Supporting Information

Biological electricity generation system based on mitochondria-nanochannel - red blood cells

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Keywords: Mitochondria, Biological electricity generation system, Oxygen-carrying RBCs, Nanochannel

Content of Supporting Information

1. Extraction of mitochondria by gradient density centrifugation [s1]

The mitochondrial extraction kit was purchased from Nanjing Jiancheng Bioengineering Institute. Sprague-Daley (SD) rat was provided by experimental animal house of Peking University.

Firstly, fresh liver tissue was obtained from SD rats. 200mg of fresh liver tissue was weighed and washed with saline solution to remove excess blood. Then liver tissue was broken into tiny fragments and added 1.5 ml of 4 ℃ cell lysis solution (obtained from the extraction kit). The cells were ground 20 times with a close-clearance pestle to fully disrupted. The tissue homogenate was transferred to a 4 °C tube, and centrifuged at 800×g for 5 min. Nucleus, large membrane fragments and unlysed cells was at the bottom of the tube. Thirdly, carefully added 0.5 ml supernatant along a 4 °C tube wall, which has been pre-filled with 0.5 ml 60%, 30% and 18% Percoll density gradient solution respectively (obtained from the extraction kit), and make it cover the top layer. Centrifuge the mixture at 15000×g for 10 min in 4 °C. The supernatants were the cytoplasmic components and the precipitation between 60% and 30% was mitochondria. Finally, the purified mitochondrial precipitates were re-suspended with 50-100μL mitochondrial suspension (obtained from the extraction kit) and stored at -70 °C.

The solution components in the kit:

Cell lysis solution (pH 7.2): 300 mM sucrose, 5 mM N-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) and 0.2 mM Ethylene glycol tetraacetic acid (EGTA).

Percoll density gradient solution: Percoll density gradient solution involves three layered solutions of 18% Percoll (6.55 ml Percoll A, 1.45 ml Percoll stoste), 30% Percoll (5.55 ml Percoll B, 2.45 ml Percoll stoste) and 60% Percoll (3.2 ml Percoll C, 4.8 ml Percoll stoste). A Percoll buffer (pH 6.9) containing 300 mM sucrose, 10 mM TES and 0.2 mM EGTA was used as medium solution, from which three solutions of Percoll A (90 ml Percoll buffer and 2.05 g sucrose), Percoll B (90 ml Percoll buffer and 3.96 g sucrose) and Percoll C (90 ml Percoll buffer and 13.86 g sucrose) were obtained.

Mitochondrial suspension: 210 mM Mannitol, 70 mM sucrose, 5 mM Hydroxyethyl piperazine ethyl sulfonic acid (HEPES) and 0.1% bovine serum albumin (BSA).

2. The preparation process of sulfonated poly (ether ether ketone) (SPEEK) [s2]

Firstly, 8.2 g dried Poly (ether ether ketone) (PEEK, Mw = 38300 g/mol) powder was 2added to 100 mL sulfuric acid and stir at room temperature for 10 min. Then, heat the mixture to 50 ℃ and stir for 3.5 h. Ice water was added to the solution and quench out reaction to obtain white SPEEK powder. The mixture was continuously stirred at room temperature for 24 h. The white powder was washed with water to remove the residual sulfuric acid, and the residual moisture was dried at room temperature. Finally, SPEEK products was obtained after drying in oven at 60 ℃ for 48 h.

