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Supporting information

Application of bacterial-derived long cellulose nanofiber to suspension culture of mammalian cells as a shear protectant

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Characterization of HP-NFBC

To evaluate fiber width, 50 μ L of 1 ppm hydroxypropyl cellulose-adsorbed nanofibrillated bacterial cellulose (HP-NFBC) was first dropped onto a mica substrate. After natural drying, the substrate was observed under a microscope using an AFM (AFM5200S, HITACHI High Tech Corp., Tokyo, Japan). FiberApp software [1] (<https://fsm.ethz.ch/publications-list/software.html>) was used to analyze the obtained images and calculate the fiber width. A total of 25 fibers were analyzed.

To determine HPC adsorption, approximately 30 mg of lyophilized HP-NFBC dispersed in water was transferred into a vial and dried for 5 h at 105 °C. After the dried HP-NFBC had completely cooled, 60 % tetrabutylphosphonium hydride (Hokko Chemical Industry Co. Ltd., Tokyo, Japan) was added to the vial, and the mixture was stirred overnight at 30 °C. After HP-NFBC was completely dissolved, the solution was mixed with Milli-Q water (100 mL) and stirred for 30 min, allowing NFBC to precipitate while HPC remained dissolved. Finally, the NFBC residue was collected by vacuum filtration and dried at 105 °C for 2 h. After drying, the yield was measured to determine the amount of HPC adsorbed based on the weight difference. The fiber width, fiber length, and HPC content of HP-NFBC are shown in Table S1. The experiments were performed in duplicate ($n = 2$), and average values were calculated.

Table S1. Characterization of HP-NFBC used in this study

	Value
Fiber width [nm]	22.48 ± 8.533
Fiber length [μm] ^{*1}	> 15
HPC content [wt%]	17.9 ± 2.5

*1) from reference [3]

