Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

A polymer-based chemical tongue for the non-invasive monitoring of osteogenic stem-cell differentiation by pattern recognition of serum-supplemented spent media

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Experimental section

Materials

Poly-L-lysine trifluoroacetate (PLL; degree of polymerization PLL: 10, 55, and 258) and methoxy-poly(ethylene glycol)-*block*-PLL trifluoroacetate (PEG-*b*-PLL; degree of polymerization PEG: 113; degree of polymerization PLL: 52) were obtained from Alamanda Polymers, Inc. Polyamidoamine (PAMAM) dendrimer (generation 4.0), 3-morpholinopropanesulfonic acid (MOPS), 2-morpholinoethanesulfonic acid (MES), β-lactoglobulin from bovine milk (LacG), glucose oxidase from *Aspergillus niger* (GOX), β-galactosidase from *Escherichia coli* (Gal), albumin from bovine serum (BSA), fibrinogen from human serum (Fib), albumin from human serum (HSA), catalase from bovine liver (Cat), myoglobin from equine heart (Myo), L-glutamine, Bradford Reagent, dexamethasone, and (+)-sodium L-ascorbate were obtained from Sigma Chemical Co. Dansyl chloride (Dnc-Cl) was obtained from Tokyo Chemical Industry Co., Ltd. Tetrahydrofuran (THF), methanol, triethylamine, Dulbecco's modified eagle medium (DMEM), Dulbecco's phosphate-buffered saline (DPBS), β-glycerophosphate disodium salt hydrate, dimethyl sulfoxide (DMSO), 4% paraformaldehyde, Alizarin Red S, and KOH were obtained from Wako Pure Chemical Ind. Penicillin-streptomycin-neomycin (PSN) antibiotic mixture and chemically defined serum-free CDCHO medium were obtained from Thermo Fisher Scientific Inc. Fetal bovine serum (FBS) was obtained from GE Healthcare Life Science. The human cervix carcinoma cell line (HeLa) and the human breast cancer cell line (MCF-7) were obtained from the RIKEN BioResource Center. The human lung adenocarcinoma epithelial cell line (A549), human osteosarcoma cell line (MG63), human hepatoma carcinoma cell line (HepG2), and human bone marrow-derived mesenchymal stem cell line (UE7T-13) were obtained from the Japanese Collection of Research Bioresources. Concentrations of proteins were determined from their absorbance using a Shimadzu UV-1800 spectrophotometer with the extinction coefficients shown in Table S1.

Synthesis

Dansylated-polymers (Dnc-polymers) were prepared according to slightly modified literature procedures.[1] **P1**: A solution of Dnc-Cl (8.4 mg, 31.3 μmol) in THF (1.6 mL) was quickly added to a stirred solution of PLL_{10} (50.0 mg, 208.3 μmol for amino groups) and triethylamine (145.2 μL, 1042 μmol) in methanol (6.3 mL) at room temperature. **P2**: A solution of Dnc-Cl (25.1 mg, 93.0 μmol) in THF (4.7 mL) was quickly added to a stirred solution of PLL_{55} (150.0 mg, 620.3 μmol for amino groups) and triethylamine (432.3 μL, 3102 μmol) in methanol (18.8 mL) at room temperature. **P3**: A solution of Dnc-Cl (8.4 mg, 31.0 μmol) in THF (1.6 mL) was quickly added to a stirred solution of PLL_{258} (50.0 mg, 206.7 μmol for amino groups) and triethylamine (144.1 μL, 1034 μmol) in methanol (6.3 mL) at room temperature. **P4**: A solution of Dnc-Cl (8.6 mg, 31.7 μmol) in THF (1.6 mL) was quickly added to a stirred solution of PEG_{114}-*b*-PLL_{52} (70.0 mg, 211.6 μmol for amino groups) and triethylamine (147.5 μL, 1058 μmol) in methanol (8.8 mL) at room temperature. **P5**: A solution of Dnc-Cl (3.4 mg, 12.6 μmol) in THF (0.6 mL) was quickly added to a stirred solution of PAMAM dendrimer (20.0 mg, 90.1 μmol for amino groups) and triethylamine (62.8 μL, 450 μmol) in methanol (5.0 mL) at room temperature. The reaction mixtures were stirred for 24 hours at room temperature and then dialyzed against: 1) 10% methanol (2 h), 2) deionized water (2 h), 3) 1 mM aqueous HCl (overnight), 4) 1 mM aqueous HCl (2 h), and 5) deionized water (2 h). The final solutions were lyophilized to obtain **P1**–**P5** as the chloride salt. The number of Dnc moieties conjugated to each polymer was determined from the absorbance at 330 nm

(Abs_{330}) of the obtained polymers in deionized water, using the equation [Dnc] = (Abs_{330}/4570); 1.2 (**P1**), 6.6 (**P2**) 28.5 (**P3**), 6.2 (**P4**), and 7.6 (**P5**) Dnc moieties were conjugated to each polymer.

