Biochemical and Biophysical Research Communications 800 (2026) 153272

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Triple helix formation of a collagen model peptide induces cell aggregation formation

Kazuki Yoshida [a], Edward Vermeersch [b], Sandra Van Vlierberghe [b], Michiya Matsusaki [a,*]

[a] Division of Applied Chemistry, Graduate School of Engineering, The University of Osaka, 2-1 Yamadaoka, Suita, Osaka, 565-0871, Japan
[b] Polymer Chemistry and Biomaterials Group, Centre of Macromolecular Chemistry, Department of Organic and Macromolecular Chemistry, Ghent University, Krijgslaan 281, S4, 9000, Ghent, Belgium

ARTICLE INFO

Keywords:
Collagen model peptide
Triple helix
Cell assembly

ABSTRACT

Biofabrication refers to the creation of tissues and organs from biological materials such as cells and proteins, and is often applied in transplantation therapy and drug efficacy evaluation. For such a construct to function as a tissue, it is important for individual cells to interact closely with each other, so tailor-made approaches for the fabrication of cell aggregates to facilitate intercellular interactions are needed. Some researchers are using the DNA chain as a crosslinking site to make cell-cell assemblies, but DNA degradation and the potential risk of an immune response are concerns. A collagen triple helix is resistant to most proteases and is expected to function as a suitable cell assembling motif in a cell assembly method. Herein, we reported a thermoresponsive cell assembly method driven by triple-helix formation of a collagen model peptide (CMP). We synthesized a 4-armed poly (ethylene glycol) (PEG) terminally co-modified with (proline-proline-glycine)$_{10}$ ((PPG)$_{10}$) and deoxycholic acid (DCA) (4-arm-PEG-DCA/-(PPG)$_{10}$) and evaluated the characteristics and the cell-assembling property. It was found that 4.3 times more cell aggregates were formed after cooling under conditions with 4-arm-PEG-DCA/-(PPG)$_{10}$ than without 4-arm-PEG-DCA/-(PPG)$_{10}$. Accordingly, we developed a thermoresponsive cell assembly method driven by triple-helix formation of CMP. The use of CMP is expected to be applied in a new cell assembling approach.

1. Introduction

Biofabrication refers to the creation of tissues and organs from biological materials such as cells and proteins, and artificial tissues and organs that reproduce complex structures and functions are expected to be applied in transplantation therapy and drug efficacy evaluation. Recently, the fabrication of tissue constructs that mimic the complex three-dimensional structures of biological tissues using hydrogels as scaffold materials have been reported [1,2]. However, it is still challenging to completely reproduce the functions of real tissues. One of the reasons is that the abundant presence of scaffold materials probably hinders cell-cell interactions [3]. For such a construct to function as a tissue, it is important for individual cells to interact closely with each other to induce differentiation. Therefore, tailor-made approaches for the fabrication of cell aggregates to facilitate intercellular interactions are needed.

Various methods for constructing higher-order tissue structures from cell aggregates are currently being studied [4]. In particular, cell surface modification approaches have attracted much attention because they can manipulate and control cell interactions and phenotypes for various biomedical applications [5]. As biologically-driven cell surface modifications, genetic engineering methods and metabolic engineering methods [6] that introduce functional groups through sugar metabolism have been reported. However, they have drawbacks such as cell-type dependency, risk of triggering immunogenic responses and tumorigenesis, and chemical insertion efficiency that can vary depending on cell metabolism [5]. Accordingly, chemically-driven cell modifications have also been studied. Among them, covalent-bond formation is considered a simple and straightforward method, but it lacks targeting specificity and is cytotoxic. On the other hand, hydrophobic insertion in the cell surface using phospholipids, alkyl chains, and cholesterol have low cytotoxicity [7,8]. We have also previously reported hydrophobic insertion using a cholesterol [9].

Some researchers are using the DNA chain as a crosslinking site to make cell-cell assemblies [7,10] but there are some concerns about the use of DNA due to its degradation and the potential risk of an immune

* Corresponding author.
E-mail address: m-matsus@chem.eng.osaka-u.ac.jp (M. Matsusaki).

https://doi.org/10.1016/j.bbrc.2026.153272
Received 23 December 2025; Received in revised form 8 January 2026; Accepted 10 January 2026
Available online 12 January 2026

response [11]. On the other hand, it is known that a collagen-like triple helix is resistant to most proteases except for collagen-specific enzymes [12] so the application of collagen-like triple helices is considered promising.

