Supplementary Information (SI) for Analytical Methods.

Supporting Information of

Single-Fiber versus Macroscale Electrodes: Enzyme Loading and Impacts on Bioelectronic Applications in Flexible Biodevices

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1. **Enzyme immobilization procedure used to produce FCF/ADH/Nafion, ½ FCF/ADH/Nafion, and ¼ FCF/ADH/Nafion electrodes**

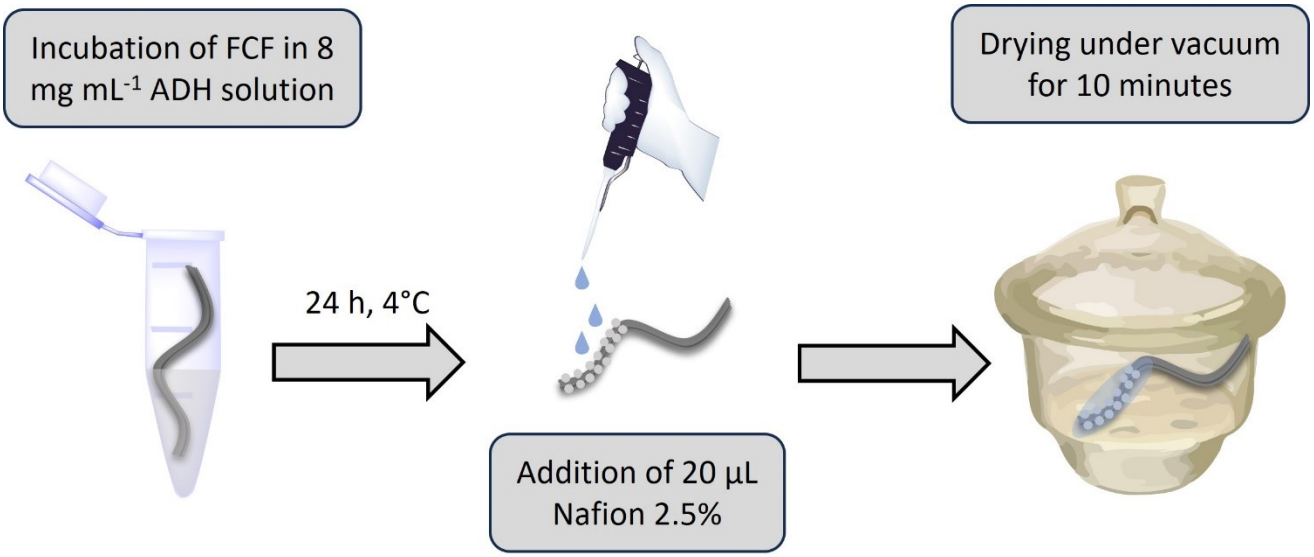

Figure S1. Enzyme immobilization procedure used for producing FCF/ADH/Nafion, ½ FCF/ADH/Nafion, and ¼ FCF/ADH/Nafion bioelectrodes. The small little-gray balls indicate the ADH enzyme physically adsorbed to the FCF.

