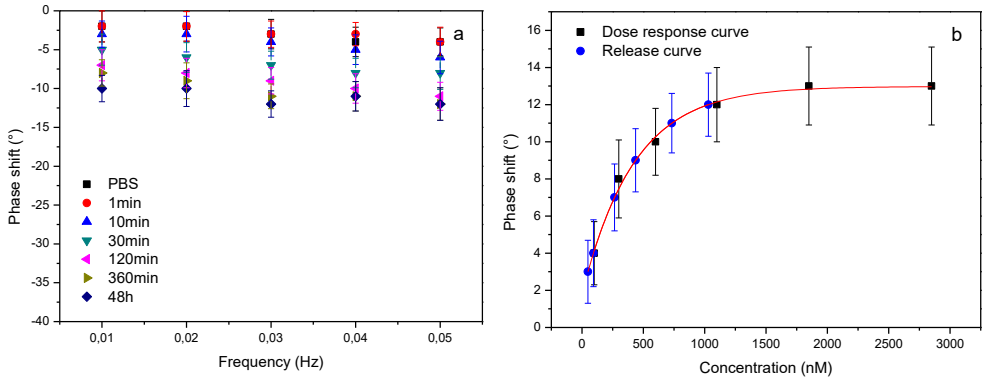

Figure 4. Drug elution analysis. The drug elution was studied using TWTA and the resulting Bode plot shows a concentration-dependent phase shift indicating that the concentration of aspirin gradually increases with time (a). The dose-response curve was used to determine the concentration in the eluted solutions (b).

The results obtained in Figure 4 were corrected for the dilution factor to create an elution profile that validated using a gold standard reference technique, *i.e.* UV-Visible spectroscopy. The elution profiles of both techniques demonstrate a similar behavior (Figure 5a): a sharp increase within the first two hours after which a stable plateau is reached that does not significantly change over the next two days. This indicates that the nanosponge releases the drugs in a relatively quick burst which is suitable for some applications requiring immediate effect. However, to actually achieve sustained, prolonged delivery of drugs the nanosponge should be functionalized with molecules that bind the drug and actually release it slowly over time.

When analyzing the elution profile obtained by UV-Visible spectroscopy, a small decrease in the concentration of aspirin can be observed over the course of two days. This can be explained by the fact that some of the acetylsalicylic acid will be converted into salicylic acid in PBS. This is confirmed by analyzing UV absorbance at 295.5 nm, which shows that salicylic acid is indeed present in the elution and its concentration will increase slightly over the course of two days (Figure 5b). The fact that this is not shown in the TWTA data is due to the fact that both compounds will bind to the MIP in a similar manner [35].

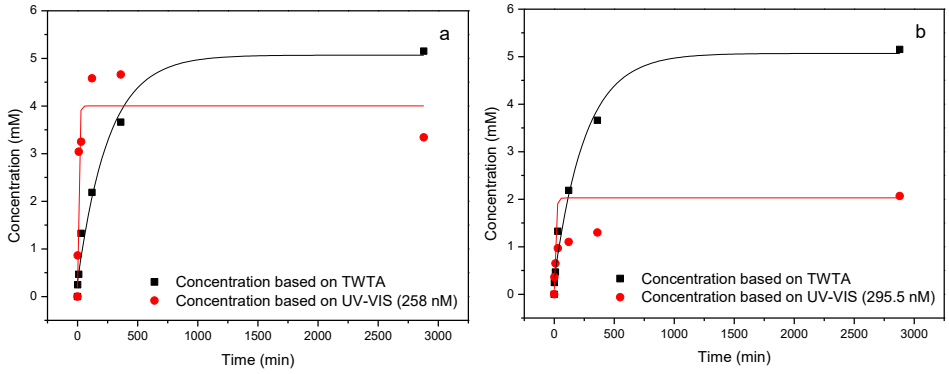

Figure 5. Validation of TWTA data by UV-Visible spectroscopy. The drug elution profile derived from the TWTA data in figure 4 were compared to the drug elution profile obtained with UV-Vis and both show a initial burst of aspirin release in the first two hours in the milimolar regime (a). The decrease in aspirin concentration for the UV-vis data shown in figure 5a can be explained by conversion of aspirin into salicylic acid which is confirmed by analyzing absorbance at 295.5 nm.