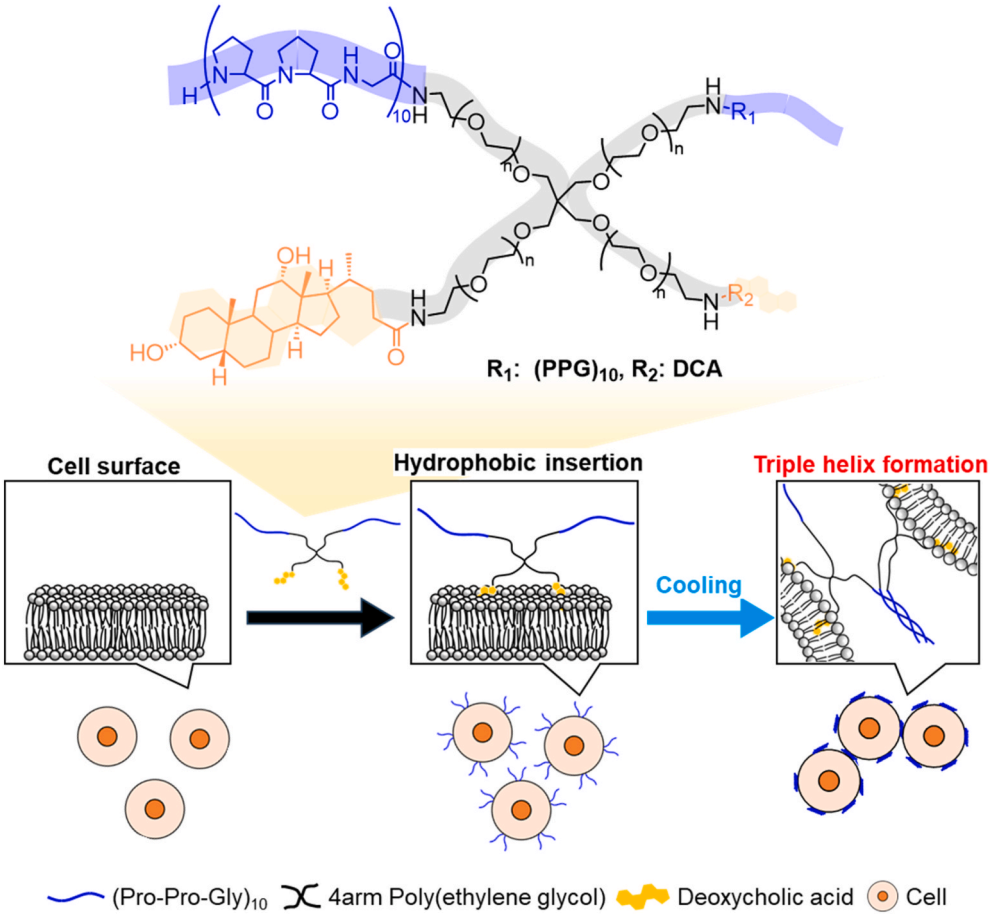
The triple-helix region of collagen has a glycine (Gly, G) repeating every three residues in the amino acid sequence (Xaa-Yaa-Gly), and proline (Pro, P) often appears selectively at Xaa and hydroxyproline (Hyp, O) at Yaa. In particular, collagen model peptides (CMPs) are peptides with repeating triple-helical amino acid sequences [13]. CMPs exhibit repeatable triple-helix formation and dissociation by heating and cooling. Moreover, the CMP's triple-helix stability can be controlled by the amino acid sequences. However, few reports have used CMP as a cell assembling motif in a cell assembly method.

In this study, a thermoresponsive cell assembly method driven by triple-helix formation of CMP was investigated. Herein, we synthesized a 4-armed poly (ethylene glycol) (PEG) terminally co-modified with $(PPG)_{10}$ and deoxycholic acid (DCA) (4-arm-PEG-DCA/-$(PPG)_{10}$) and evaluated the characteristics and the cell-assembling property (Scheme 1). It was found that more cell aggregates were formed after cooling under conditions with 4-arm-PEG-DCA/-$(PPG)_{10}$ than without 4-arm-PEG-DCA/-$(PPG)_{10}$. Accordingly, we developed a thermoresponsive cell assembly method driven by triple-helix formation of CMP. The use of CMP is expected to be applied in a cell assembling approach.

2. Experimental section

2.1. Materials

The $(PPG)_{10}$ (4006) was purchased from Peptide Institute, Inc. (Osaka, Japan). Boric acid (021-02195) ,deuterium oxide (536–7474), dimethyl sulfoxide (DMSO) (043-07216), *N*-[(9*H*-fluoren-9-ylmethoxy) carbonyloxy]succinimide (Fmoc-NHS) (064–02461), 5 mol/L hydrochloric acid (081–05435), 4 % paraformaldehyde phosphate buffer solution (163–20145), piperidine (160–02776), sodium carbonate (199–01585), sodium deoxycholate (DCA-Na) (192–08312), 5mol/L sodium hydroxide (196-05375), tetrahydrofuran (THF) (206–05106), and trypsin (9002-07-7) were purchased from Wako Chemicals (Osaka, Japan). Cell Count Reagent SF (07553–44), Dulbecco's Modified Eagle Medium (High Glucose) (08458–16), sodium bicarbonate (31,213–15), fluorescein 5-isothiocyanate (FITC) (16,151–66), D-PBS without Ca and Mg, Powder (07269–84), and Antibiotic-Antimycotic Mixed Stock Solution (100x) (02892–54) were purchased from Nacalai Tesque (Kyoto, Japan). Ethylenediaminetetraacetic acid tetrasodium salt, 2-hydrate (EDTA) (000–29295) and hexane (010–36766) was purchased from Kishida Chemical (Osaka, Japan). Dimethyl sulfoxide-d_6 (151,874-25G), and 4arm-PEG20k-NH2 HCl salt (JKA7026-1G) were purchased from Sigma-Aldrich (St. Louis, USA). 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) (D2919) was purchased from Tokyo Chemical Industry (Tokyo, Japan). 4′,6-diamidino-2-phenylindole (DAPI), fetal bovine serum (FBS) (10,270–106) and FluoroBrite™ DMEM (FluoroBriteDMEM) (A18967-01), and Trypan Blue stain 0.4 % (T10282) were purchased from Thermo Fisher Scientific Inc. (Waltham, U.S.A). Agarose (50,090) was purchased from Cambrex Bio Science Rockland, Inc. (ME, U.S.A). Epithelial fibrosarcoma (HT1080) and normal human dermal fibroblasts (NHDF) (CC-2509) were obtained from Lonza (Basel, Switzerland).