Figures and Tables

Table S1. Extinction coefficients of proteins used in this study

Protein	Source	Abbr.	ε $(mg/mL)^{-1}$ cm^{-1}	Wavelength (nm)
β-lactoglobulin	bovine milk	LacG	0.937 [a]	280
glucose oxidase	*Aspergillus niger*	GOX	1.67 [b]	280
β-galactosidase	*Escherichia coli*	Gal	2.240 [a]	280
albumin	bovine serum	BSA	0.646 [a]	280
fibrinogen	human serum	Fib	1.689 [a]	280
albumin	human serum	HSA	0.518 [a]	280
catalase	bovine liver	Cat	3.65 [b]	276
myoglobin	equine heart	Myo	0.734 [b]	555

[a] Determined based on ref. 2. [b] According to the supplier's data sheet.

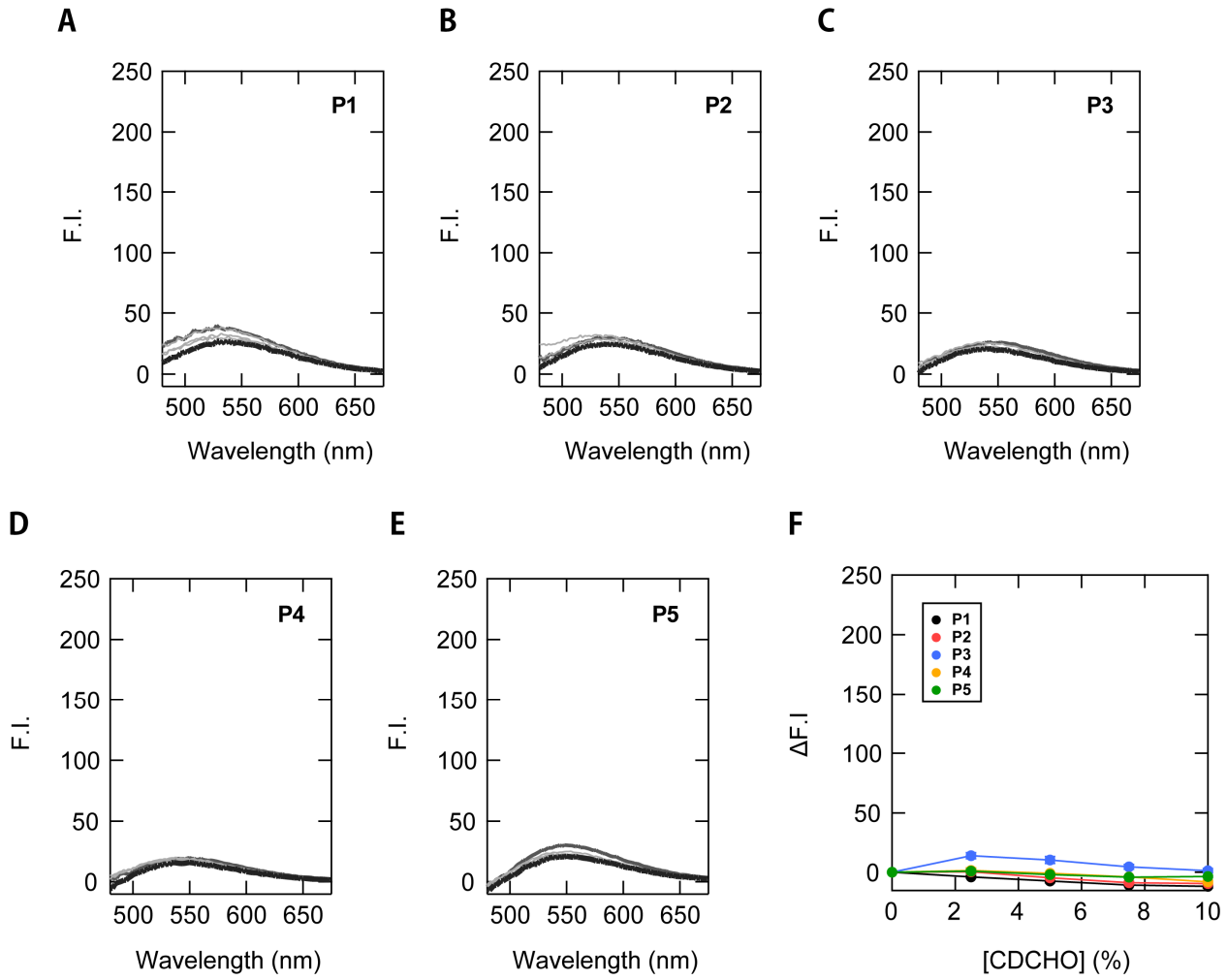

Fig. S1. Fluorescence of Dnc-polymers in the presence of CDCHO medium. (A–E) Fluorescence spectra of **P1–P5** (2.0 μg/mL) upon addition of CDCHO (0–10.0 vol%) in 18–20 mM MOPS buffer (pH = 7.4); λ_{ex} = 360 nm. (F) Fluorescence intensity of **P1**–**P5**; $\lambda_{ex}/\lambda_{em}$ = 360 nm/520 nm. The values shown represent mean values ± 1 SE from three independent experiments.