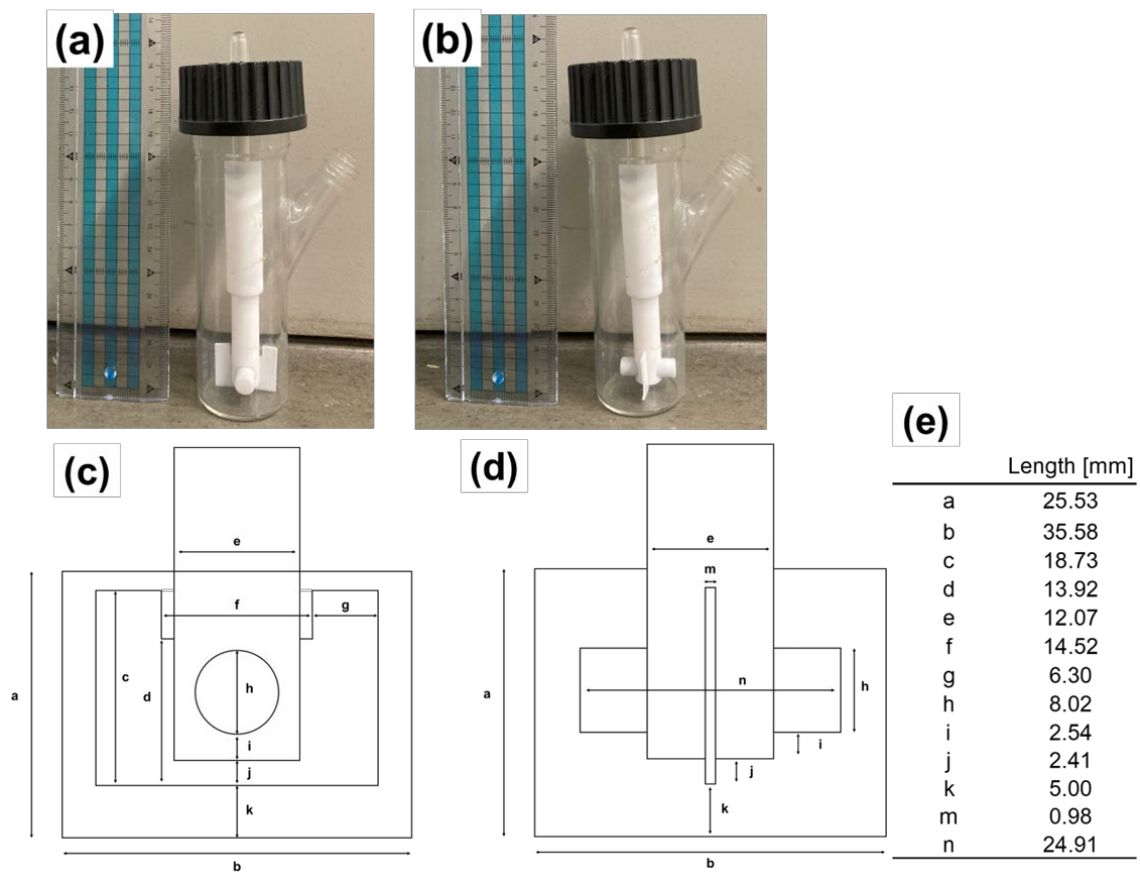


Fig. S1 Geometry of spinner flasks for suspension culture. (a) A photo of a spinner flask with a horizontally oriented impeller, (b) with a horizontally oriented magnetic stirrer bar. Drawing of a (c) horizontally oriented impeller, (d) horizontally oriented magnetic stirrer bar, and (e) the lengths of each point in (c) and (d).

Table S2. Constants of Cross model and density of each media.

	μ_0 [Pa·s]	μ_{inf} [10^{-3} Pa·s]	m [s]	n [-]	ρ [kg/m ³]
No addition	0.04360	0.7889	2.774	0.8957	1004
0.016 (w/v)% FP003	1.147	1.280	2.482	1.088	1004
0.10 (w/v)% FP003	5.571	0.3554	48.91	0.7402	1005
0.01 (w/v)% HP-NFBC	1.397	0.4363	26.18	0.8281	1004
0.05 (w/v)% HP-NFBC	34.03	0	109.3	0.8581	1005
0.10 (w/v)% HP-NFBC	18.64	0	3.927	1.012	1007
0.20 (w/v)% HP-NFBC	250.0	0.5985	10.10	1.045	1003

Cytotoxicity test of NFBC and FP003

A cytotoxicity test of HP-NFBC and FP003 was performed within the experimental concentration range used in this study on an adherent culture of CHO cells. CHO DP-12 clone#1934 (ATCC CRL-12445) was precultured for 4 d in 100 mm tissue culture dishes (Corning, NY, USA) in a CO₂ incubator under 37 °C and 5 % CO₂. The cells were seeded into 6-well plates (Violamo; AS ONE; Osaka, Japan) with 2 mL 10 % NBS Ham's F-12 medium at a cell density of 5.00×10^3 cells/cm² and incubated in a CO₂ incubator under the same conditions as the pre-culture. After 1 d of culture for cell adhesion, the media were replaced with 2 mL each of the media without additives, media with FP003 (0.016, 0.10 (w/v)%), and media with HP-NFBC (0.10, 0.20 (w/v)%). Subsequently, cells were cultured for 7 d. Cell viability and density were determined on days 1, 3, 5, and 7 after

seeding, using the trypan blue exclusion method (Fig. S2(a) and (b)). The glucose concentration in the culture medium was measured using the Glucose CII test Wako kit (FUJIFILM Wako Pure Chemical Corp., Osaka, Japan) according to the manufacturer's instructions (Fig. S2(c)).

The cell viability under each condition was nearly 100 %, with slight variations among the concentrations (Fig. S2(a)). Moreover, no significant differences were observed between the groups. Cell densities indicated that the number of cells increased exponentially until day 5, and proliferation was maintained until day 7 (Fig. S2(b)). In these growth curves, no differences were observed between the conditions. The glucose concentration in each medium was similar to that in the other media (Fig. S2(c)). Based on the above results, HP-NFBC and FP003 can be regarded as nontoxic on CHO cell proliferation at the experimental concentration range used in this study.