Scheme 1. A schematic illustration of the thermoresponsive cell assembling method driven by triple-helix formation of CMP.

2.2. Synthesis of 4-arm-PEG-DCA/-(PPG)$_{10}$

2.2.1. Synthesis of 4-arm-PEG-DCA

After dissolving DCA-Na (8.3 mg, 20 μmol) in 5 mL of 50 mM Na_2CO_3/$NaHCO_3$ (aq.) (pH 8.5), DMT-MM (27.7 mg, 100 μmol) in 0.5 mL of Milli-Q water was added and stirred in an ice bath for 10 min. After activation, the 4arm-PEG-NH2 (100 mg, 5 μmol) in 4.5 mL of 50 mM Na_2CO_3/$NaHCO_3$ was added, and then the reaction mixture was stirred for 24 h at room temperature. After the reaction, the whole reaction mixture was dialyzed (MWCO: 3.5 kDa) for 3 days against methanol and water. The resulting solution was freeze-dried. The 4arm-PEG20k-DCA was characterized by ^{1}H NMR measurement. The ^{1}H NMR spectra were measured by a Nuclear Magnetic Resonance Spectrometer (Bruker, AVANCE III 600, Massachusetts, USA).

2.2.2. Fmoc protection of (PPG)$_{10}$

The (PPG)$_{10}$ (50 mg, 19.8 μmol) was dissolved in 12.5 mL of 100 mM $NaHCO_3$ (pH 8.5) and preincubated at 80 °C for 5 min to dissociate the triple helices. After a cool-down of the solution in a refrigerator for 5 min, 66.6 mg of Fmoc-NHS in 12.5 mL THF was added to the solution immediately, and then the reaction mixture was stirred for 24 h at room temperature. After the reaction, the whole reaction mixture was dialyzed (MWCO: 1000 Da) for 3 days against methanol and water. The resulting solution was freeze-dried. The Fmoc(PPG)$_{10}$ was characterized by ^{1}H NMR and matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) Mass measurements. The MALDI-TOF Mass spectrum was measured by a Time-of-Flight Mass Spectrometer (JEOL, JMS-S3000, Tokyo, Japan).

2.2.3. Synthesis of 4-arm-PEG-DCA/-(PPG)$_{10}$

After dissolving the Fmoc(PPG)$_{10}$ (8.4 mg, 3.0 μmol) in 1 mL of 50 mM Na_2CO_3/$NaHCO_3$, the solution was preincubated at 80 °C for 5 min to dissociate the triple helices. After a cool-down of the solution in a refrigerator for 5 min, DMT-MM (4.2 mg, 15 μmol) in 0.1 mL of Milli-Q water was added and stirred in an ice bath for 5 min. After activation, the 4arm-PEG-DCA (21 mg, 1 μmol) in 0.9 mL of 50 mM Na_2CO_3/$NaHCO_3$ was added, and then the reaction mixture was stirred for 24 h at room temperature. After the reaction, the whole reaction mixture was dialyzed (MWCO: 3.5 kDa) for 3 days against water at 37 °C. The resulting solution was freeze-dried. The obtained product was characterized by ^{1}H NMR and UV–vis measurements. The UV–vis spectrum was measured by a UV–Vis Spectrophotometer (JASCO, V-670, Tokyo, Japan). The UV–vis spectra of Fmoc-NHS in DMSO at 250, 100, 75, 50, 25, 10, and 1 μM at 25 °C were also measured to produce a calibration curve.

Subsequently, after the 4arm-PEG20k-DCA/-Fmoc (PPG)$_{10}$ (15 mg, 0.65 μmol) was dissolved in 4 mL of DMSO, excess piperidine (1 mL) was added dropwise and stirred for 2 h. After deprotection, the whole reaction mixture was washed three times with hexane using a separating funnel. 5 mL of Milli-Q water was added to the lower phase solution, and then dialysis (MWCO: 3.5 kDa) for 3 days against methanol at room temperature and water at 37 °C was conducted. The resulting solution was freeze-dried and the obtained product was characterized by ^{1}H NMR measurement.