3. The preparation process of polyimide (PI) nanofiber mats[s3]

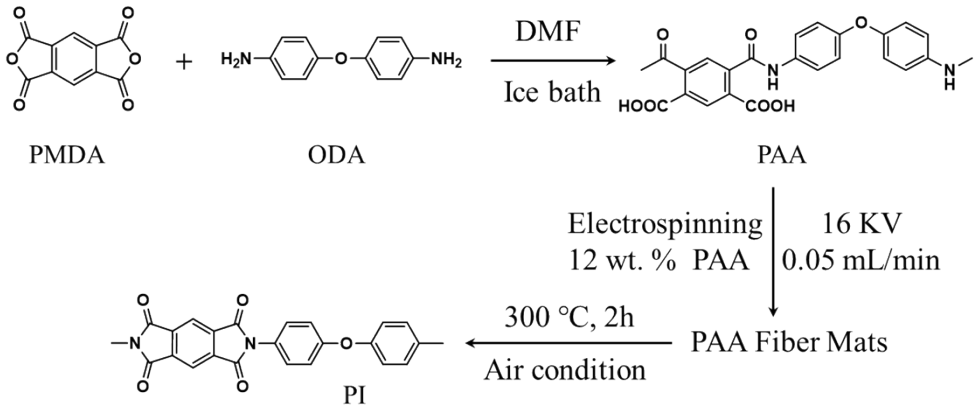

Fig. S1 The preparation process of PI nanofiber mats

The fabrication process of PI nanofiber mats was performed in the **Fig. S1**. 1.88 g 4,4'-oxidianiline (ODA) and 2.01g Pyromellitic dianhydride (PMDA) were slowly dissolved in 30ml N,N-dimethylformamide (DMF) and stirred continuously for 2 h under ice bath conditions to obtain poly (amic acid) (PAA) solution. Then, PAA nanofiber mats were obtained by electrospinning with a flow rate of 0.5 mm/min at 16kV. The distance between the syringe and the receiver was 15 cm. Finally, PAA nanofibers were thermal treated at 300 ℃ for 2 h to converted into PI nanofiber mats.

4. The FTIR spectrum of PAA and PI nanofibers

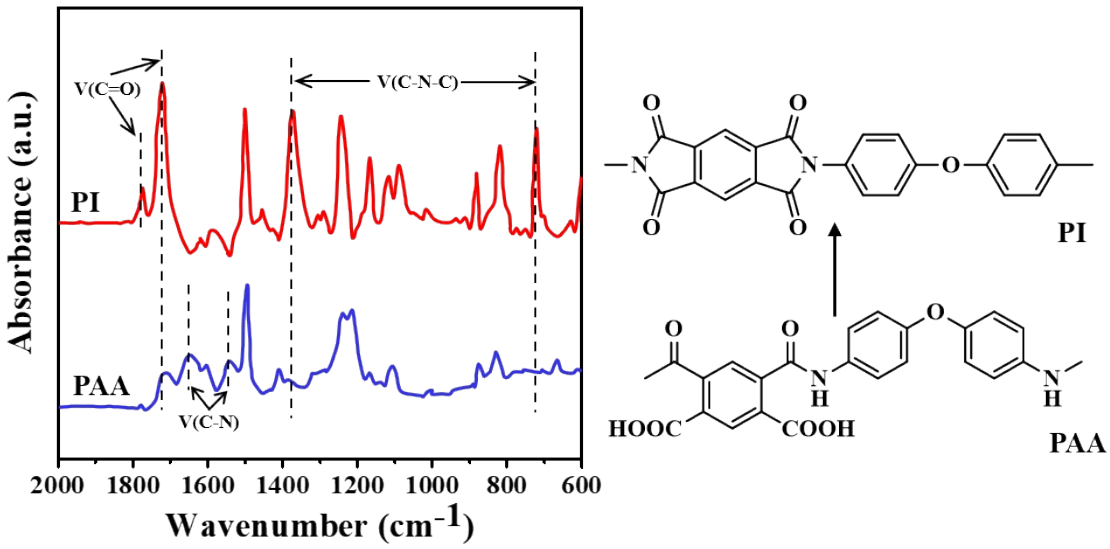

Fig. S2 The FTIR spectrum of PAA and PI nanofibers.