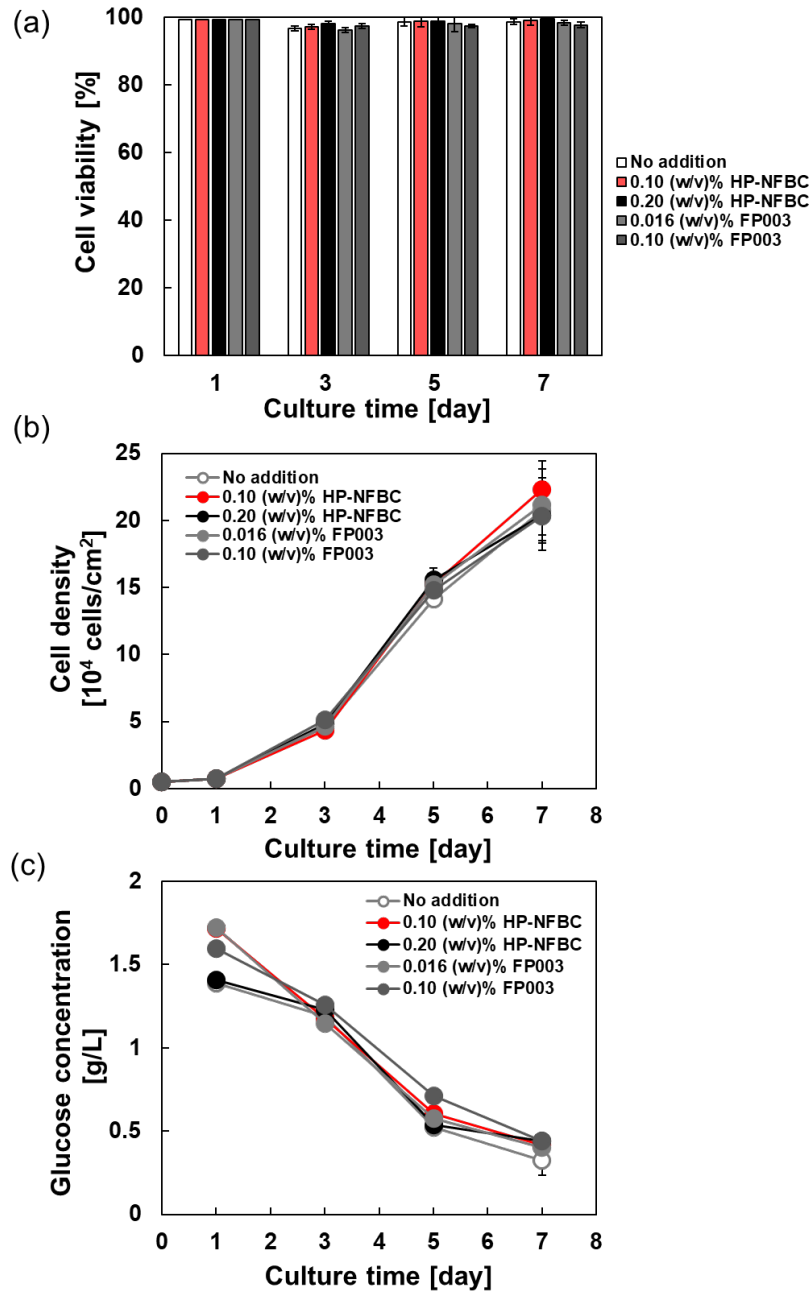


Fig. S2. Cytotoxicity tests of HP-NFBC and FP003 at the same concentrations as a suspension culture in the adherent culture of CHO cells. (a) Cell viability, (b) cell density, and (c) glucose concentration. Experiments were performed at $n = 3$, and error bars were indicated by S.D.

Effects of NFBC addition and stirring rate in suspension culture

The effects of HP-NFBC addition and stirring rate on CHO cell growth in suspension culture were investigated using media with 0.10 (w/v)% HP-NFBC and without HP-NFBC under stirring rates of 70 and 120 rpm (Fig. S3).

Results in Fig. S3 are described in Section 3.2.

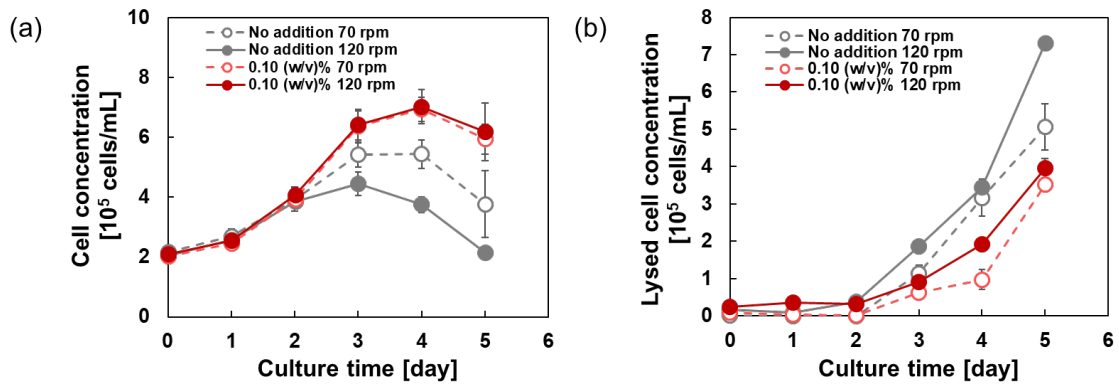


Fig. S3. Viable and lysed cell concentrations in the media with and without HP-NFBC under two different stirring rates (70 and 120 rpm). (a) Growth curves and (b) lysed cell concentration estimated by lactate dehydrogenase concentrations in the culture media. Experiments were performed at $n = 3$, and error bars were indicated by S.D.