2.3. Functional characterizations of 4-arm-PEG-DCA/-(PPG)$_{10}$

The 4-arm-PEG-DCA/-(PPG)$_{10}$ was dissolved in PBS at 20 mg/mL. The solution was incubated at 25 °C, heated to 60 °C for 15 min, and re-equilibrated at 4 °C for 1 day. The circular dichroism (CD) spectra were measured by a CD spectrometer (JASCO, J-1100, Tokyo, Japan) at 25 °C, 80 °C, and 4 °C, respectively. Thermal transition curves were obtained by recording the ellipticity at 228 nm using a J−1100 (JASCO, Tokyo, Japan), while the temperature was continuously increased in the range 25–90 °C at a heating rate of 1.0 °C min^{-1}. The melting temperature (T_m) was determined by the following equation: $F(T)=(\theta(T)-\theta_U(T))/(\theta_F(T)-\theta_U(T))$. $F(T)$ is the fraction folded. $\theta(T)$, $\theta_U(T)$, and $\theta_F(T)$ are the observed ellipticity, the ellipticities calculated from the linear approximation of the region at triple-helix dissociation and formation, respectively. T_m was defined as the temperature at which $F(T_m) = 1/2$.

2.4. Evaluation of cell assembly formation

2.4.1. Evaluation of cytotoxicity response

NHDF was maintained in DMEM supplemented with 10 % FBS and 1 % antibiotics. Cells were cultured in 5 % CO_2 and 95 % humidified air at 37 °C. NHDF was seeded on a 96-well plate at 5×10^3 cells/well. After 24 h, 100 μL of 4arm-PEG20k-DCA/-(PPG)$_{10}$ at 500, 100, 50, and 0 μM was added in DMEM medium supplemented with 10 % FBS and 1 % antibiotics at pH 7.4 in each well and incubated for 24 h at 37 °C. After incubation, the plates were washed with PBS, and cell counting kit-8 solution diluted 10 times by FluoroBriteDMEM with 1 % antibiotics was added. After 2 h at 37 °C, 80 μL of the supernatant was collected in 96-well plates, and the absorbance at 450 nm was measured using a Synergy HTX microplate reader (Agilent Technologies, Santa Clara, U.S.A).

2.4.2. Polymer adsorption to cell

A stock solution of fluorescein isothiocyanate labeled 4arm-PEG20k-DCA/-(PPG)$_{10}$ (4arm-PEG20k-DCA/-F(PPG)$_{10}$) (500 μM) in PBS was prepared. The solution was preincubated at 50 °C for 15 min and diluted 10 times by FluoroBriteDMEM with 1 % antibiotics before the addition. NHDF was seeded on a 35 mm glass-bottomed dish at 2.5×10^5 cells. After 24 h incubation, the dishes were washed with PBS, and 1 mL of the 50 μM 4arm-PEG20k-DCA/-F(PPG)$_{10}$ was added. After incubation at 37 °C for 6 h, the plates were washed with PBS, and fixed using a 4 % paraformaldehyde phosphate buffer solution. For the fluorescence imaging, the nuclei were stained with DAPI and the dishes were then observed using an FV-10i confocal laser microscope (OLYMPUS, Tokyo, Japan). Images were acquired using the same equipment.

A stock solution of 4arm-PEG20k-DCA/-Fmoc(PPG)$_{10}$ (500 μM) in PBS was also prepared. The stock solution was diluted 10 times by FluoroBriteDMEM with 1 % antibiotics before the addition. The 1.0×10^6 HT1080 cells were transferred to 1.5 mL tubes. 1 mL of the 50 μM 4arm-PEG20k-DCA/-Fmoc(PPG)$_{10}$ was added to the tubes, and kept at 37 °C for 0, 2, and 6 h. After the corresponding points, 200 μL of the samples was transferred to new tubes and centrifuged (3500 rpm, 1 min). The supernatant was removed and the precipitate was gently washed with PBS twice. After that, 100 μL of PBS was added into the tube, the cells were re-suspended and the suspension was transferred to a 96-well plate. Fluorescence intensity was then measured using a microplate reader (excitation wavelength: 275 nm, emission wavelength: 315 nm).

2.4.3. Cell-cell assembly formation

The 96-well plates were coated with 50 μL of 0.5 wt% agarose in PBS before the experiments. A stock solution of 4arm-PEG20k-DCA/-(PPG)$_{10}$ (5000 μM) in PBS was prepared. The solution was preincubated at 50 °C for 15 min and diluted 10 times by DMEM with 10 % FBS, 1 % antibiotics, and 500 μM EDTA before the addition. NHDF was seeded on a 96-well plate at 5×10^3 cells/well. 100 μL of 4arm-PEG20k-DCA/-(PPG)$_{10}$ at 500 μM was added in each well and incubated for 2 h at 37 °C. After the incubation, the plates were kept at 4 °C for 15 min, and then at room temperature for 2 h with gentle agitation. After incubation, the dishes were observed using a phase-contrast microscope (Thermo Fisher Scientific Inc., Waltham, U.S.A). Images were acquired using the same equipment.

