Electronic Supplementary Material (ESI) for Lab on a Chip

Functionalization of polycarbonate with proteins; open-tubular enzymatic microreactors

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Supporting Information

Preparation of the chips: For preparation of the each type of bioreactor we used the following initial procedure: i) 10% isopropanolic solution of PEI was passed through 400 μm × 400 μm × 328 mm microchannals with the rate of flow 4 mL/h at 70^{o}C for 2h and then isopropanol (15 min, 20 mL/h, RT) was used for purification and ii) we passed a water solution of cations ($ZnCl_2$ (1 mM) and $MgCl_2$ (1 mM) for immobilization of ALP and $Ni(NO_3)_2$ (1 mM) for immobilization of urese) through the microchannel at room temperature for 30 minutes with rate of flow 4 mL/h and we purified the microchannel in the same way as before. Saturation of PEI with cations was necessary and enabled preventing inactivation of enzyme by precluding chelate formation with cations which are present in enzyme structure (Zn^{2+} and Mg^{2+} for ALP and Ni^{2+} for urease) and are responsible for activity of enzymes. Moreover, we confirmed the chelate properties of PEI by XPS analysis.

After experiments all types of bioreactors were cleaned in the same way. We washed the microdevices for 30 minutes by passing water through it (V = 20 mL/h) and then we dried the microchannels by blowing air through them. Dry reactors were stored at +4^{o}C.

Procedure A: Coating with the enzyme via physical immobilization (ionic interactions).

For immobilization of ALP, the reactor was filled with an aqueous solution of ALP (concentration is presented in description of the figures) and the solution was passed at room temperature overnight (V = 3 μL/h). Before the first use, the bioreactor was washed for 2h by passing through working buffer (0.2 M diethanolamine buffer pH 9.5; V = 4 mL/h). The enzyme activity assay was performed in 0.2 M diethanolamine buffer (pH 9.5) using 1 mM NPP as a substrate without stabilization of temperature (RT).

Procedure B1: Coating with enzyme with the use of a linker

The general experimental procedure of modification of PC microchannels involved: connecting a linker to the PEI-modified surface of PC and subsequent immobilization of enzyme. For the layer of PEI with the linker, a 0,5% isopropanolic solution of poly(ethylene glycol) diglycidyl ether was applied at 70^{o}C for 1h and then isopropanol (15 min, 20 mL/h, RT) was used for purification. For enzyme immobilization, the reactor was filled with an aqueous solution of ALP (15 mg/mL) and the solution was passed at room temperature overnight (V = 3 μL/h) and after exchange inlet and outlet tubes, we used a post-modified cross-linking process with the use of 2% aqueous solution of glutaraldehyde for 0.5h at room temperature. Before the first use, the bioreactor was washed for 2h by passing through working buffer (0.2 M diethanolamine buffer pH 9.5; V = 4 mL/h). The enzyme activity assay was performed in 0.2 M diethanolamine buffer (pH 9.5) using 1 mM NPP as a substrate without stabilization of temperature (RT).

Procedure B2: Coating with enzyme with the use of a linker and a protective competitive inhibitor.

The procedure was performed in the same way as *Procedure B1* except that for enzyme immobilization the reactor was filled with an aqueous solution of ALP (15 mg/mL) with addition of 100 μM NH_4VO_3.

Procedure C: Immobilization of the enzyme with the use of an activator EDC

For enzyme immobilization, the reactor was filled with an aqueous solution of ALP, EDC and NHS (1 mg of ALP in 1 mL of water was mixing with 0.5 mg of EDC for 0.5h and then 0.5 mg of NHS was added) and the mixture was passed at room temperature overnight (V = 3 μL/h). The addition of NHS improved efficiency of EDC coupling reactions. Before the first use, we exchanged inlet and outlet tubes and then the bioreactor was washed for 1h by passing through working buffer (0.2 M diethanolamine buffer (DEA) pH 9.5; V = 20 mL/h). The enzyme activity assay was performed in 0.2 M DEA buffer (pH 9.5) using 1 mM NPP as a substrate without stabilization of temperature (RT).

Procedure for immobilization of urease with the use of EDC

The procedure was performed in the same way as *Procedure C* except that for saturation of PEI with cations we used an aqueous solution of $Ni(NO_3)_2$ (1 mM) and, for enzyme immobilization, we used an aqueous solution of urease (urease from *Canavalia ensiformis* (Jack bean) Type IX, powder, 50,000-100,000 units/g solid). The enzyme activity assays were performed in 5 mM phosphate buffer composed of equal amounts of KH_2PO_4 and K_2HPO_4 in 0.1 M NaCl using 10 mM urea.

