Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

A multi-channel microfluidic platform based on human flavin-containing monooxygenase 3 for personalised medicine

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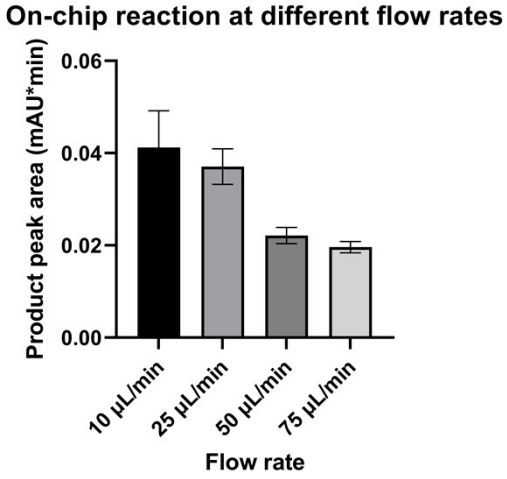

Fig. S1. Tamoxifen N-oxide production at different flow rates applied to the same microfluidic platform. Wild-type FMO3 was immobilized in all four serpentines of the chip, and after washing the unbound enzyme (100 µL/min, 4 min, with 50 mM KPi, pH 7.4), tamoxifen conversion was performed at 75 µL/min flow rate. Subsequently, channels were washed again, and the reaction was then performed at 50 µL/min flow rate. The same procedure was repeated for 25 µL/min and 10 µL/min flow rates. Product peak areas resulted to be comparable to the concentrations showed in **Figure 6B**, where data points were obtained from single use chips. These results suggest that FMO3 remains not only bound to the surface but also active for multiple usages in a reproducible manner.

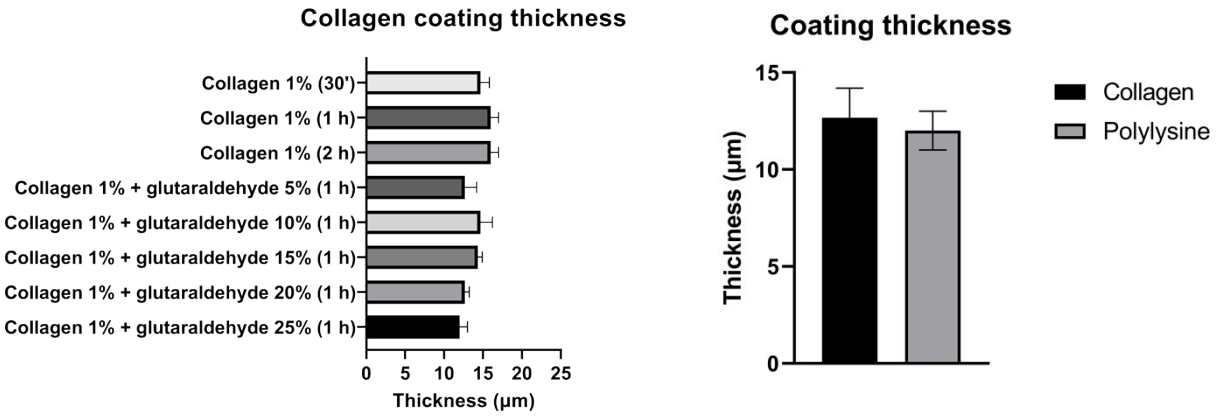

Fig. S2. Characterization of the glass surface functionalized for cross-linking. Green-fluorescent beads (diluted 1:1000 in PBS) were mixed with either collagen or polylysine (1:1 ratio) before adding glutaraldehyde to be able to look at the coating with the microscope. Different incubation times and glutaraldehyde concentrations were used for collagen, and the coating thickness was measured using z-stack (1 µm step size) (left graph). The 5% glutaraldehyde concentration was used for all the subsequent experiments. The coating thickness does not change when using polylysine instead of collagen (right graph).