2.5. Statistical analysis

The results are expressed as means ± standard deviation (SD). Student's *t*-tests were used for data analysis, with $p < 0.05$ being considered

statistically significant.

3. Results and discussion

3.1. Synthesis of 4-arm-PEG-DCA/-$(PPG)_{10}$

$(PPG)_{10}$ and DCA were introduced in 4arm-PEG-NH2 by amide coupling reaction using DMT-MM as a coupling reagent (Fig. 1). First, 4arm-PEG20k-DCA_X was synthesized, and the incorporation amount of DCA was calculated to be 62 % from the ^{1}H NMR spectra (Fig. 1a and S1). Before introducing $(PPG)_{10}$ in 4arm-PEG20k-DCA_X, for the purpose of preventing a side reaction, Fmoc-protection of $(PPG)_{10}$ was carried out by Fmoc-NHS (Fig. 1b). The obtained Fmoc$(PPG)_{10}$ was characterized by ^{1}H NMR and MALDI-TOF Mass spectra (Figs. S2 and S3). The

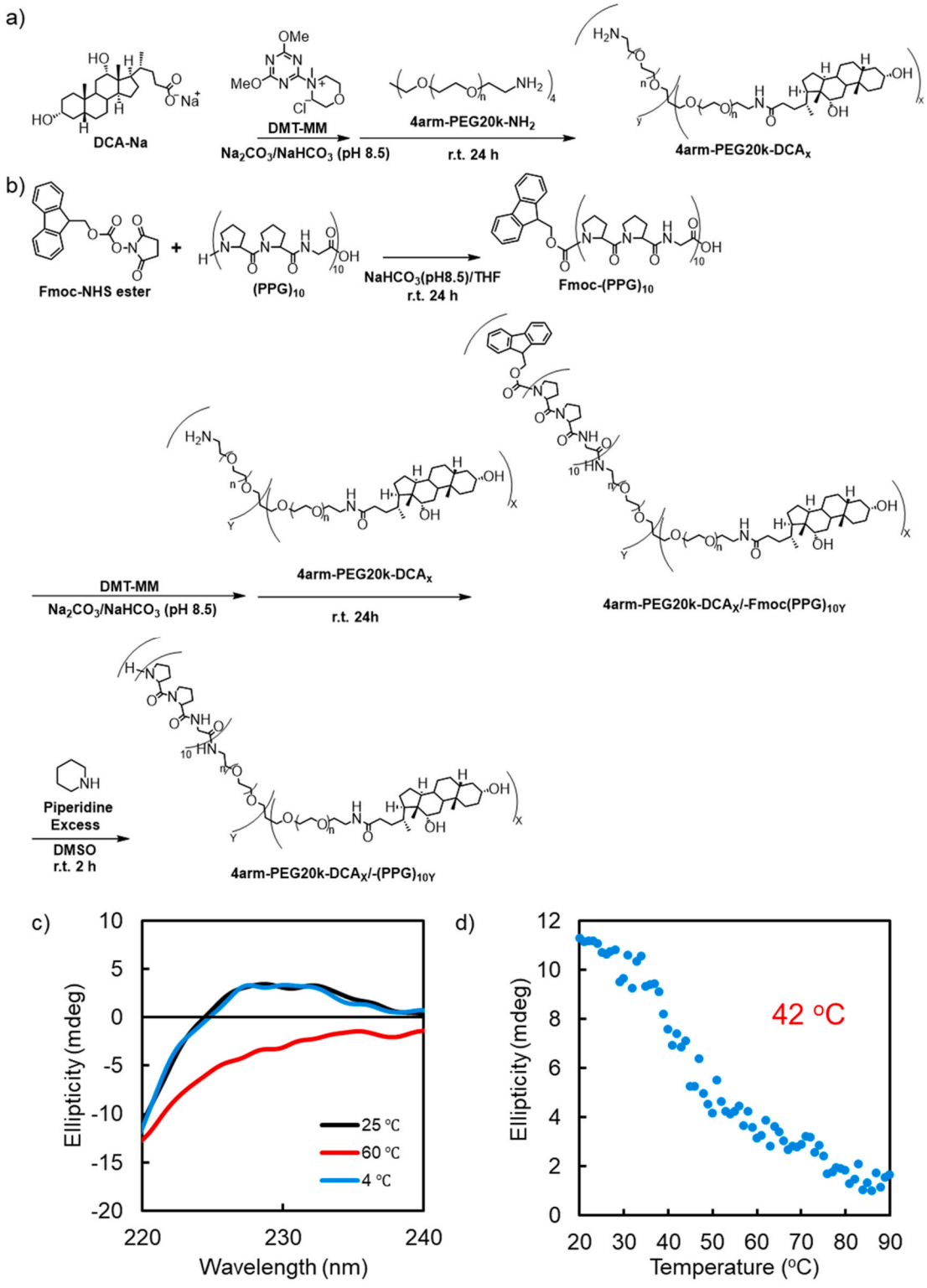


Fig. 1. Preparation of 4arm-PEG20k-DCA/-$(PPG)_{10}$. a) Synthesis of 4armPEG20k-DCA_x by amide coupling. b) Synthesis of 4armPEG20k-DCA/-$(PPG)_{10}$. c) CD profiles of 4arm-PEG20k-DCA/-$(PPG)_{10}$ at 25 °C, 60 °C, and 4 °C. d) The melting profile of 4arm-PEG20k-DCA/-$(PPG)_{10}$ obtained by CD measurement at 228 nm.