Effect of dispersion state at the same viscosity on cell growth

The effect of the dispersion states of HP-NFBC and FP003 on cell growth was investigated using media with the same viscosities, one supplemented with HP-NFBC and the other with 0.016 (w/v)% FP003. After measuring the viscosities of media with various concentrations of HP-NFBC using a rheometer (MCR702), it was found that 0.02 (w/v)% HP-NFBC had a similar viscosity to 0.016 (w/v)% FP003 in the range of 10–30 s⁻¹ as determined by computational fluid dynamics analysis (Fig. S4).

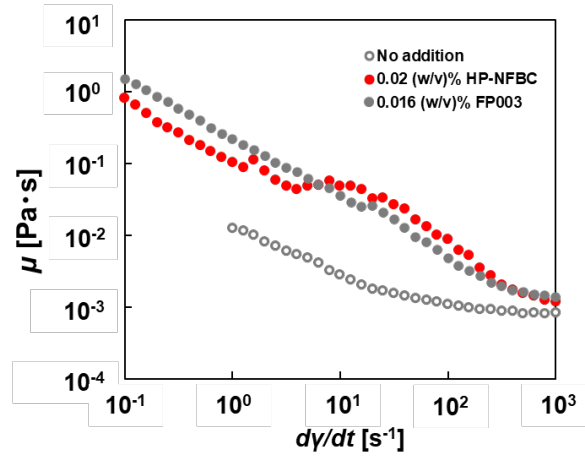


Fig. S4. Shear viscosity of media containing 0.02 (w/v)% HP-NFBC and 0.016 (w/v)% FP003.

As similar shear viscosities in Fig. S4, 0.02 (w/v)% HP-NFBC and 0.016 (w/v)% FP003 were compared in the suspension culture of CHO cells under the same conditions in Materials and methods. No significant differences in cell proliferation (Fig. S5(a)) and lysis (Fig. S5(b)) were observed between the two, indicating a negligible impact of the dispersion state on cell growth.

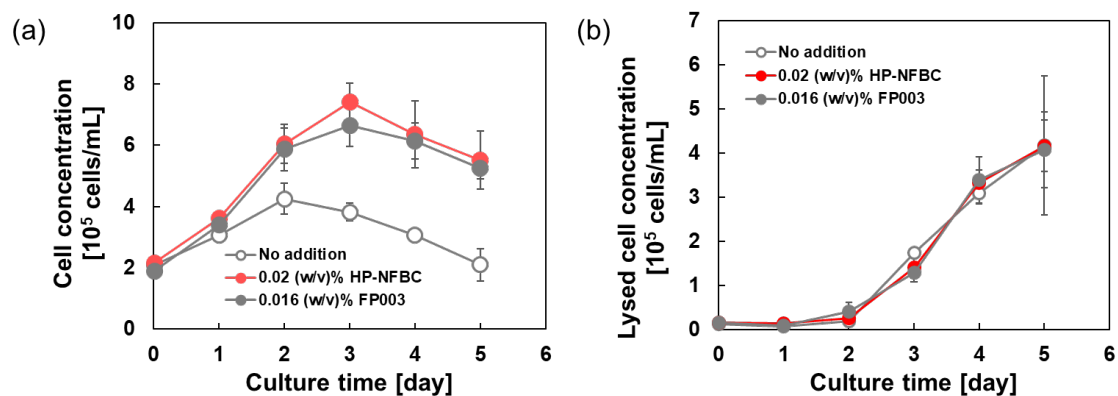


Fig. S5. Viable and lysed cell concentrations in the media with 0.02 (w/v)% HP-NFBC and 0.016 (w/v)% FP003 with similar viscosity in the range of 10–30 s⁻¹ and without both additives. (a) Growth curves and (b) lysed cell concentration estimated by lactate dehydrogenase concentrations in the culture media. Experiments were performed at $n = 3$, and error bars were indicated by S.D.