4. Conclusions

The data shown in this article illustrate the potential use of a MIP-based thermal detection platform, which has previously been used for diagnostic purposes, for analyzing the drug release kinetics of drug delivery matrices. A proof-of-principle was demonstrated by validating the results obtained with the biomimetic sensor using UV-Visible spectroscopy, demonstrating a similar profile in the same concentration range. The metalorganic framework synthesized in this work appears to release the model drug, aspirin, within the first two hours. Future research should be aimed at functionalizing the framework to get to a more gradual release of the drug. In addition, loading and elution of other, potentially more relevant drugs should also be studied. As the MIP platform is generic, it can be used to study a wide variety of targets in a wide variety of matrices, by changing the MIP receptors or optimizing their selectivity or performance in more challenging media and chemical conditions (pH, temperature,…). Additionally, the platform has been recently tested for detecting molecules directly on a thermocouple wire [36], this opens up perspectives in terms of analyzing drug release *in vivo* using the proposed platform.

Acknowledgments: The authors are grateful for financial report received from the Province of Limburg (Limburg Meet project).

Author Contributions: The thermal wave transport device was designed by B.v.G. and T.J.C. TWTA measurements, data processing and interpretation were performed by B.v.G. and K.E. All nanosponges and MIPs were synthesized by C.J.P. and K.F. while input on the nanosponge synthesis and loading was provided by A.P.G. Insights on the MIP synthesis and template removal protocol was provided by E.S.R. and H.D. Input on possible experimental design were provided by G.T., C.J.P. and A.P.G. The manuscript was jointly written by P.J.W., K.E and A.P.G. All authors have given approval to the final version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Mosbach, K.; Mosbach, R. Entrapment of enzymes and microorganisms in synthetic cross-linked polymers and their application in column techniques. *Acta Chem. Scand.*, **1966**, 20, 2807-2810.
2. Sellergen, B.; Ekberg, B.; Mosbach, K. Molecular imprinting of amino acid derivatives in macroporous polymers : Demonstration of substrate- and enantio-selectivity by chromatographic resolution of racemic mixtures of amino acid derivatives. *J. Chromatogr.*, **1985**, 347, 1-10.
3. Schirhagl, R.; Hall, E.W.; Fuereder, I.; Zare, R.N. Separation of bacteria with imprinted polymeric films. *Analyst*, **2012**, 137, 1495-1499.