Figures:

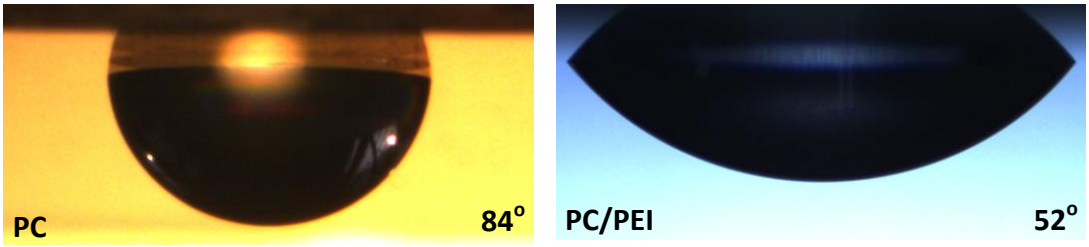

Fig.SI_1. Contact angles of water on unmodified (left) and modified with the use of PEI polycarbonate (right).

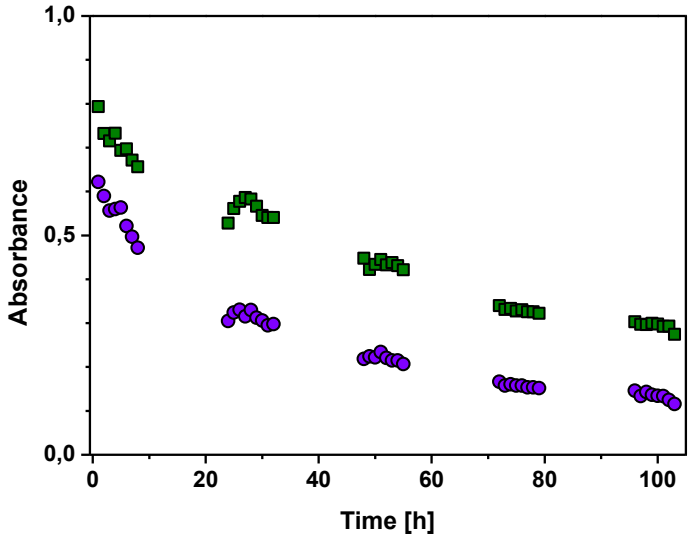

Fig.SI_2. The long-term test of operational stability of the two types of enzymatic bioreactors: a bioreactor made by the *Procedure B1*, solid squares; and a bioreactor made by the *Procedure B1* without a post-modification cross-linking, solid circles. The enzyme activity assays were performed in 0.2 M diethanolamine buffer (pH 9.5) using 1 mM NPP at room temperature and a fixed 10 mL/h rate of flow for 5 days (9h in the first and second day and 8h in the third, fourth and fifth day; in the meantime the dry bioreactors were stored in the refrigerator at +4^{o}C).

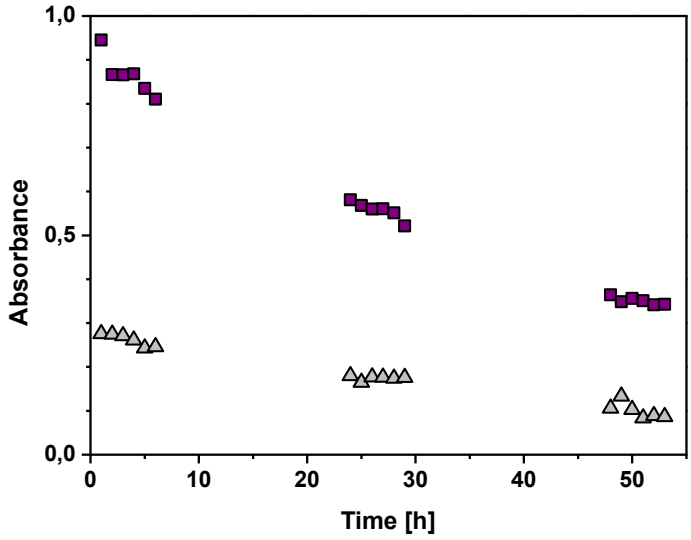

Fig.SI_3. The long-term test of operational stability of the two types of enzymatic bioreactors made by: *Procedure B2*, solid squares and *Procedure B2*and further incubated under more drastic conditions (pH 9.5, 24h), solid triangles. The enzyme activity assays were performed in 0.2 M diethanolamine buffer (pH 9.5) using 1 mM NPP at room temperature and a fixed 10 mL/h rate of flow for 3 days (6h per day; in the meantime the dry bioreactors were stored in the refrigerator at +4^{o}C).

