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Fuel-independent and membrane-less self-charging biosupercapacitor

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We present a fuel-independent self-charging biosupercapacitor comprising an oxygen reducing enzymatic biocathode and an opposing bioelectrode, in which the supercapacitive properties of immobilised protein were utilised. Our findings disclose a novel hybrid type of bioelectrochemical systems, which can potentially be employed as autonomous power suppliers in substrate-deficient conditions.

The rapidly differentiating field of portable and autonomous miniature electronics operating under *ex vivo* and *in vivo* conditions requires power sources capable of efficient operation in close contact with physiological fluids or inside the human body.

Enzymatic fuel cells (EFCs) are bioelectrochemical devices, which directly convert chemical energy into electric power, while fuel and oxidant are continuously supplied to the electrodes. As distinct from the conventional fuel cells, where employment of abiotic catalysts usually requires harsh environment for efficient operation (*e.g.* strong acidic or basic media, high temperature), EFCs are capable to function at human physiological pH and temperature. EFCs utilize enzymes (oxidoreductases) to facilitate oxidation or/and reduction reactions occurring on the bioanode and biocathode, respectively. These miniature power sources enable delivering power densities up to several mW cm^{-2} and attract specific attention as autonomous power suppliers for implantable or disposable medical devices for monitoring of diseases during short periods up to several months.² The performance of EFCs was examined *in vivo* in rats,³ rabbits⁴⁻⁵ and *ex vivo* in human blood,⁶ demonstrating the perspectives of their practical application.

In spite of all possible advantages of EFCs, they cannot be efficiently applied, when the concentration of oxidant or/and

fuel is not sufficient to provide satisfactory power output or performance of the electrode is underachieved to establish the necessary level of chemical energy conversion. Several strategies have been proposed to overcome these restrictions. Switching from continuous to intermittent mode in supercapacitive EFCs allows accumulation of charge during long-term low-power operation, which can be instantaneously extracted in short high-power pulses.⁷⁻⁸ Another approach is related to the development of hybrid devices, where a low-performance bioelectrode can be replaced with a rechargeable battery-type or pseudocapacitive abiotic electrodes based on Prussian Blue,⁹ $\text{Ag}_2\text{O}/\text{Ag}$ ¹⁰ or MnO_2 ¹¹ for the cathodic side. However, any practical long-term applicability of inorganic materials in implantable conditions is debatable due to the inflammatory response and possible poisoning with leaking heavy metal ions. Recently a novel concept, denoted protein-based biosupercapacitors (BSCs), has been demonstrated by Mosa et al.,¹² where different protein films were layer-by-layer assembled with reduced graphene oxide. Biomodification with protein leads to a significant enhancement of the electrode capacitance due to adsorption/desorption of electrolyte ions and protonation/deprotonation of the amino acid side chains. The potential employment of surface-confined acid/base reactions of organic molecules for charge-storing purposes has also been recently demonstrated.¹³ Since the processes determining the charge-storing features of immobilised protein molecules are reversible, one can expect the recovery of the electrical double layer structure after the discharging pulse, *i.e.* so-called self-charging behavior, previously reported for enzyme-based electrodes, rapidly recharging after current pulses or when an external load was applied.⁸

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Fig. 1 (A, C) Typical cyclic voltammograms of bare (dashed curves) and biomodified (solid curves) NP⁽⁻⁾ (red curves), NP⁽⁺⁾ (black curves) and NP₃₆ (blue curves) electrodes in air saturated PB (pH 7.5), scan rate 5 mV s⁻¹. (B, D) Scanning electron microscopy images of a glassy carbon electrode modified with NP⁽⁻⁾ (B) and NP₃₆ electrochemically treated in 0.5 M H₂SO₄ (D); magnification: ×50 000.

In this work, we aimed to develop a self-charging bioelectrode based on the supercapacitive properties of myoglobin (Mb) immobilised on the surface of functionalised gold nanoparticles (AuNPs) to substitute a fuel-dependent bioanode in a conventional charge-storing EFC operating in a pulse mode for potential application under implantable or wearable conditions. Mb has a significant ratio of charged residue/molar mass, which determines a higher capacitance of Mb-based electrodes compared to bioelectrodes based on hemoglobin, cytochrome c, glucose oxidase and catalase.¹² Being a neutral protein with an isoelectric point close to 7.0,¹⁴ Mb has an overall negative charge at pH 7.5. To create the very first example of a hybrid bioelectrochemical device, combining energy-converting and electrostatic supercapacitive rechargeable bioelectrodes, an Mb/AuNPs electrode has been further coupled with a biocathode based on bilirubin oxidase (BOx), inversely charged due to oxygen reducing activity of immobilised enzyme.