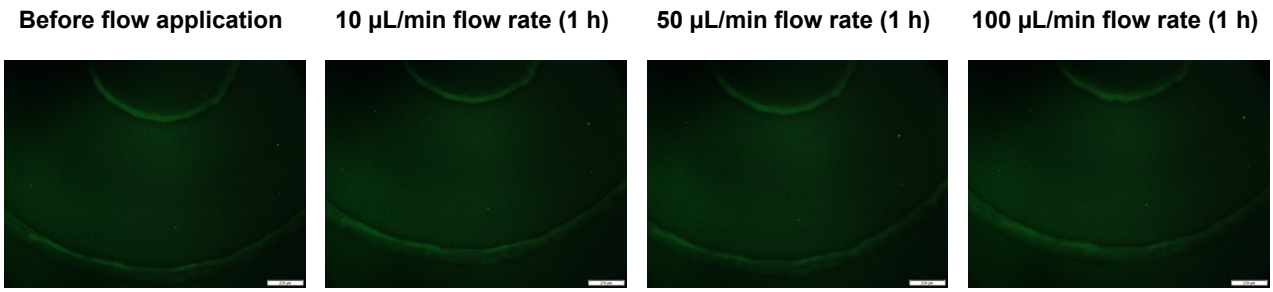

Fig. S3. Microscopic images of a serpentine's turn after coating with glutaraldehyde-functionalized polylysine. To make sure that the coating is stable, and it is not disrupted by the flow, several pictures were taken before and after the application of different flow rates (10, 50, 100 µL/min) for 1 hour. The stability of the coating was checked on a serpentine's turn, where it was more likely to detach or break. The coating resulted to be stable for each investigated flow rate, since no sign of detaching or breaking was detected, and the fluorescence remained the same.

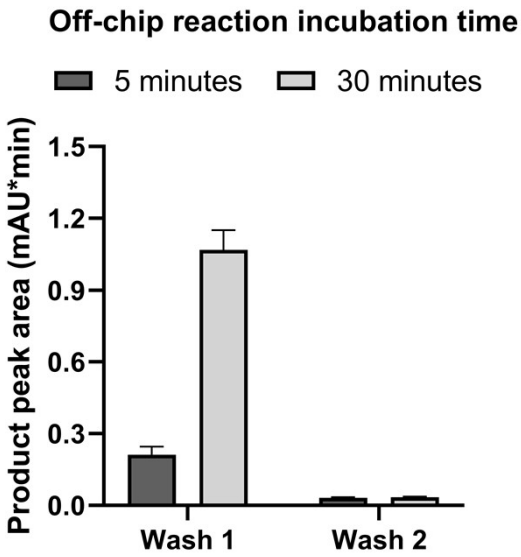

Fig. S4. Amount of Tamoxifen N-oxide produced by unbound FMO3 washed-out from polylysine-coated serpentines after immobilization via cross-linking. After removing the excess enzymatic solution from the serpentines with a micropipette, a syringe pump was connected to the chip and a solution with 100 µM tamoxifen and 1 mM NADPH in 50 mM potassium phosphate buffer, pH 7.4 was pumped through the channels at 100 µL/min. One set of samples was collected during the first 4 minutes of washing (wash 1); a second set of samples was collected 4 minutes after the first one (wash 2). If free enzyme is present in the samples, then it will oxygenate the substrate molecules that passed through the channels. The more enzyme is washed out from the chip, the more tamoxifen is converted into its N-oxide product over time. Both set of samples (wash 1 and wash 2) were kept at room temperature to let the reaction proceed for 5 minutes. The same procedure was then repeated on another functionalized microreactor, and both set of samples (wash 1 and wash 2) were kept at room temperature to let the reaction proceed for 30 minutes. Results are shown in Figure S1, in the Supplementary Information file now available. Product peak areas resulted to be higher in wash 1 samples for both incubation times. In addition, a five-fold increase is observed in the 30 minutes sample when compared to the corresponding 5 minutes sample, indicating that wash 1 contains free FMO3 which can convert tamoxifen over time. On the other hand, very small product peaks could be detected in wash 2 samples, and the peak area did not increase with increasing incubation time. These results suggest that there is no washed-out enzyme in the samples and the detected product is formed by the immobilized FMO3. In conclusion, a 4 minutes wash at 100 µL/min is enough to remove all the excess enzyme from the polylysine-coated serpentines and make sure that the observed product peak only depends on the catalytic activity of the immobilized FMO3.