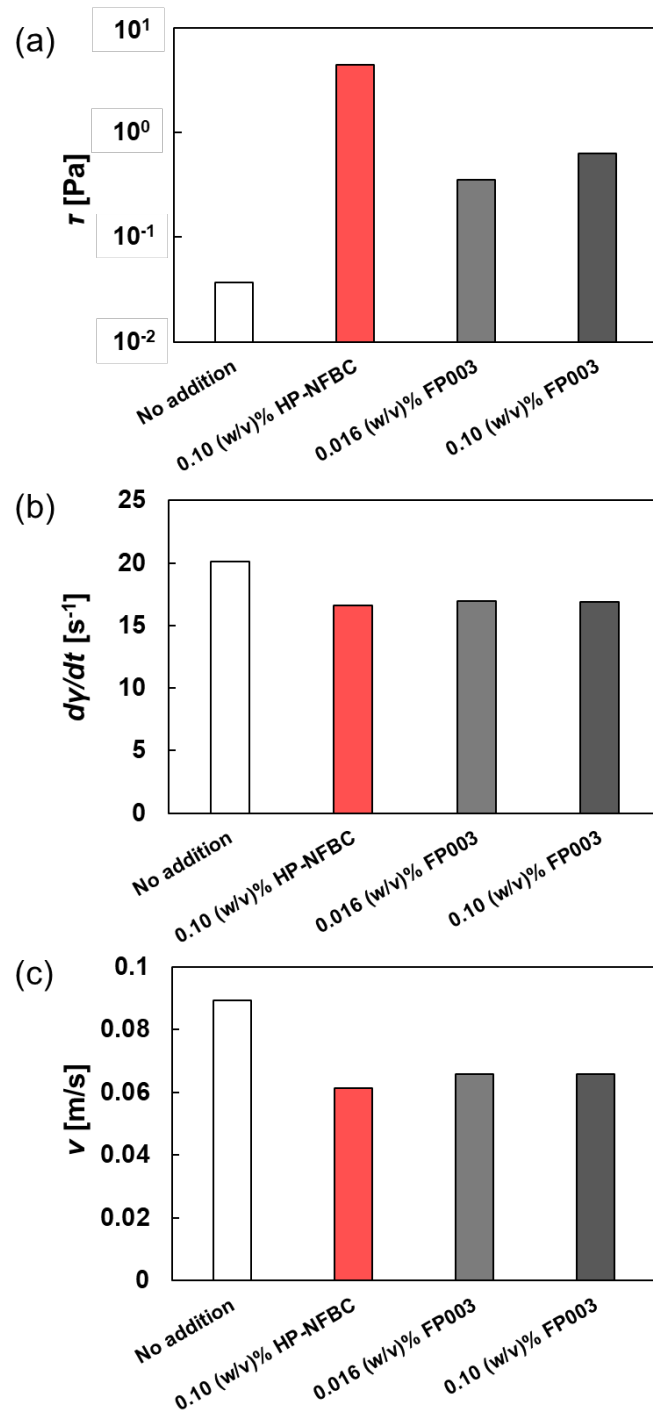


Fig. S6. Average values of the hydrodynamic parameters of the media with and without HP-NFBC and FP003 under stirring rate at 120 rpm. (a) Shear stress, (b) shear rate, and (c) flow velocity.



Fig. S7. Streamline of the media with and without HP-NFBC and FP003 under stirring rate at 120 rpm.

Integral viable cell concentration during suspension culture

The integral viable cell concentration (IVCC) during suspension culture for i days was calculated using trapezoidal integration, as shown in Eq. S1,

$$\text{IVCC} [\text{cells} \cdot \text{day/mL}] = \int_0^t \text{VCC} dt \doteq \sum_{i=1}^n \frac{\text{VCC}_i + \text{VCC}_{i-1}}{2} \times (t_i - t_{i-1}) \quad (\text{S1})$$

where VCC_i indicates the viable cell concentration on day i , and t_i indicates the culture time [days] on day i .

The IVCCs calculated from the cell concentrations in Fig. 7(a) were plotted against the corresponding HP-NFBC concentrations (Fig. S8). Results in Fig. S8 are described in Section 3.5.