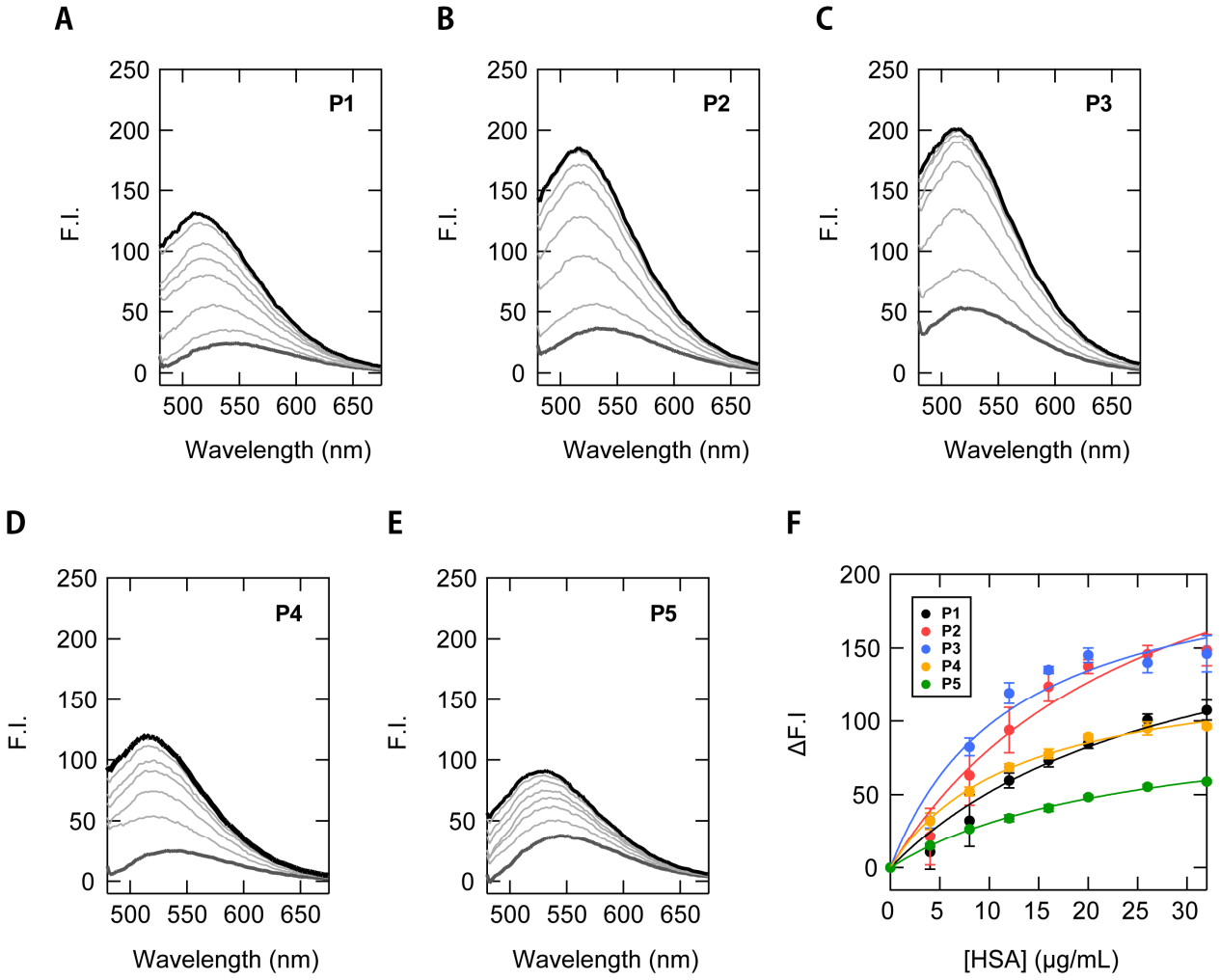

Fig. S2. Fluorescence of Dnc-polymers in the presence of HSA. (A–E) Fluorescence spectra of **P1–P5** (2.0 μg/mL) upon addition of HSA (0–30.0 μg/mL) in 19 mM MOPS buffer (pH = 7.4) with 5.0 vol% CDCHO; λ_{ex} = 360 nm. (F) Fluorescence intensity of **P1–P5**; $\lambda_{ex}/\lambda_{em}$ = 360 nm/520 nm. The values shown represent mean values ± 1 SE from three independent experiments.