AuNPs with an average size of 9.2 (NP₉) and of 35.9 nm (NP₃₆) determined by atomic force microscopy (Fig. S1, ESI†) were prepared by the common citrate-reduction procedure. As-prepared NP₉ were electrostatically coated with polycations polyethyleneimine (NP⁽⁺⁾) or poly(allylamine hydrochloride) with a subsequent coverage with polyanion poly(sodium 4-styrenesulfonate) (NP⁽⁻⁾) to obtain a positively or a negatively charged surface in neutral media following previously reported protocols.¹⁵ Detailed description of the experimental procedures is provided in the Supporting Information.

Mb was immobilised on glassy carbon electrodes modified with NP⁽⁻⁾ or NP⁽⁺⁾ to create diverse surface charges for suitable protein orientation. Both NP⁽⁻⁾ and NP⁽⁺⁾ modified electrodes have comparable capacitance of 30 ± 2 and 35 ± 2 μF cm⁻², respectively, in an air-saturated 12 mM phosphate buffer

Scheme 1. Schematic representation of the assembled fuel-independent and membrane-less self-charging biosupercapacitor in self-charging and discharging states (left and right, respectively). The structures of Mb and BOx were created using PDB 3WFT and 2XLL, respectively.

solution containing 137 mM NaCl and 2.7 mM KCl (PB, pH 7.5), which was used as the electrolyte for all electrochemical experiments (Fig. 1A). The relatively low capacitance values were attributed to the sub-monolayer coverage of coated NP₉ (Fig. 1B) used to distinguish any possible pseudocapacitive features of the charged AuNP surface suitable for adsorption/desorption of the ions from the supporting electrolyte and the charge-storing features of Mb itself. Biomodification of NP⁽⁻⁾ with Mb leads to a growth of the electrode capacitance up to 82 ± 5 μF cm⁻², in contrast to the biomodified NP⁽⁺⁾ electrodes, where a 1.1-fold reduction of the electrode capacitance was indicated. Utilization of the denatured Mb results in a significant decrease in the capacitance compared to the native protein (Fig. S2A, ESI†), confirming correlation between the protein structure and electrostatic features. The distinctive rectangular shape of the cyclic voltammogram (CV) indicates a typical pseudocapacitive behavior of the immobilised protein, similar to Mb-modified graphene electrodes,¹² azurin-modified Au(111) electrodes¹⁶ and some inorganic materials, such as RuO₂ or MnO₂.¹⁷

To assemble a fuel-independent membrane-less self-charging biosupercapacitor (SCBSC), a Mb-modified NP⁽⁻⁾ bioelectrode (Mb/NP⁽⁻⁾) possessing no anodic bioelectrocatalytic ability (Fig. S3, ESI†) was combined with an oxygen-reducing biocathode based on BOx, adsorbed on a highly developed three-dimensional gold matrix (Fig. 1D) of NP₃₆ treated in 0.5 M sulfuric acid (Scheme 1). Since the capacitance of the biocathode was 4.8-fold higher than that for the Mb-modified NP⁽⁻⁾ electrode (Fig. 1C), the charge-storing ability of the hybrid biodevice was limited by the Mb/NP⁽⁻⁾. Utilization of three-dimensional BOx/AuNPs biocathodes for bioelectrocatalytic oxygen reduction has been well established in recent works related to supercapacitive enzymatic fuel cells¹⁸ and biosolar cells¹⁹ and thoroughly evaluated in a recent review by Mano and de Poulpique,²⁰ therefore a detailed investigation of its performance was beyond the scope of this particular paper.

The open circuit voltage (OCV) of the SCBSC in equilibrium state was 0.42 ± 0.03 V, corresponding to the fully charged biocathode (open circuit potential (OCP) of 0.72 ± 0.01) and

Fig. 2 Representative charge/discharge curves of the SCBCS (A) and separate electrodes (B), enzymatic biocathode (blue curve) and a protein bioanode (red curve), in air saturated PB (pH 7.5). Discharge was carried out by applying a pulse current of $10 \mu\text{A cm}^{-2}$ for 1 s. Inset in (B): magnification of initial charge/discharge cycles.