4. Vlatakis, G.; Andersson, L.I.; Müller, R.; Mosbach, K. Drug assay using antibody mimics made by molecular imprinting. *Nature*, **1993**, 361, 645-647.
5. Andersson, L.I.; Mosbach, K. Molecular imprinting of the coenzyme-substrate analogue N-pyridoxyl-L-phenylalaninanilide. *Makromol. Chem., Rapid Commun.*, **1989**, 10, 491-495.
6. Chianella, I.; Guerreiro, A.; Moczko, E.; Caygill, J.S.; Piletska, E.V.; De Vargas Sansalvador, I.M.P.; Whitcombe, M.J.; Piletsky, S.A. Direct Replacement of Antibodies with Molecularly Imprinted Polymer Nanoparticles in ELISA—Development of a Novel Assay for Vancomycin. *Anal. Chem.* **2013**, *85*, 8462-8468.
7. Haupt, K.; Mosbach, K. Molecularly Imprinted Polymers and Their Use in Biomimetic Sensors. *Chem. Rev.* **2000**, *100*, 2495-2504.
8. Ye, L.; Haupt, K. Molecularly Imprinted Polymers as Antibody and Receptor mimics for Assays, Sensors and Drug Discovery. *Anal. Bioanal. Chem.* **2004**, *378*, 1887-1897.
9. Whitcombe, M.J.; Kirsch, N.; Nicholls, I.A. Molecular Imprinting Science and Technology: A Survey of the Literature for the Years 2004-2011. *J. Mol. Recogn.* **2014**, *27*, 297-401.
10. Cennamo, N.; D'Agostino, G.; Pesavento, M.; Zeni, L. High selectivity and sensitivity sensor based on MIP and SPR in tapered plastic optical fibers for the detection of l-nicotine. *Sens. Actuators B Chem.* **2014**, 191, 529-536.
11. Altintas, Z.; Gittens, M.; Guerreiro, A.; Thompson, K.; Walker, J.; Piletsky, S.; Tothill, I.E. Detection of Waterborne Viruses Using High Affinity Molecularly Imprinted Polymers. *Anal. Chem.* **2015**, *87 (13)*, 6801–6807.
12. Ramanaviciene, A.; Ramanavicius, A. Molecularly Imprinted Polypyrrole-Based Synthetic Receptor for Direct Detection of Bovine Leukemia Virus Glycoproteins. *Biosens. Bioelectron*. **2004**, *20 (6)*, 1076–1082.
13. Lakshimi, D.; Bossi, A.; Whitcombe, M.J.; Chianella, I.; Fowler, S.A.; Subrahmanyam, S.; Piletska E.V. Piletsky, S.A. Electrochemical Sensor for Catechol and Dopamine Based on a Catalytic Molecularly Imprinted Polymer-Conducting Polymer Hybrid Recognition Element. *Anal. Chem.* **2009**, *81*, 3576-3584.
14. D., Cai; Ren, L.; Zhao, H.; Xu, C.; Zhang, L.; Ying, Y.; Wang, H.; Lan, Y.; Roberts, M.F.; Chuang, J.H.; Naughton, M.J.; Ren, Z.; Chiles, T.C. A Molecular-Imprint Nanosensor for Ultrasensitive Detection of Proteins. *Nat. Nanotechnol*. **2010**, *5*, 597–601.
15. Bajwa, S.Z.; Lieberzeit, P.A. Recognition principle of Cu2+-imprinted polymers—Assessing interactions by combined spectroscopic and mass-sensitive measurements. *Sens. Actuator. B Chem.* **2015**, *207*, 976-980.
16. Ratautaite, V.; Plausinaitis, D.; Baleviciute, I.; Mikoliunaite, L.; Ramanaviciene, A.; Ramanavicius, A. Characterization of Caffeine-Imprinted Polypyrrole by a Quartz Crystal Microbalance and Electrochemical Impedance Spectroscopy. *Sens. Actuator. B Chem.* **2015**, *212*, 63–71.
17. Hayden, O.; Dickert, F.L. Selective Microorganism Detection with Cell Surface Imprinted Polymers. *Adv. Mater*. **2001**, *13 (19)*, 1480–1483.
18. Wackers, G.; Vandenryt, T.; Cornelis, P.; Kellens, E.; Thoelen, R.; De Ceuninck; W.; Losada Pérez, P.; van Grinsven, B.; Peeters, M.; Wagner, P. Array Formatting of the Heat-Transfer Method (HTM) for the Detection of Small Organic Molecules by Molecularly Imprinted Polymers. *Sensors* **2014**, *14*, 11016-11030.
19. Eersels, K.; van Grinsven, B.; Khorshid, M.; Somers, V.; Püttmann, C.; Stein, C.; Barth, S.; Diliën, H.; Bos, G.M.J.; Germeraad, W.T.V.; Cleij, T.J., Thoelen, R.; De Ceuninck, W.; Wagner, P. Heat-Transfer-Method-Based Cell Culture Quality Assay through Cell Detection by Surface Imprinted Polymers. *Langmuir* **2015**, *31 (6)*, 2043−2050.
20. van Grinsven, B.; Eersels, K.; Akkermans, O.; Ellermann, S.; Kordek, A.; Peeters, M.; Deschaume, O.; Bartic, C.; Diliën, H.; Steen Redeker, E.; Wagner, P.; Cleij, T.J. Label-Free Detection of Escherichia Coli Based on Thermal Transport Through Surface Imprinted Polymers. *ACS Sens.* **2016**, *1*, 1140-1147.
21. Peeters, M.M.; van Grinsven, B.; Foster, C.W.; Cleij, T.J.; Banks, C.E. Introducing Thermal Wave Transport Analysis (TWTA): A Thermal Technique for Dopamine Detection by Screen-Printed Electrodes Functionalized with Molecularly Imprinted Polymer (MIP) Particles. *Molecules* **2016**, *21*, 552.
22. Steen Redeker, E.; Eersels, K.; Akkermans, O.; Royakkers, J.; Dyson, D.; Nurekeyeva, K.; Ferrando, B.; Cornelis, P.; Peeters, M.; Wagner, P.; Diliën, H.; van Grinsven, B.; Cleij, T.J. Biomimetic Bacterial Identification Platform Based on Thermal Wave Transport Analysis (TWTA) through Surface-Imprinted Polymers. *ACS Inf. Dis*. **2017**, *3*, 388-397.
23. Selvolini, G.; Marrazza, G. MIP-Based Sensors: Promising New Tools for Cancer Biomarker Determination. *Sensors* **2017**, 17, 718.