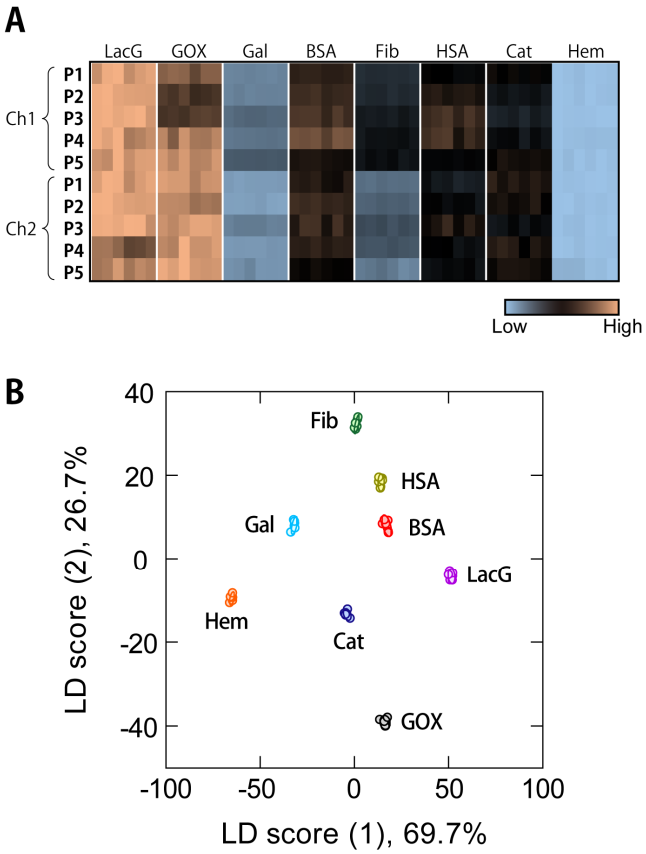

Fig. S3. Optical pattern recognition of proteins in the presence of CDCHO medium. (A) Heat map of the fluorescence response patterns of the eight proteins (20.0 μg/mL) in 19 mM MOPS buffer (pH = 7.4) and 5.0 vol% CDCHO medium. For each analyte, six independent experimental values are shown. (B) LDA score plot for the proteins. The ellipsoids represent the confidence intervals (±1 SD) for each analyte.