presence of Fmoc$(PPG)_{10}$ was confirmed by the mass spectrum and the grafting degree was determined to be around 100 % from ^{1}H NMR spectra. Subsequently, the Fmoc$(PPG)_{10}$ was grafted to 4arm-PEG20k-DCA_X by amide coupling reaction, and then the Fmoc group was deprotected by piperidine (Fig. 1b and S4). The introduction of Fmoc$(PPG)_{10}$ was suggested by the presence of the Fmoc-derived peak at 7–8 ppm (Fig. S4a) and at around 300 nm (Fig. S5a). The grafting degree was calculated to be around 53.8 % from UV–vis measurement (Fig. S5a), and this was expected given the presence of remaining non-grafted Fmoc$(PPG)_{10}$. The Fmoc-derived peak seen in Fig. S4a was not visible in Fig. S4b after the Fmoc-deprotection reaction. From these results, the synthesis of 4arm-PEG20k-$DCA_{(62)}$/-$(PPG)_{10(38)}$ was confirmed.

3.2. Functional characterizations of 4-arm-PEG-DCA/-$(PPG)_{10}$

Next, we characterized the triple-helix formation property of 4-arm-PEG-DCA/-$(PPG)_{10}$ by CD measurement (Fig. 1c and d). To confirm the dissociation and formation of the triple helix at different temperatures, CD spectra at 25 °C, 60 °C and 4 °C were measured. As shown in Fig. 1c, positive ellipticity associated with a triple helix was observed at 228 nm at 25 °C, but disappeared after incubation at 60 °C, and recovery of positive ellipticity was observed after cooling at 4 °C. These results indicated that 4-arm-PEG-DCA/-$(PPG)_{10}$ exhibits triple-helix formation and dissociation properties. The triple-helix stability was also evaluated by CD denaturation measurement (Fig. 1d). In the spectrum, the denaturation behavior of the $(PPG)_{10}$ moiety was observed, and the melting