2. Results of Ethanol bioelectrocatalysis using ½ FCF/ADH/Nafion and ¼ FCF/ADH/Nafion bioelectrodes

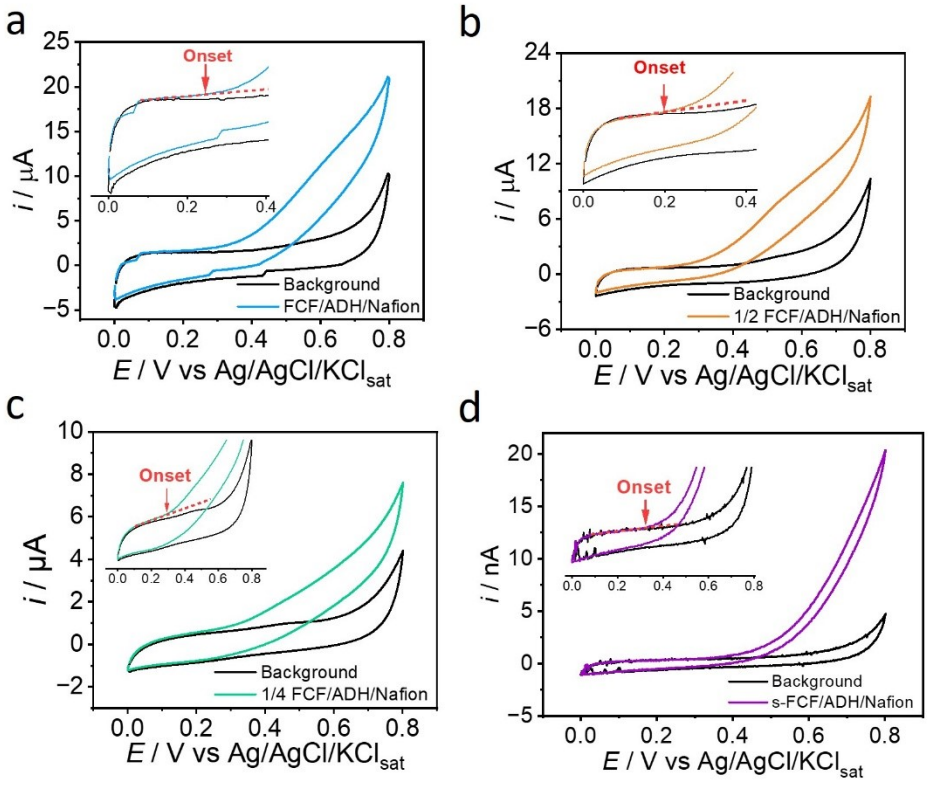

Figure S2. CVs of (a) ½ FCF/ADH/Nafion and (b) ¼ FCF/ADH/Nafion in the absence (black) and presence of 0.25 mol L^{-1} ethanol (red or green). Zoomed view from onset potential of ethanol bioelectrooxidation for (c) FCF/ADH/Nafion, (d) s-FCF/ADH/Nafion, (e) ½ FCF/ADH/Nafion, and (f) ¼ FCF/ADH/Nafion. All CV were collected using 0.2 mol L^{-1} phosphate buffer pH 7.5 added of 0.6 mmol L^{-1} NAD^+ as electrolyte, 5 mV s^{-1} as scan rate, and room temperature.

The onset values for each electrode were obtained by visual evaluation of the cyclic voltammogram as well as background current. We considered the onset potential as the first where a clear difference between the background and bioelectrooxidation currens occurs (**see arrows at Figure S7**).

3. Calculation of the electrodes´ area

The area calculation for each electrode used the Randles-Sevcik equation (**Equation 1**) considering the number of exchanged electrons (n) and diffusion coefficient (D) of $[Fe(CN)^6]^{-3/-4}$ as 1 and 4.9×10^{-6}, respectively. The other parameters have their common meaning in electrochemistry. The peak currents for each electrode were extracted from the raw CVs obtained with $[Fe(CN)6]^{-3/-4}$ and used to obtain the area of each electrode. As electrode s-FCF does not display any peak, the steady-state current was employed instead. **Table S1** summarizes the obtained area values for each electrode. It is clear from the results that the proposed weigh-based miniaturization procedure leads to a decrease in the area of electrodes.

$$I_p = (2.687 \times 10^5) n^{3/2} v^{1/2} D^{1/2} AC$$ **Equation S1**

Table S1. Calculates area of 1 FCF, ½ FCF, ¼ FCF, and s-FCF electrodes.

Electrode type	Weight (mg)	Area (cm^2)
1 FCF	17.4	3.40 ± 0.14
½ FCF	8.7	2.20 ± 0.75
¼ FCF	4.3	0.90 ± 0.39
s-FCF	Not measured	0.04