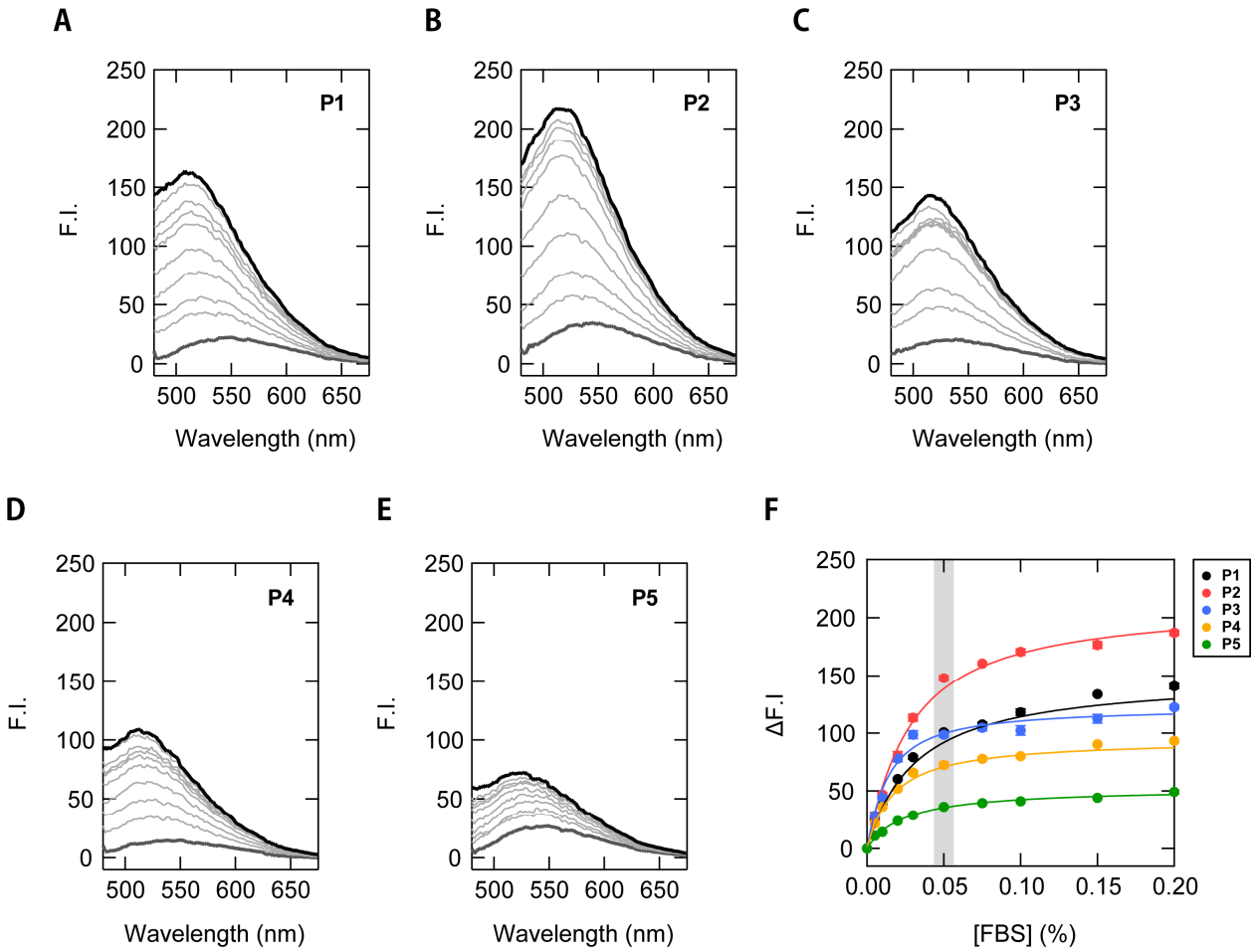

Fig. S4. Fluorescence of Dnc-polymers in the presence of FBS. (A–E) Fluorescence spectra of **P1–P5** (2.0 μg/mL) upon addition of FBS (0–0.2 vol%) in 19 mM MOPS buffer (pH = 7.4) with 5.0 vol% CDCHO; λ_{ex} = 360 nm. (F) Fluorescence intensities of **P1–P5**; $\lambda_{ex}/\lambda_{em}$ = 360 nm/520 nm. The values shown represent mean values ± 1 SE from three independent experiments. The volume fraction in the shaded area, i.e., 5.0 vol% of spent CDCHO media supplemented with 1.0 vol% FBS, was chosen as the final concentration of the spent medium to be added in the chemical-tongue analyses.