Mb/NP⁽⁻⁾ (OCP of 0.30 ± 0.02 V) (Fig. 2). The OCV value achieved in this work is close to the OCV of most examples of supercapacitive EFCs,^{18, 21–23} but 0.15 V higher compared to a supercapacitive glucose/oxygen EFC intended to operate at micromolar concentrations of fuel.²⁴ The stabilised SCBCS was discharged by applying a current pulse of $10 \mu\text{A cm}^{-2}$ for 1 s, which leads to a voltage drop of 0.19 ± 0.02 V, which remains constant for at least 20 following cycles of continuous charge/discharge operation. The overall capacitance of the SCBCS calculated from fast galvanostatic discharge was 25% lower than the expected value considering the capacitance of the separate bioelectrodes calculated from CVs, following a common antithetical correlation between the capacitance and scan (or discharge) rate for pseudocapacitive materials.²⁵ An average peak power output of $\text{ca. } 3 \mu\text{W cm}^{-2}$ achieved for the fuel-independent membrane-less SCBCS is similar to that for lactate/oxygen EFCs^{26–27} and glucose/oxygen EFCs designed for operation at low concentrations of fuel.^{28–29}

The self-recharging process was carried out for 900 ± 30 s after the pulse was applied and led to a 91%, 98% and 99% recovery of the OCV after the 1st, 10th and 20th discharging pulses, respectively, indicating a dynamic stabilization of the performance of the SCBCS on an OCV of *ca.* 0.24 V (Fig. 2). This OCV value obtained for the fuel-independent SCBCS is twice as high as the value achieved in a recent report in the presence of glucose,²⁴ demonstrating the advantages of the proposed concept over conventional EFCs adapted to operate in the pulse mode.

Taking into account that a low potential drop during the discharging process and the oxygen reducing activity determine the rapid recovery of the OCP of the cathode, the self-recharging ability and stability of the assembled SCBCS are mainly determined by the Mb/NP⁽⁻⁾ (Fig. 2B). Since there were no significant changes in the capacitance, this suggests that no Mb desorption occurred during the experiments, but rather a gradual flattening and compression of the enzyme layer could be considered as the origin of the decrease in the OCV value (and growth of OCP of the Mb/NP⁽⁻⁾), similarly to the effect demonstrated for the biocathodes based on BOx physisorbed on the Au surface.³⁰

The design of a supercapacitive protein electrode based on a sub-monolayer coverage with AuNPs can easily be adapted to create transparent electrodes to substitute enzymatic bioanodes for potential utilization without any significant losses in transparency (Fig. S4, ESI[†]), in contrast to reported analogues.^{24, 26–27}

Taking into account the concept of a self-rechargeable protein electrode and the fuel-independent SCBCS presented herein, it should be emphasised that classification of biosupercapacitors into conventional and self-charging based on their operational principles⁸ cannot be justified. Even the so-called *conventional* BSCs possess complete or a particular ability to recover their surface charge in solution not only due to “active” enzymatic energy conversion ability, but also because of “passive” electrostatic equilibration, therefore classification of biological power sources to *energy converting*, *charge storing* and *hybrid* devices looks more reasonable, taking into account recent progress in the field. Energy converting systems, *viz.* conventional and supercapacitive EFCs, photobioelectrochemical cells, are designed for irreversible conversion of light or chemical energy into electric power and require substrates for operation, charge-storing BSCs and biobatteries employ electrostatic, pseudocapacitive or faradaic properties of immobilised biomaterials for reversible charge-storing purposes, whereas hybrid devices combine electrodes of different types, such as the SCBCS presented in this work.

In conclusion, the present study has revealed a novel concept of a fuel-independent and membrane-less self-charging biosupercapacitor, combining an enzymatic biocathode employing the oxygen-reducing activity of BOx and a protein electrode gaining the advantages of the pseudocapacitive properties of immobilised Mb. The characteristics of the SCBCS achieved in pulse mode together with a notable operational stability compared to recently reported analogues, allow to consider this kind of hybrid biological power source as a

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possible alternative to conventional and supercapacitive EFCs, which require substrates for active charging of enzymatic electrodes.

The findings reported herein may be a significant step towards development of more efficient and biocompatible biological power sources and self-powered biosensors intermittently operating in physiological fluids under lack of fuel, e.g. in tears or saliva.³¹ Furthermore, such an approach opens new perspectives of employment of human proteins as a bioelement in supercapacitive electrodes for potentially implantable hybrid bioelectrochemical systems with reduced inflammatory response, where redox enzymes (and human oxidoreductases in particular) cannot be efficiently utilised for energy conversion purposes.

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Conflicts of interest

There are no conflicts to declare.

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