24. Passarella, R.J.; Spratt, D.E.; Van der Ende, A.E.; Phillips, J.G.; Wu, H.M.; Sathiyakumar, V.; Zhou, L.; Hallahan, D.E.; Harth, E.; Diaz, R. Targeted Nanoparticles That Deliver a Sustained, Specific Release of Paclitaxel to Irradiated Tumors. *Cancer Res.* **2010**, 70, 4550-4559.
25. Subramanian, S.; Singireddy, A.; Krishnamoorthy, K.; Rajappan, M. Nanosponges: A Novel Class of Drug Delivery System – Review. *J. Pharm. Pharmaceut. Sci.* **2012**, 15, 103-111.
26. Cavalli, R.; Trotta, F.; Tumiatti, W. Cyclodextrin-based Nanosponges for Drug Delivery. *J. Incl. Phenom. Macrocycl. Chem.* **2007**, 56, 209-213.
27. Horcajada, P.; Serre, C.; Vallet-Regi, M.; Sebban, M.; Taulelle, F.; Férey, G. Metal–Organic Frameworks as Efficient Materials for Drug Delivery. *Angew. Chem. Int. Ed.* **2006**, 45, 5974-5978.
28. Horcajada, P.; Chalati, T.; Serre, C.; Gillet, B.; Sebrie, C.; Baati, T.; Eubank, J.F.; Hertaux, D.; Kreuz, C.; Chang, J.S.; Hwang, Y.K.; Marsaud, V.; Bories, P.N.; Cynober, L.; Gil, S.; Férey, G.; Couvreur, P.; Gref, R. Porous metal–organic-framework nanoscale carriers as a potential platform for drug delivery and imaging. *Nat. Mater.* **2010**, 9, 172-178.
29. Bagalkot, V.; Zhang, L.; Levy-Nissenbaum, E.; Jon, S.; Kantoff, P.W.; Langer, R.; Farokhzad, O.C. Quantum Dot-Aptamer Conjugates for Synchronous Cancer Imaging, Therapy, and Sensing of Drug Delivery Based on Bi-Fluorescence Resonance Energy Transfer. *Nano Lett.* **2007**, 7, 3065-3070.
30. Wang, Q.; Ma, D.; Higgins, P.J. Analytical method selection for drug product dissolution testing. *Dissolut. Technol.* **2006**, 13, 6-13.
31. Kamberi, M.; Tran, T.N. UV-visible spectroscopy as an alternative to liquid chromatography for determination of everolimus in surfactant-containing dissolution media: a useful approach based on solid-phase extraction. *J. Pharm. Biomed. Anal.* **2012**, 70, 94-100.
32. Lee, R.; Jo, D.H.; Chung, S.J.; Na, H.K.; Kim, J.H.; Lee, T.G. Real-time and label-free monitoring of nanoparticle cellular uptake using capacitance-based assays. *Sci. Rep.* **2016**, 6, 33668.
33. Karlsson, J.; Atefyekta, S.; Andersson, M. Controlling drug delivery kinetics from mesoporous titania thin films by pore size and surface energy. *Int. J. Nanomed.* **2015**, 10, 4425-4436.
34. Miner, J.; Hoffhines, A. The Discovery of Aspirin's Antithrombotic Effects. *Tex. Heart Inst. J.* **2007**, 34, 179-186.
35. Meischl, F.; Schemeth, D.; Harder, M.; Köpfle, N.; Tessadri, R.; Rainer, M. Synthesis and evaluation of a novel molecularly imprinted polymer for the selective isolation of acetylsalicylic acid from aqueous solutions. *J. Enivorn. Chem.* **2016**, 4, 4083-4090.
36. Diliën, H.; Peeters, M.; Royakkers, J.; Harings, J.; Cornelis, P.; Wagner, P.; Steen Redeker, E.; Banks, C.E.; Eersels, K.; van Grinsven, B.; Cleij, T.J. Label-Free Detection of Small Organic Molecules by Molecularly Imprinted Polymer Functionalized Thermocouples: Toward In Vivo Applications. *ACS Sens.* **2017**, 2, 583-589.