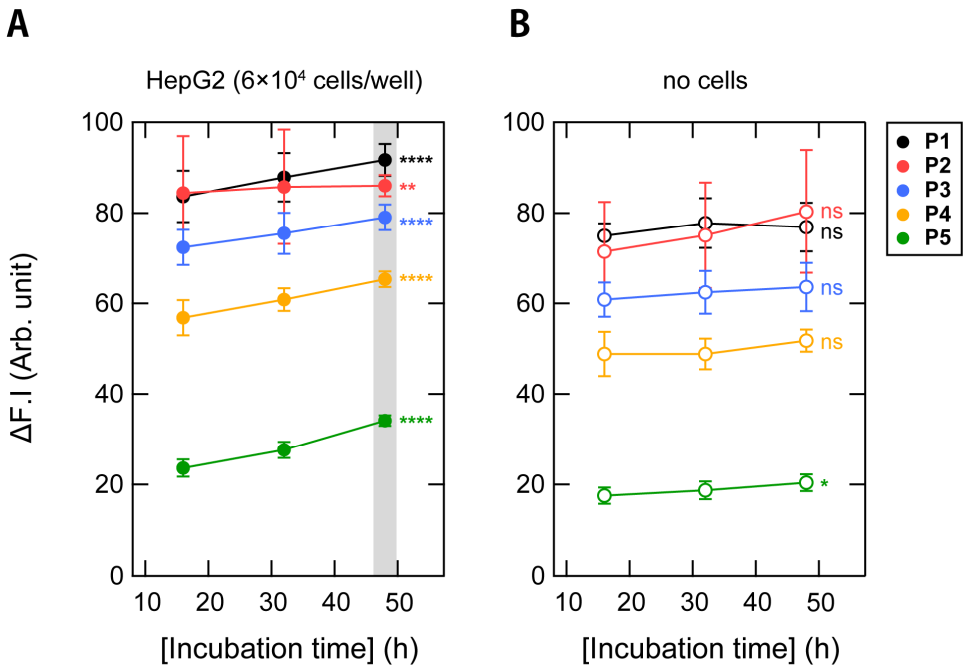

Fig. S5. Fluorescence responses of Dnc-polymers (2.0 μg/mL) to 5.0 vol% spent media (collected after 16–48 h incubation with (A) or without (B) A549 cells (6.0 × 10^4 cells/well)) supplemented with 1.0 vol% FBS in 19 mM MOPS buffer (pH = 7.4); $\lambda_{ex}/\lambda_{em}$ = 360 nm/480 nm. Mean values ± SD (n = 6; two-tailed, unpaired, Student's t-test; *P < 0.05, **P < 0.01, ****P < 0.0001, ns: not significant; 48 h case *vs*. 16 h without cells). The incubation time shown in the shaded area (48 h) was chosen for use in the chemical-tongue analyses.

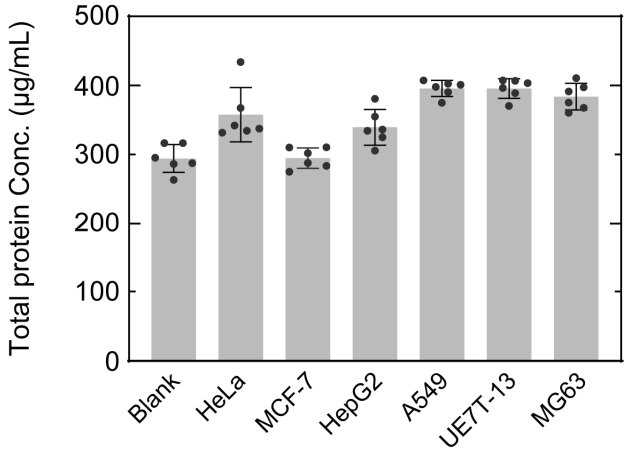

Fig. S6. Total protein concentrations in the spent media collected after culturing different cell lines (6.0 × 10^4 cells/well) and fresh media (mean values ± 1 SD; n = 6).

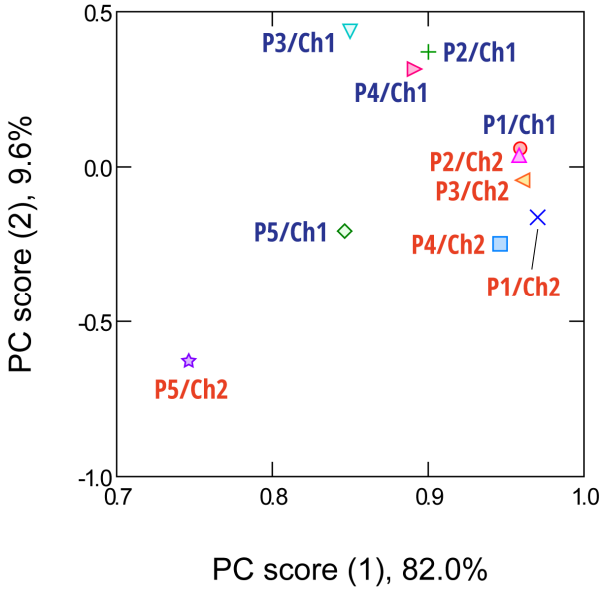

Fig. S7. Loading plot for the fluorescence response patterns of the six spent media collected after culturing different cell lines (6.0×10^4 cells/well) and one fresh medium (5.0 vol%) in 19 mM MOPS buffer (pH = 7.4) generated by the chemical tongue.

