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The macroscopic structure of RADA16 peptide hydrogel stimulates monocyte/macrophage differentiation in HL60 cells via cholesterol synthesis

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ABSTRACT

Cells can sense physical properties of surrounding 3-dimensional (3D) culture substrata; however, the physiological influences of such sensing are not fully understood. Here, we studied the physiological characteristics and activities of the macroscopic structure of a routinely used 3D culture substrata, the RADA16 self-assembling peptide scaffold. We found that RADA16 exhibited three distinct assembly patterns depending on its concentration, and one of these assemblies, formed with 0.01% (w/v) RADA16, was capable of inducing differentiation of human myelocytic leukemia HL-60 cells into monocytes/macrophages. This activity was largely reduced by destroying the 3D structure of the assembly, suggesting that the assembly intrinsically retained the ability to induce HL-60 differentiation. When cultured in the RADA16 scaffold, HL-60 cells accumulated intracellular cholesterol about 10 times more than normally cultured cells. Both the RADA16 culture and cholesterol loading brought about similar gene expression profiles. These results showed that HL-60 cells can sense the physical properties of the RADA16 scaffold through a mechanism that may involve intracellular pathways of cholesterol synthesis and/or transport.

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1. Introduction

Cells often exhibit innate properties when cultured within a 3-dimensional (3D) environment better than when cultured in a 2-dimensional (2D) environment [1]. Based on this finding, a variety of 3D culture substrata have been exploited [2]. It has become clear that 3D cultured cells are influenced by the physical properties of the 3D substrata, such as stiffness or topology of the material surface [3]. These facts suggest that cells are capable of sensing the physical properties of the 3D substrata, at either the microscopic or macroscopic level. However, the detailed molecular mechanisms of such sensing activities have not been elucidated.

Peptide hydrogels represent promising 3D materials for the study of sensing machinery for the following reasons. First, a wide variety of synthetic peptide hydrogels with diverse structures and physical and/or chemical properties have been developed using different combinations and orders of amino acids [4,5]. Second, the structural assemblies of the peptide hydrogels are able to be manipulated on a large scale (μm order) [6,7] as well as a small scale (sub-nm order) [8] depending on the conditions and peptide species.

Here, we evaluated a peptide hydrogel, 16-mer peptide RADA16 (RADARADARADARADA), according to the intrinsic physical properties of the hydrogel [9] and examined its ability to induce cell differentiation.

2. Materials and methods

2.1. Chemicals

RADA16 peptide was supplied by 3D Matrix Japan Co., Ltd. (Tokyo, Japan) as a 1% solution in water. Hydroxypropyl-β-cyclodextrin (CD) was provided by Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Congo red, cholesterol, methylcellulose (25 cp), and TPA were purchased from Wako Chemical (Tokyo, Japan). The RGDS peptide was purchased from Sigma Chemicals (Tokyo, Japan).

2.2. Cell line, culture conditions, and 3D environment analysis

Human promyelocytic leukemia cells (HL-60 cells) were obtained from the Riken Cell Bank (RCB0041). Cells were grown in RPMI-1640 (Nissui, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum (S1610, Biowest, Florida, USA), pas-

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Abbreviations: CD, hydroxypropyl-β-cyclodextrin; FT, fourier transformation.

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saged every 4 days at 0.5×10^5 cells/mL. For cell culture assays, cells were cultured in 24-well cell culture plates (3047, Becton Dickinson, Tokyo, Japan) at 1×10^6 cells/mL. For 3D culture assay, cells were recovered by centrifugation (150×*g* for 3 min), washed with 10% sucrose in water, and resuspended in an appropriate volume of 10% sucrose. The cell solution was mixed with an equal volume of RADA16 solution, which was diluted beforehand to a given concentration with 10% sucrose. Four hundred microliters of the mixture was poured into a well of the culture plate, and 1 mL of RPMI-1640 medium with 10% FBS was immediately applied onto the mixture. Thus, the final concentration of FBS was 7.1% in the 3D culture, and the concentration of FBS in the control culture was adjusted to the same concentration. The macroscopic structure of the RADA16 gel was ascertained by microscopy after staining with 10 μg/mL Congo red. To destroy the macroscopic gel structure, 1.4 mL of the culture setup (400 μL of gel and 1 mL of culture media) was gently pipetted four times with a pipetter set at 800 μL. During the culture assay, this manipulation was repeated every 12 h. Pipetting was also performed for cells in the control culture. In order to avoid the formation of cell clusters, cells were recovered 48 h after the onset of culture and subjected to mRNA extraction. For all assays, cell viability was evaluated by trypan blue exclusion.