4. Micrographs of FCF/ADH/Nafion bioelectrode

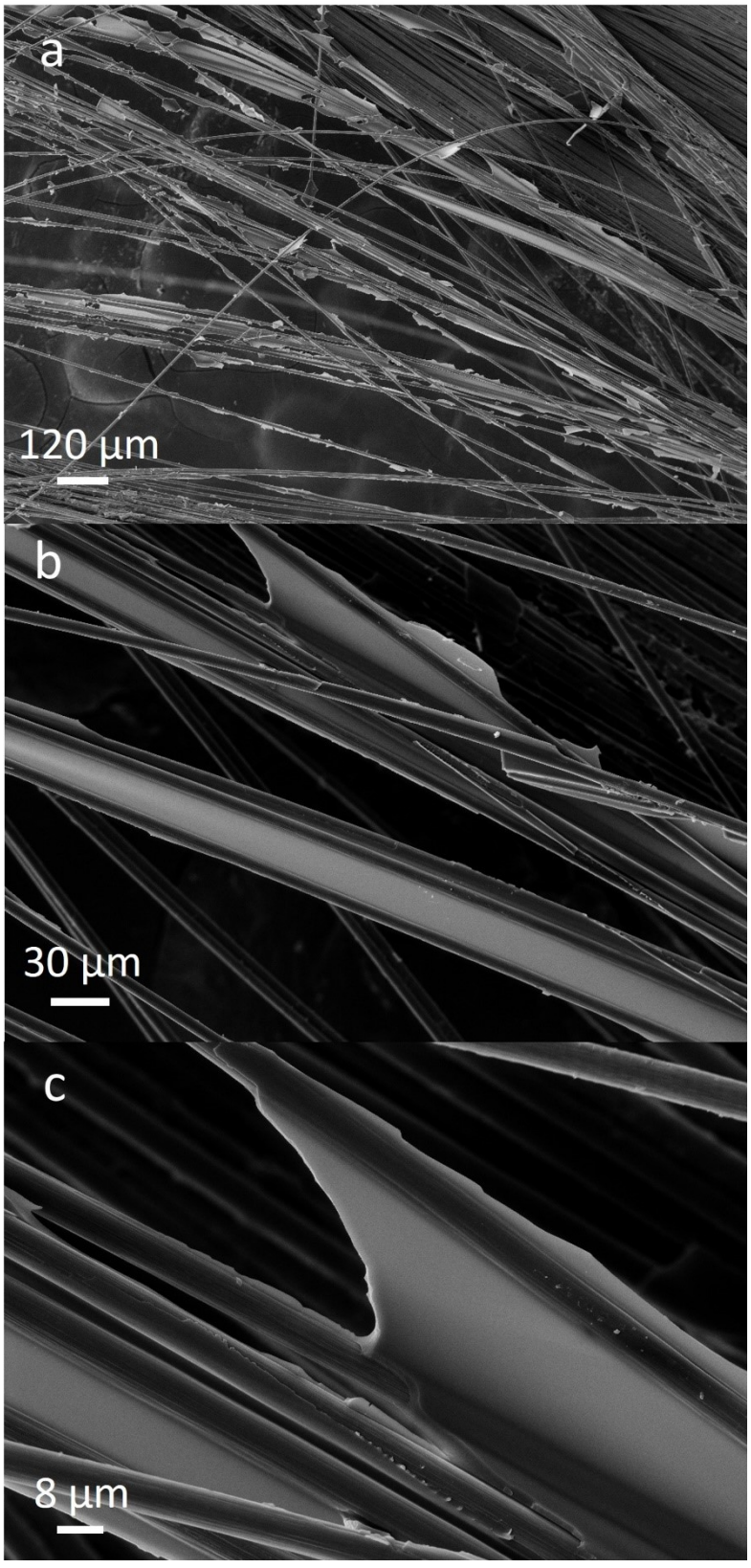

Figure S3. Micrographs of FCF/ADH/Nafion with different magnification.

5. Calculation of ADH adsorbed on the surface of ½ FCF/ADH/Nafion bioelectrode

The calculation of the amount of ADH adsorbed on ½ FCF was carried out by using a 1.6 mg mL^{-1} (x5 diluted 8 mg mL^{-1}) solution as control (black line in **Figure S4**). The samples were submitted to the immobilization process and had their absorbance measured (colored lines). We calculated the amount of adsorbed enzyme by subtracting the measured samples from the control. The molar mass employed for ADH was 1.50×10^5 g mol^{-1}. **Table S2** shows the obtained values for 5 different samples.

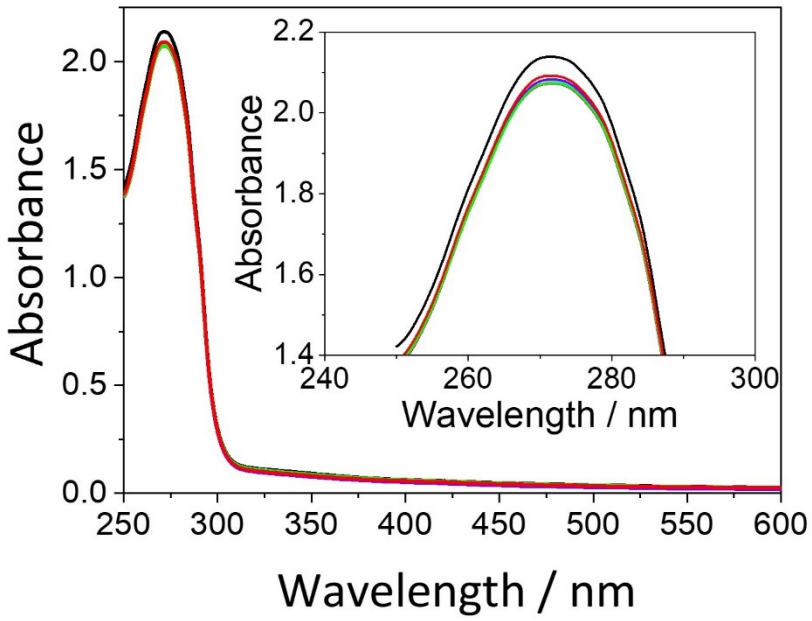

Figure S4. UV-Vis spectra of ADH solutions. The black line indicates the spectra of fresh 8 mg mL^{-1} ADH solution and the colored lines indicate the spectra of 8 different solutions after the enzyme immobilization process.