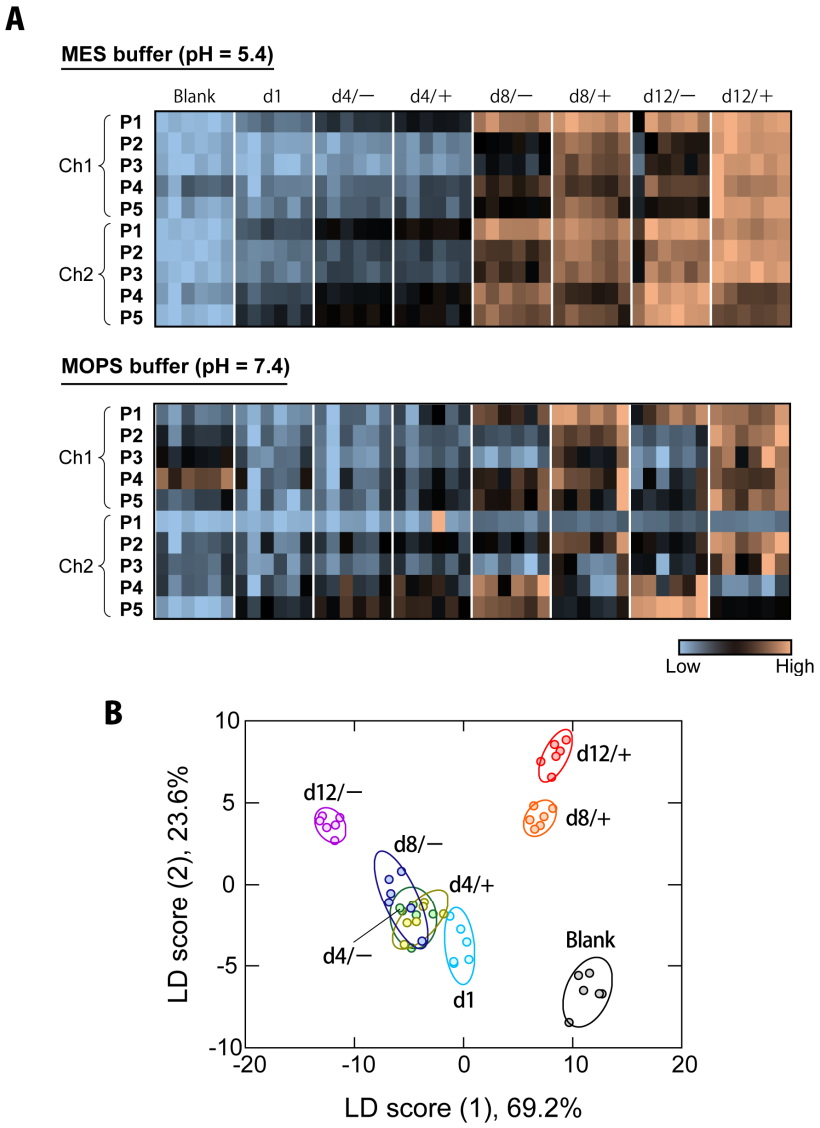

Fig. S8. Optical pattern recognition of serum-supplemented spent media collected during differentiation induction. (A) Heat maps of the fluorescence response patterns of the spent media collected during the osteogenic differentiation culture and one fresh medium (5.0 vol%) in 19 mM MES buffer (pH = 5.4) (upper panel) or 19 mM MOPS buffer (pH = 7.4) (lower panel). For each analyte, six independent experimental values are shown. (B) LDA score plot for the stem cell differentiation in 19 mM MOPS buffer (pH = 7.4). The ellipsoids represent the confidence intervals (± 1 SD) for each analyte.

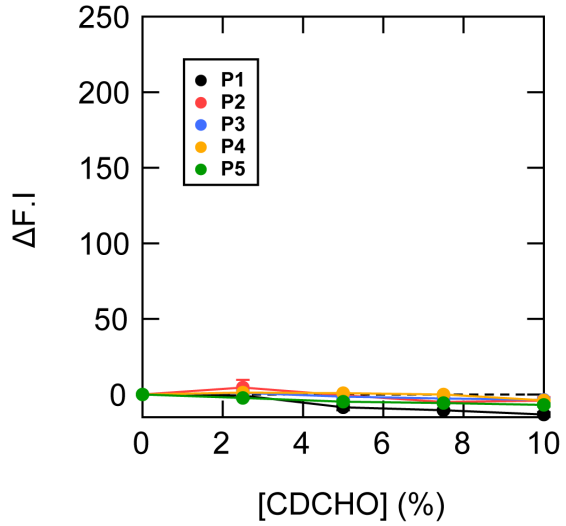

Fig. S9. Fluorescence intensity of Dnc-polymers (2.0 μg/mL) upon addition of CDCHO (0–10.0 vol%) in 18–20 mM MES buffer (pH = 5.4); $\lambda_{ex}/\lambda_{em}$ = 360 nm/520 nm. The values shown represent mean values ± 1 SE from three independent experiments.