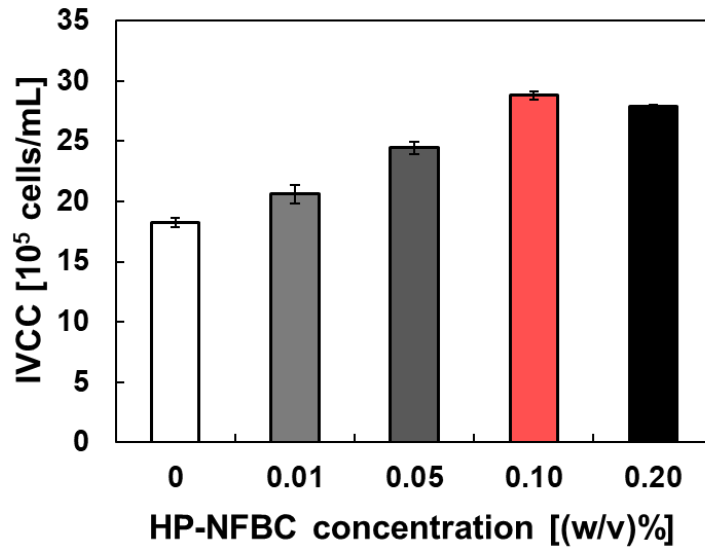


Fig. S8. Correlations between the concentration of HP-NFBC and IVCC. Experiments were performed at $n = 2$; error bars indicated the minimum or maximum value.

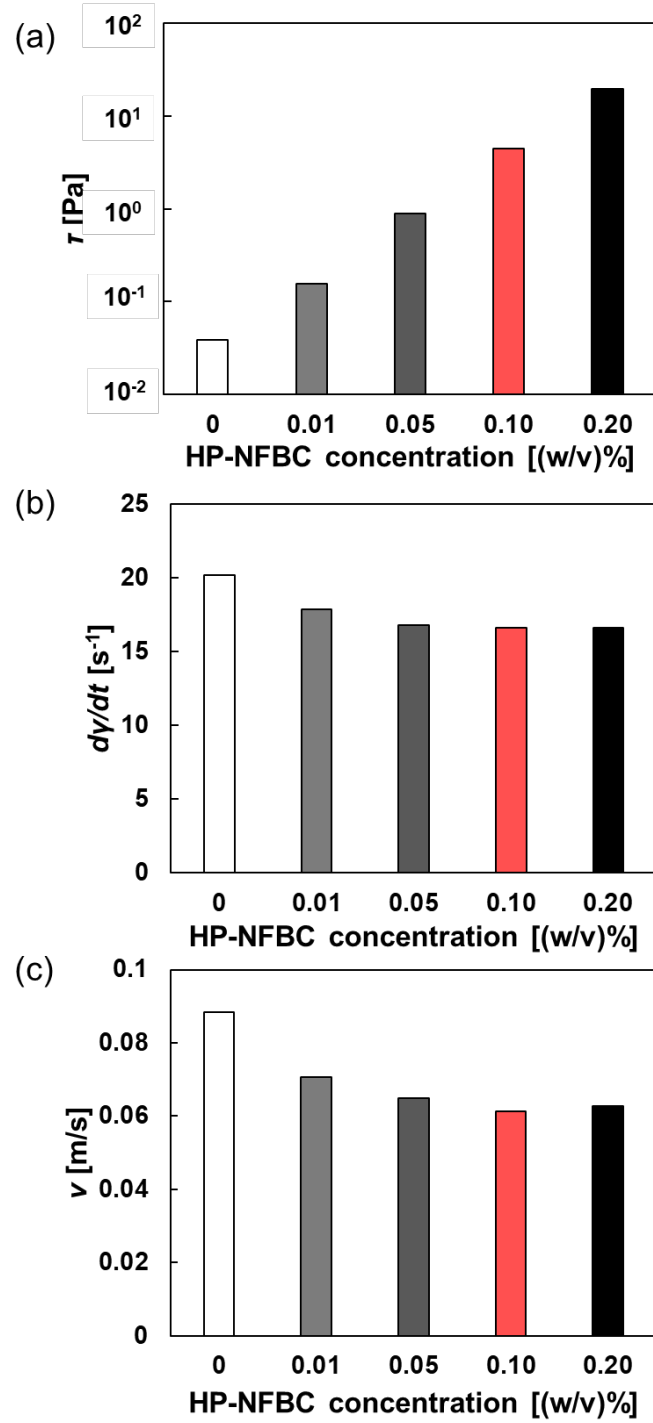


Fig. S9. Average values of the hydrodynamic parameters of the media containing various concentrations of HP-NFBC under stirring rate at 120 rpm. (a) Shear stress, (b) shear rate, and (c) flow velocity.