Table S2. Calculation of adsorbed enzyme on the surface of ½ FCF/ADH/Nafion.

Sample	Electrode area (cm^2)	Absorbance (278 nm)	ADH adsorbed / mg cm^{-2}	ADH adsorbed / nmol cm^{-2}
Control	---	2.05	---	---
1	0.090	1.99	0.27	1.79
2	0.090	2.00	0.23	1.53
3	0.090	2.00	0.23	1.51
4	0.084	1.99	0.28	1.85
5	0.087	2.00	0.20	1.32
Average	**----**	**---**	**0.24 ± 0.03**	**1.60 ± 0.22**

6. Micrographs of s-FCF/ADH/Nafion bioelectrode

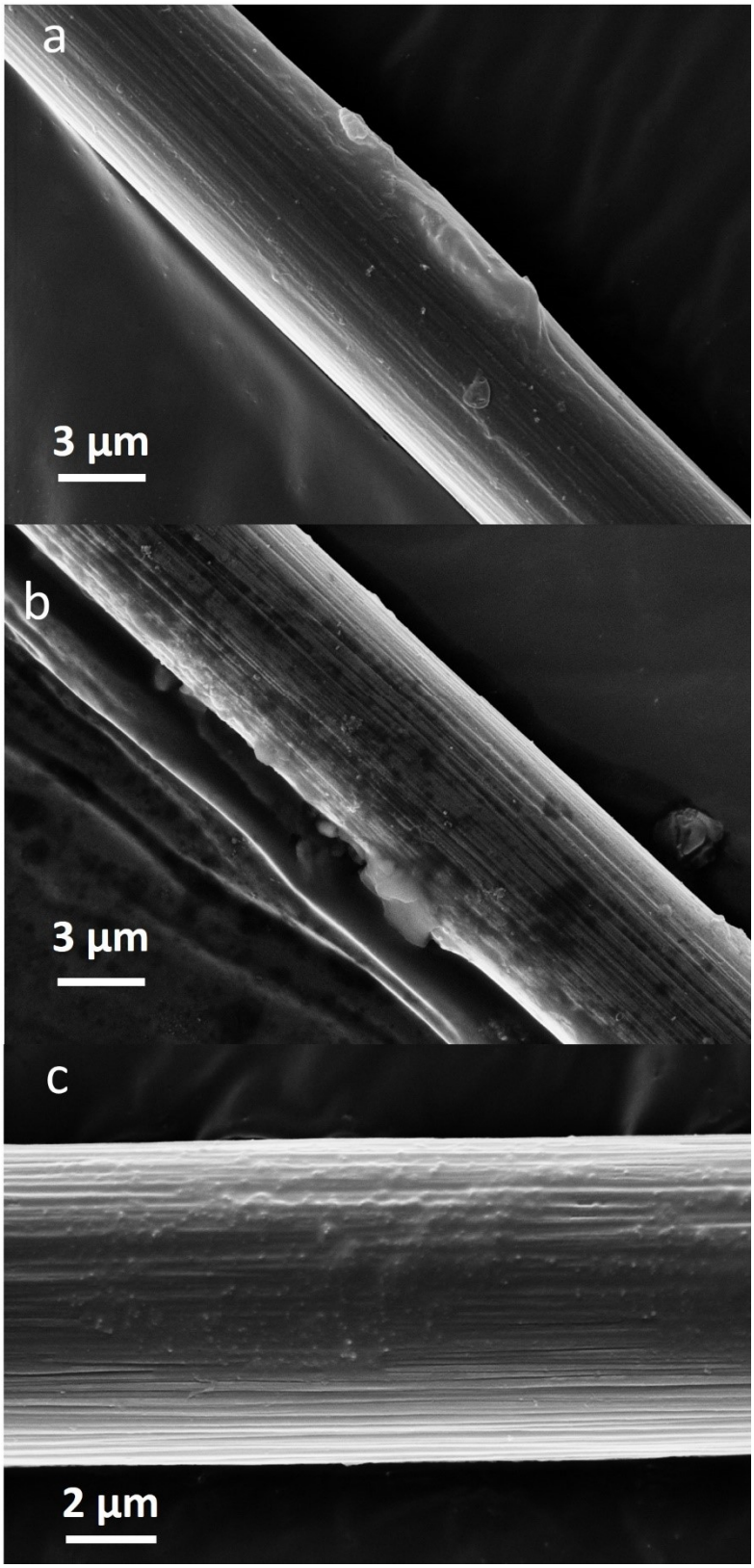

Figure S5. Micrographs of s-FCF/ADH/Nafion with different magnification.