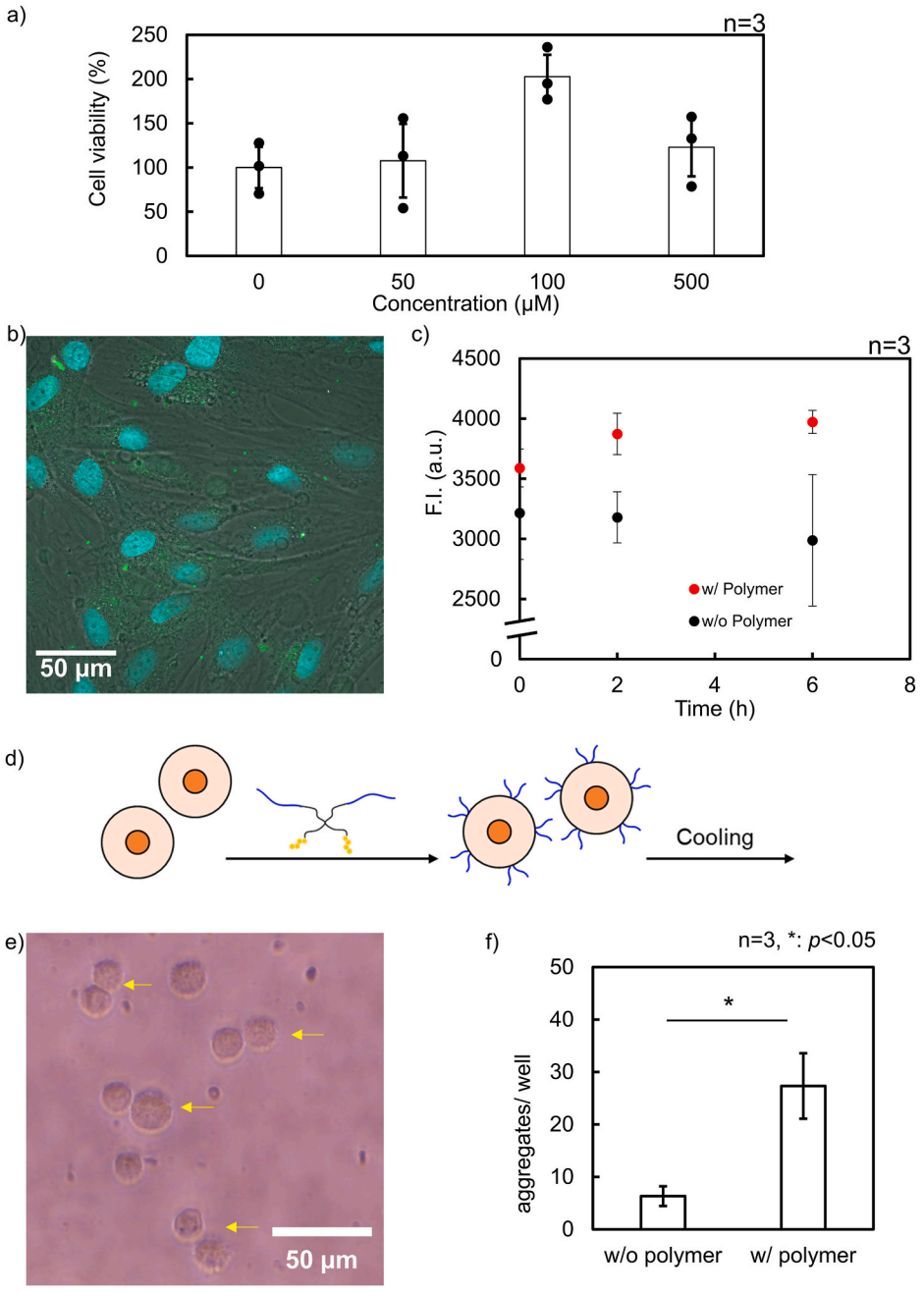


Fig. 2. a) The cell viability test after incubation with the 500, 100, 50, and 0 μM 4arm-PEG20k-DCA/-$(PPG)_{10}$ for 24 h. b) Fluorescence image of cells with 4arm-PEG20k-DCA/-F$(PPG)_{10}$ after incubation for 6 h. c) Fluorescence profiles of cell suspension with 4arm-PEG20k-DCA/-Fmoc$(PPG)_{10}$ or without 4arm-PEG20k-DCA/-Fmoc $(PPG)_{10}$ at 0, 2, and 6 h after incubation. d) A schematic illustration of cell modification and aggregation formation. e) A phase contrast image of cell aggregates with 4arm-PEG20k-DCA/-$(PPG)_{10}$. f) Number of cell aggregates with 4arm-PEG20k-DCA/-$(PPG)_{10}$ or without 4arm-PEG20k-DCA/-$(PPG)_{10}$.

point was calculated to be 42 °C. From these results, the triple-helix formation property of 4-arm-PEG-DCA/-$(PPG)_{10}$ was confirmed.

3.3. Evaluation of cell assembly formation

3.3.1. Evaluation of cytotoxicity response

Toward applications in cell modification, the cytotoxicity effect of 4arm-PEG20k-DCA/-$(PPG)_{10}$ was assessed on *in vitro* monolayer cultures of NHDF by incubating 4arm-PEG20k-DCA/-$(PPG)_{10}$ at 500, 100, 50, and 0 μM for 24 h (Fig. 2a). Importantly, 4arm-PEG20k-DCA/-$(PPG)_{10}$ showed a low cytotoxicity at 500, 100, and 50 μM (Fig. 2a). The results suggested that the 4arm-PEG20k-DCA/-$(PPG)_{10}$ had a low cytotoxicity at concentrations of no more than 500 μM.

3.3.2. Polymer adsorption to cell

To check the polymer adsorption property to cell, first, fluorescein isothiocyanate labeled 4arm-PEG20k-DCA/-$(PPG)_{10}$ (4arm-PEG20k-DCA/-F$(PPG)_{10}$) was synthesized and characterized by ^{1}H NMR (Fig. S6). After incubation for 6 h, a confocal laser microscopy image of the NHDF cells showed the fluorescence intensity derived from the 4arm-PEG20k-DCA/-F$(PPG)_{10}$ on the cells (Fig. 2b). Furthermore, the polymer adsorption property was evaluated using 4arm-PEG20k-DCA/-Fmoc$(PPG)_{10}$ with HT1080 cell (Fig. 2c). Compared with the control samples, the fluorescence intensity of the cell suspension in PBS was increased after 2 and 6 h incubation with 4arm-PEG20k-DCA/-Fmoc$(PPG)_{10}$ (Fig. 2c). These results indicated that the 4arm-PEG20k-DCA/-$(PPG)_{10}$ was adsorbed to cells.

3.3.3. Cell-cell assembly formation

Cell-cell assembly formation was assessed by evaluating the amount of cell aggregates using phase contrast microscopy observation. A brief scheme was shown in Fig. 2d. As shown in Fig. 2e, a phase contrast image of the 4arm-PEG20k-DCA/-$(PPG)_{10}$-modified NHDF showed the presence of cell aggregates after cooling at room temperature for 2 h. The amounts of cell aggregates with 4arm-PEG20k-DCA/-$(PPG)_{10}$-modification were 4.3 times higher than the samples without 4arm-PEG20k-DCA/-$(PPG)_{10}$-modification (Fig. 2f). From these results, a thermoresponsive cell assembly driven by the triple-helix formation of CMP was suggested.