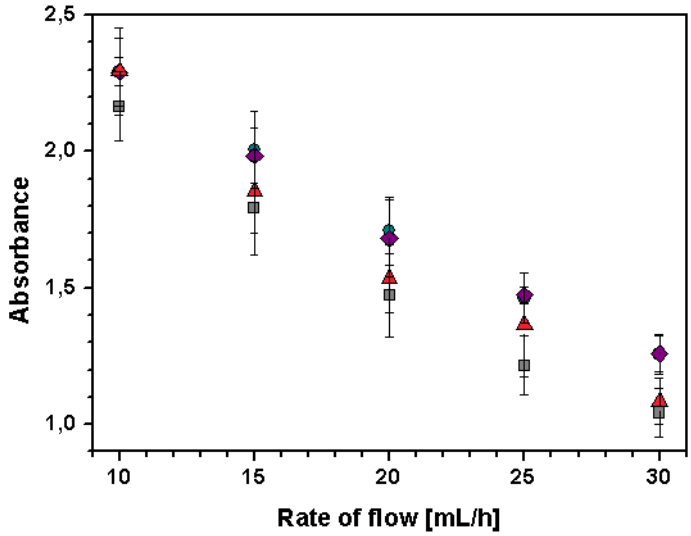

Fig.SI_4. The activity tests of immobilized enzyme (15 mg/mL solution of ALP) with the use of different mass proportion of EDC : NHS: 1:0, solid squares, 1:2, solid circles and 1:1, solid diamonds and triangles, as a function of the rate of flow of solution of NPP. Procedure of preparation includes mixing solution of ALP (15 mg/mL) with EDC for 0.5h and then addition of NHS with the exception of the last experiment (solid triangles), where ALP, EDC and NHS were mixed at the same time. The enzyme activity assays were performed in 0.2M diethanolamine buffer (pH 9.5) using 1 mM NPP at room temperature. Each data point represents an average of three series of measurements.

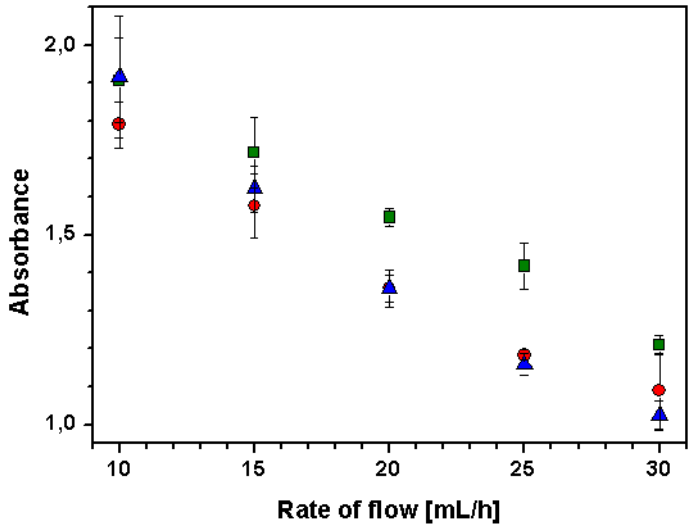

Fig.SI_5. The activity tests of immobilized enzyme (15 mg/mL solution of ALP) with the use of different mass proportion of ALP : EDC : NHS: 20:1:1, solid squares, 2:1:1, solid triangles and 1:1:1, solid circles, as a function of the rate of flow of solution of NPP. Procedure of preparation includes mixing solution of ALP (15 mg/mL) with EDC for 0.5h and then addition

of NHS. The enzyme activity assays were performed in 0.2 M diethanolamine buffer (pH 9.5) using 1 mM NPP at room temperature. Each data point represents an average of three series of measurements.

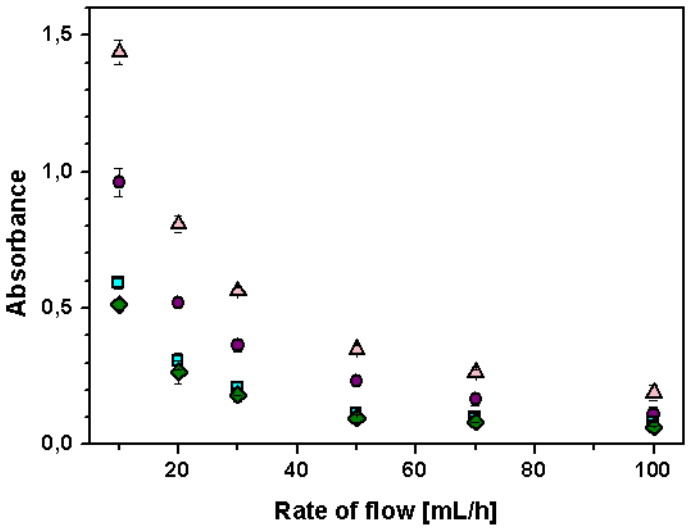

Fig.SI_6. The activity tests of immobilized ALP as a function of the rate of flow of solution of NPP for a type of bioreactor, which was prepared in a simplified way by applying ALP directly on a layer of PEI with the use of an aqueous solution of ALP: 0.1 mg/mL, solid triangles, 1 mg/mL, solid circles, 10 mg/mL, solid squares and 15 mg/mL, solid diamonds (*Procedure A*). The enzyme activity assays were performed in 0.2 M diethanolamine buffer (pH 9.5) using 1 mM NPP at room temperature. Each data point represents an average of three series of measurements.