7. Bioelectrooxidation of NADH using s-FCF electrode

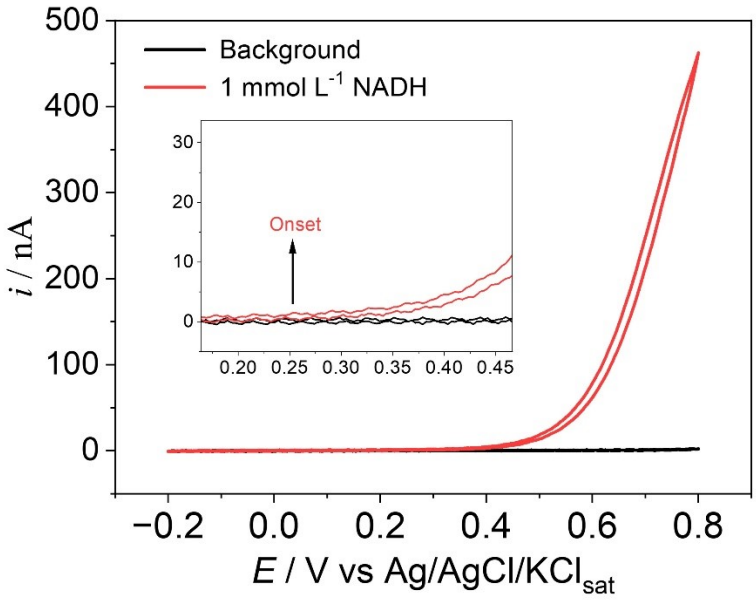

Figure S6. Cyclic voltammogram recorded using s-FCF electrode in 1 mmol L^{-1} NADH in 0.1 mol L^{-1} phosphate buffer (pH 7.5). Scan rate, 5 mV s^{-1}, room temperature, N_2 atmosphere.

8. Lineweaver-Burk equation

The Lineweaver-Burk equation is a linearized form of Michalis-Menten formalism that allows convenient calculation of some enzymatic parameters, like the maximum reaction rate (V_{max}) and the Michaelis constant (K_m). This curve relates the reciprocal values of reaction rate and substrate concentration giving Vmax and K_m as intercept and slope, respectively. A bioelectrochemical version of this equation is possible by substituting the reaction rate with the current obtained in the system, as shown in **Equation S1**. In this equation, I_{SS} is the steady-state catalytic current, I_{max} is the maximum current, and c is the bulk concentration.

$$\frac{1}{I_{SS}} = \frac{1}{I_{max}} + \frac{K_M^{app} 1}{I_{max} c}$$

Equation S1

9. Michaelis Menten Plot

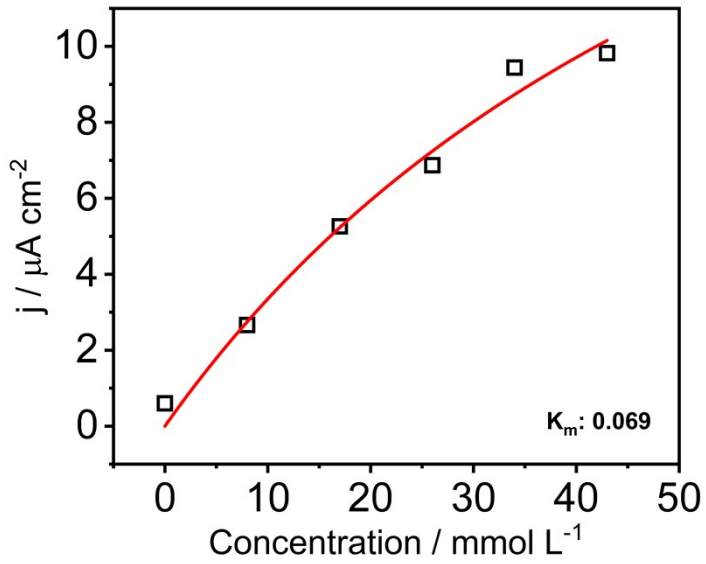

Figure S7. Michaelis-Menten plot using the current output extracted from the cyclic voltammograms in Figure 5.