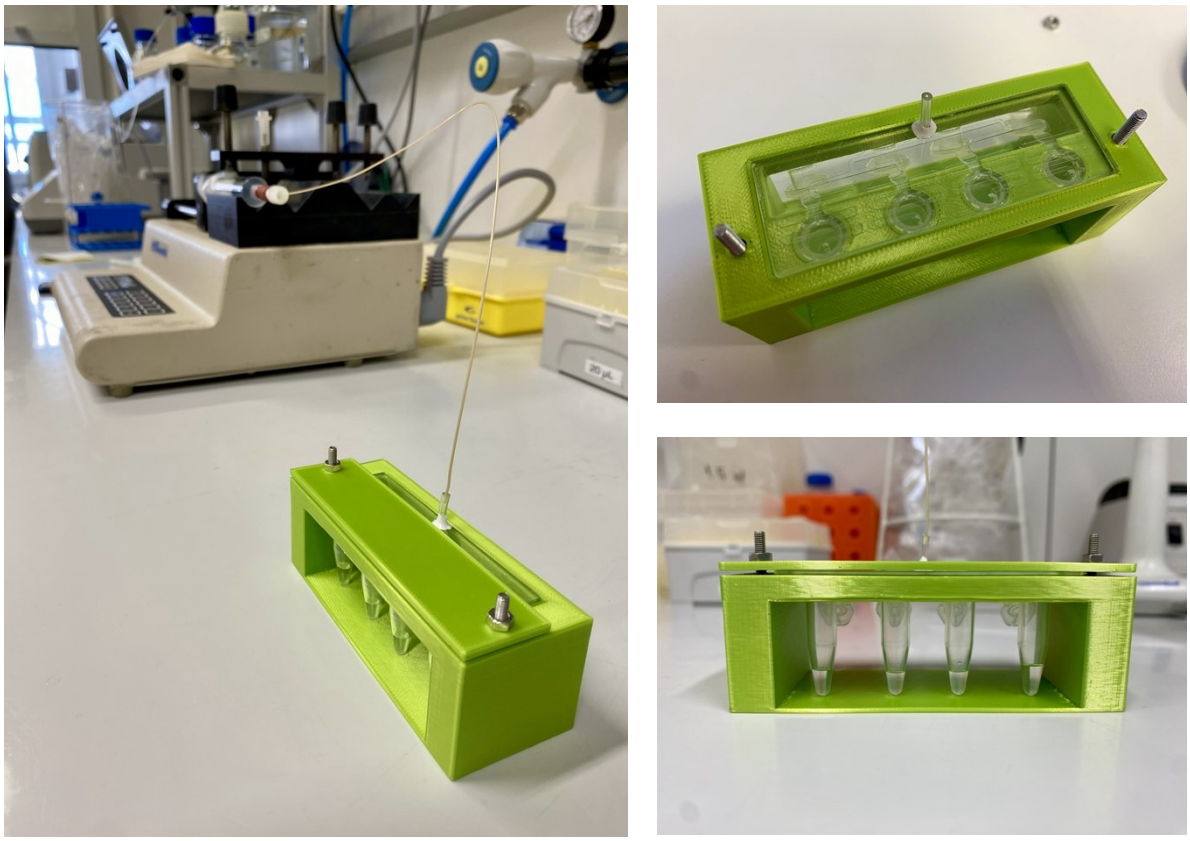

Fig. S5. Real experimental setup images for data collection with the microfluidic platform. A port is glued to the chip's inlet to allow the connection to the syringe pump. A 3D-printed PLA (polylactic acid) structure (designed with AutoCAD) holds the chip and four Eppendorfs placed in correspondence with the outlets to facilitate sample collection. Two screws hold in place a small lid that covers the chip and make the structure more robust.