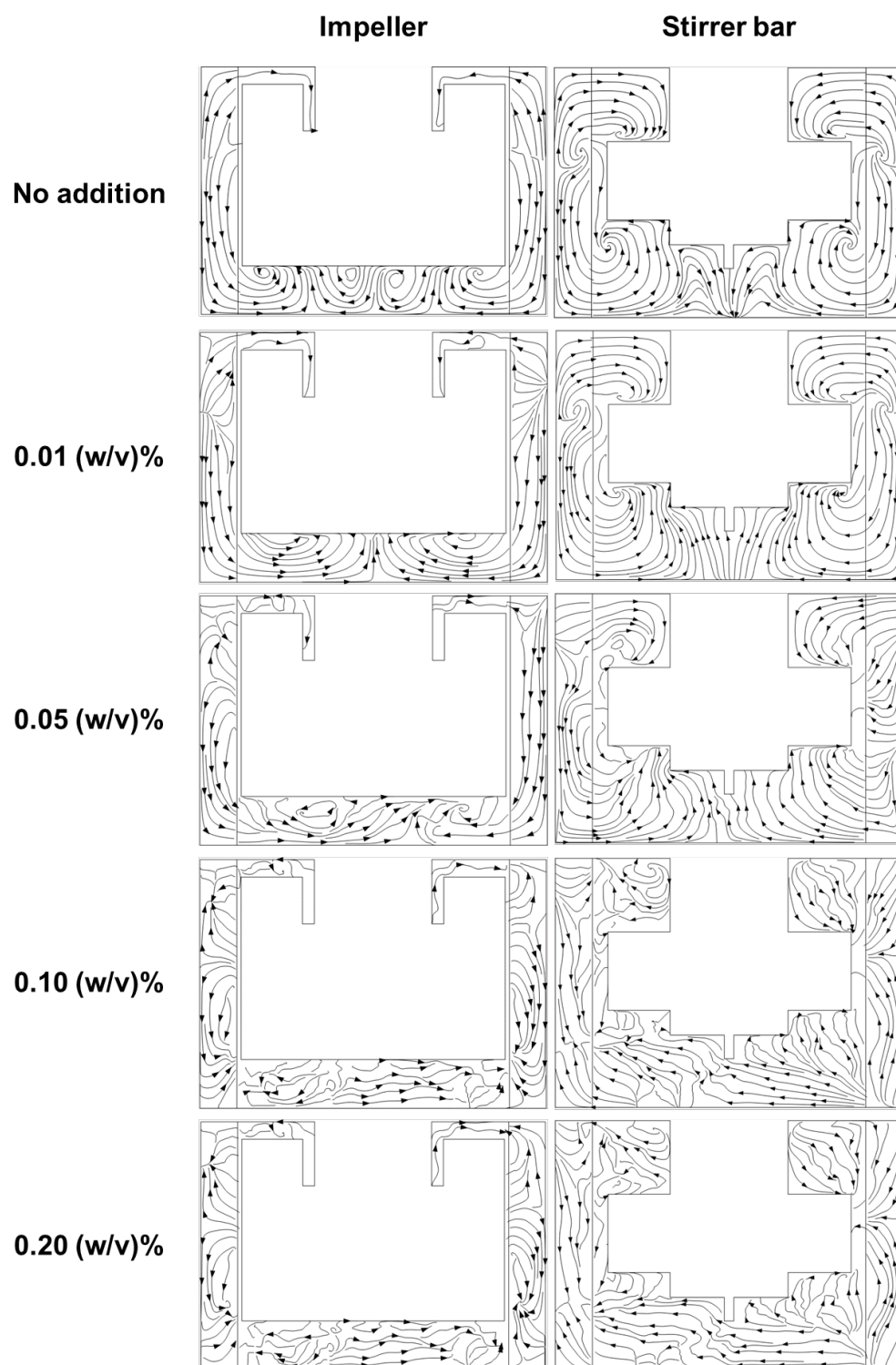


Fig. S10. Streamline of the media with various concentrations of HP-NFBC under stirring rate at 120 rpm.

Statistical analyses

Statistical significance was tested for $n = 3$ experiments using Tukey's test with

R version 4.1.0. [2].

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