2.3. Immunofluorescent staining

After rinsing with PBS, 100 μL of the cell solution was dropped in 1 mL ice-cold 0.5% paraformaldehyde in PBS. After 20 min, fixation was arrested by adding 50 μL of 1 M NH_4Cl. After rinsing with PBS, 100 μL of the cell suspension was added to 5 μL of anti-CD14 antibodies conjugated with R-phycoerythrin (P5435: clone UCHM-1; Sigma).

2.4. Flow cytometry

Flow cytometry was performed for the measurement of cell volume (electronic cell volume), side scatter intensity, and fluorescence intensity using a microtiter plate-based flow cytometer (Cell Lab Quanta SC, Beckman Coulter, Tokyo, Japan) according to the manufacturer's instructions. One milliliter containing at least 5×10^5 living cells or immunostained fixed cells was applied to the analysis.

2.5. Quantitative RT-PCR

Expression levels of mRNAs were determined by fluorescent real-time PCR. After removal of the RADA16 peptide by centrifugation at 120×*g*, total RNA was extracted from the cultured cells using an RNA extraction kit (MagExtractor RNA, Toyobo, Tokyo, Japan), and cDNA was synthesized from 1 to 4 mg of total RNA using a cDNA synthesis kit (Omniscript RT, Qiagen, Tokyo, Japan). Real-time PCR was performed with Premix Ex-Taq (Takara, Tokyo, Japan) on a Prism 7300 instrument (ABI, Tokyo, Japan) using the following conditions: preheat at 50.0 °C for 2 min, hot start at 95.0 °C for 20 s, and 40 cycles of denaturation at 95.0 °C for 5 s and annealing at 60.0 °C for 31 s. The relative amount of cDNA was determined by the standard curve method, followed by normalization to the expression of five internal control genes (glyceraldehyde 3-phosphate dehydrogenase, hypoxanthine phosphoribosyltransferase 1, succinate dehydrogenase complex, subunit A, TATA box binding protein, and 14-3-3 zeta) by the geometric averaging method [10]. Real-time PCR results were expressed as fold change ($2^{-\Delta\Delta CT}$) relative to the expression level in the untreated control. Primer sequences used in the PCR procedure will be provided upon request.

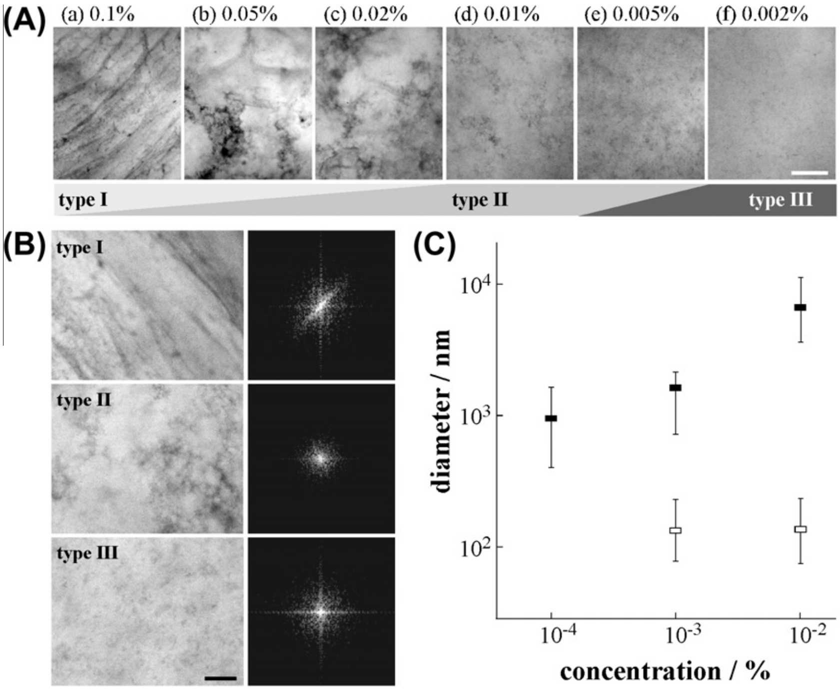

Fig. 1. Macroscopic structure of RADA16 hydrogels with different RADA16 concentrations. (A) Microscopic images of RADA16 gels with concentrations at 0.1% (a), 0.05% (b), 0.02% (c), 0.01% (d), 0.005% (e), and 0.002% (f). The bar represents 200 μm. For type I, type II, and type III structures of RADA16, see Results and discussion. The horizontal bar below the images indicates observed types of RADA16 assembly at each concentration. (B) Representative microscopic images (left) and corresponding 2-dimensional Fourier transformation (right) of type I, II, and III structures. The bar represents 100 μm. (C) Particle size distribution of RADA16 with concentrations at 0.0001%, 0.001%, and 0.01% prepared with (filled boxes) or without (open boxes) NaCl. Top and bottom of bars represent the 25th and 75th percentiles, respectively, and boxes within the bars represent the median.

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