4. Conclusion

In summary, we developed a thermoresponsive cell assembly method driven by triple-helix formation of CMP. It was found that more cell aggregates were formed after cooling under conditions with 4-arm-PEG-DCA/-$(PPG)_{10}$ than without 4-arm-PEG-DCA/-$(PPG)_{10}$. The use of CMP is expected to be applied in a cell assembling approach for tissue engineering.

CRediT authorship contribution statement

Kazuki Yoshida: Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Edward Vermeersch:** Methodology, Writing - review & editing. **Sandra Van Vlierberghe:** Supervision, Resources. **Michiya Matsusaki:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was financially supported by JST SPRING (JPMJSP2138) and COI-NEXT (JPMJPF2009) from JST, by New Energy and Industrial Technology Development Organization (NEDO) (23201377-0, 23201347-0, and 21W2K022), and the fund of AIST-Osaka University Advanced Photonics and Biosensing Open Innovation Laboratory (PhotoBio-OIL). The authors also acknowledge the financial support of the Research Foundation Flanders (FWO) for the financial support of this joint mobility project between Flanders and Japan (VS02925N). EV would like to acknowledge the financial support from FWO under the form of an FWO SB PhD fellowship (1S89426N).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2026.153272.

References

[1] S. Choi, K.Y. Lee, S.L. Kim, L.A. MacQueen, H. Chang, J.F. Zimmerman, Q. Jin, M. M. Peters, H.A.M. Ardoña, X. Liu, A.-C. Heiler, R. Gabardi, C. Richardson, W.T. Pu, A.R. Bausch, K.K. Parker, Fibre-infused gel scaffolds guide cardiomyocyte alignment in 3D-printed ventricles, Nat. Mater. 22 (2023) 1039–1046, https://doi.org/10.1038/s41563-023-01611-3.

[2] N. Noor, A. Shapira, R. Edri, I. Gal, L. Wertheim, T. Dvir, 3D printing of personalized thick and perfusable cardiac patches and hearts, Adv. Sci. 6 (2019) 1900344, https://doi.org/10.1002/advs.201900344.

[3] O. Jeon, Y.B. Lee, H. Jeong, S.J. Lee, D. Wells, E. Alsberg, Individual cell-only bioink and photocurable supporting medium for 3D printing and generation of engineered tissues with complex geometries, Mater. Horiz. 6 (2019) 1625–1631. https://doi.org/10.1039/C9MH00375D.

[4] V.M. Gaspar, P. Lavrador, J. Borges, M.B. Oliveira, J.F. Mano, Advanced Bottom-Up engineering of living architectures, Adv. Mater. 32 (2020) 1903975, https://doi.org/10.1002/adma.201903975.

[5] J. Almeida-Pinto, M.R. Lagarto, P. Lavrador, J.F. Mano, V.M. Gaspar, Cell surface engineering tools for programming living assemblies, Adv. Sci. 10 (2023) 2304040, https://doi.org/10.1002/advs.202304040.

[6] J.A. Prescher, D.H. Dube, C.R. Bertozzi, Chemical remodelling of cell surfaces in living animals, Nature 430 (2004) 873–877, https://doi.org/10.1038/nature02791.

[7] Y. Teramura, Cell surface modification with ssDNA–PEG–lipid for analysing intercellular interactions between different cells, Biomaterials 48 (2015) 119–128, https://doi.org/10.1016/j.biomaterials.2015.01.032.

[8] Y. Teramura, K.N. Ekdahl, K. Fromell, B. Nilsson, K. Ishihara, Potential of cell surface engineering with biocompatible polymers for biomedical applications, Langmuir 36 (2020) 12088–12106, https://doi.org/10.1021/acs.langmuir.0c01678.

[9] H. Nakatsuji, Y. Shioji, N. Hiraoka, Y. Okada, N. Kato, S. Shibata, I. Aoki, M. Matsusaki, Cancer-microenvironment triggered self-assembling therapy with molecular blocks, Mater. Horiz. 8 (2021) 1216–1221, https://doi.org/10.1039/D0MH02058C.

[10] Z.J. Gartner, C.R. Bertozzi, Programmed assembly of 3-dimensional microtissues with defined cellular connectivity, Proc. Natl. Acad. Sci. 106 (2009) 4606–4610, https://doi.org/10.1073/pnas.0900717106.

[11] P. Shi, Y. Wang, Synthetic DNA for cell-surface engineering, Angew. Chem. Int. Ed. 60 (2021) 11580–11591, https://doi.org/10.1002/anie.202010278.

[12] L.L. Bennink, D.J. Smith, C.A. Foss, M.G. Pomper, Y. Li, S.M. Yu, High serum stability of collagen hybridizing peptides and their fluorophore conjugates, Mol. Pharm. 14 (2017) 1906–1915, https://doi.org/10.1021/acs.molpharmaceut.7b00009.

[13] S. Sakakibara, Y. Kishida, Y. Kikuchi, R. Sakai, K. Kakiuchi, Synthesis of Poly-(L-prolyl-L-prolylglycyl) of defined molecular weights, BCSJ 41 (1968), https://doi.org/10.1246/bcsj.41.1273, 1273–1273.