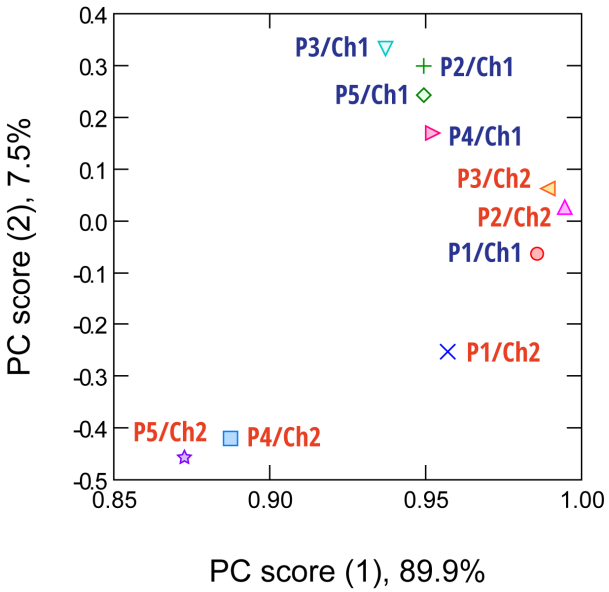

Fig. S10. Loading plot for the fluorescence response patterns of the seven spent media collected during differentiation induction and one fresh medium (5.0 vol%) in 19 mM MES buffer (pH = 5.4) generated by the chemical tongue.

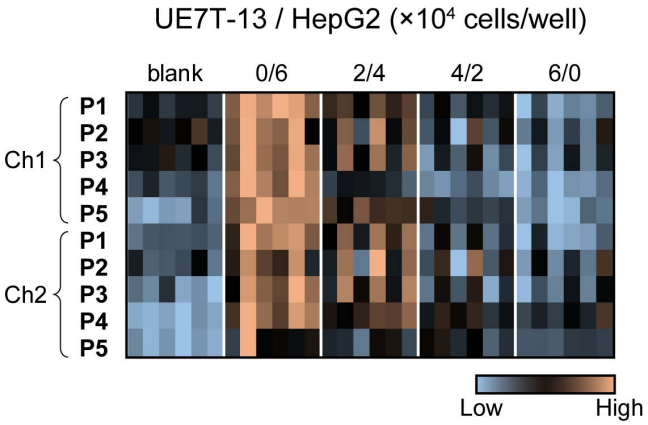

Fig. S11. Heat map of the fluorescence response patterns of the four spent media collected after culturing mixtures of UE7T-13 and HepG2 (total density: 6.0×10^4 cells/well) and one fresh medium (5.0 vol%) in 19 mM MOPS buffer (pH = 7.4). For each analyte, six independent experimental values are shown.

Dataset S1 (separate file). Data set matrix of the differences in fluorescence intensity before and after the addition of proteins ($I–I_0$, 20.0 μg/mL) generated from the chemical tongue. The jackknife test afforded 100% accuracy.

Dataset S2 (separate file). Data set matrix of the differences in fluorescence intensity before and after the addition of serum-supplemented spent media used to culture human cell lines ($I–I_0$) generated from the chemical tongue. The jackknife test afforded 98% accuracy. The rightmost column indicates whether the data in the corresponding row was used as training data (denoted by "–") or test data (results of the verification are shown) in the holdout test.

Dataset S3 (separate file). Data set matrix of the differences in fluorescence intensity before and after the addition of serum-supplemented spent media collected during differentiation induction ($I–I_0$) generated from the chemical tongue in 19 mM MOPS buffer (pH = 7.4). The jackknife test afforded 83% accuracy. The rightmost column indicates whether the data in the corresponding row was used as training data (denoted by "–") or test data (results of the verification are shown) in the holdout test.

Dataset S4 (separate file). Data set matrix of the differences in fluorescence intensity before and after the addition of serum-supplemented spent media collected during differentiation induction ($I–I_0$) generated from the chemical tongue in 19 mM MES buffer (pH = 5.4). The jackknife test afforded 96% accuracy. The rightmost column indicates whether the data in the corresponding row was used as training data (denoted by "–") or test data (results of the verification are shown) in the holdout test.

Dataset S5 (separate file). Data set matrix of the differences in fluorescence intensity before and after the addition of serum-supplemented spent media used to culture mixtures of stem cells and cancer cells ($I–I_0$) generated from the chemical tongue. The jackknife test afforded 97% accuracy. The rightmost column indicates whether the data in the corresponding row was used as training data (denoted by "–") or test data (results of the verification are shown) in the holdout test.

References

1 S. Tomita, S. Ishihara and R. Kurita, *ACS Appl. Mater. Interfaces*, 2017, **9**, 22970–22976.

2 C. N. Pace, F. Vajdos, L. Fee, G. Grimsley and T. Gray, *Protein Sci.*, 1995, **4**, 2411–2423.