Based on chemical structure, the asymmetrical vibration peak of C=O in the carboxylic acid group was at 1721 cm^{-1} for PAA nanofibers, and 1721 and 1776 cm^{-1} for PI nanofibers. The amide group by 1653 and 1541 cm^{-1} can be found in PAA nanofibers, but it was disappear in PI nanofibers. C-N-C existed in the PI nanofibers could be found be found at 1376 and 724 cm^{-1} for its axial stretching vibration and bending vibration.

5. Calculation of sulfonation degree (SD) and proton exchange capacity (PEC) [s4]

The sulfonation degree (SD) was calculated by acid-base titration method and the proton exchange capacity (PEC) value was calculated according to equation s1:

$$PEC\ (mmol/g)\ = \frac{C_{NAOH}\ \times\ V_{NaOH}}{W_{dry}} \times 100\%\ (s1)$$

Where C_{NaOH} was 0.01 mol/L, V_{NaOH} was the volume of NaOH solution, W_{dry} was the weight of the dry membranes. The PEC value of SPEEK, PI/SPEEK/PI and Nafion 117 was performed in Table s1.

Tab. s1. PEC value of SPEEK, PI/SPEEK/PI and Nafion 117 membranes.

Tested PEM	SPEEK	PI/SPEEK/PI	Nafion 117
PEC (mmol/g)	1.53	1.48	0.85

The sulfonation degree refers to the percentage of hydrogen on PEEK replaced by the sulfonate group during sulfonation. Before titration by 0.01 mol/L NaOH solution, the SPEEK powder were taken into 2 mol/L NaCl solution to substitute H^+ with Na^+. The indicator was Phenolphthalein. The SD value could be calculated by equation s2:

$$\mathrm{SD} = \frac{288\ \times\ PEC}{1000\ -80\ \times PEC} \times 100\% \quad (s2)$$

The monomer molar mass of PEEK was 288 g/mol and -H replaced by -SO_3H was 80 g/mol. 1000 was introduced to uniform the units. The calculated SD value of SPEEK membranes was 50.2%.

6. Lifetime of the biologicial electricity generation system

The bio-energy conversion system (ITO/Mt + NADH | Pt/RBCs) underwent 10 days of power density output testing at room temperature. To ensure mitochondrial activity, mitochondrial anodes are 4 ℃ preserved after daily testing. Anode cell respiratory solution and NADH concentrations (1 mM) were remained stable by continuous perfusion, and cathode cell concentration was maintained at 40% volume fraction of RBCs. The lifetime testing of power density was performed in Fig. s3.

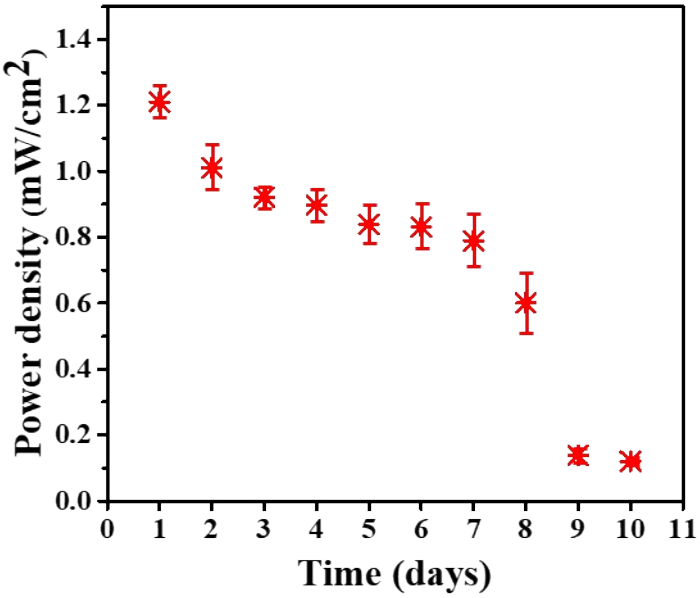

Fig. S3. The lifetime testing of power density with bio-energy conversion system (ITO/Mt + NADH | Pt/RBCs).

References

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