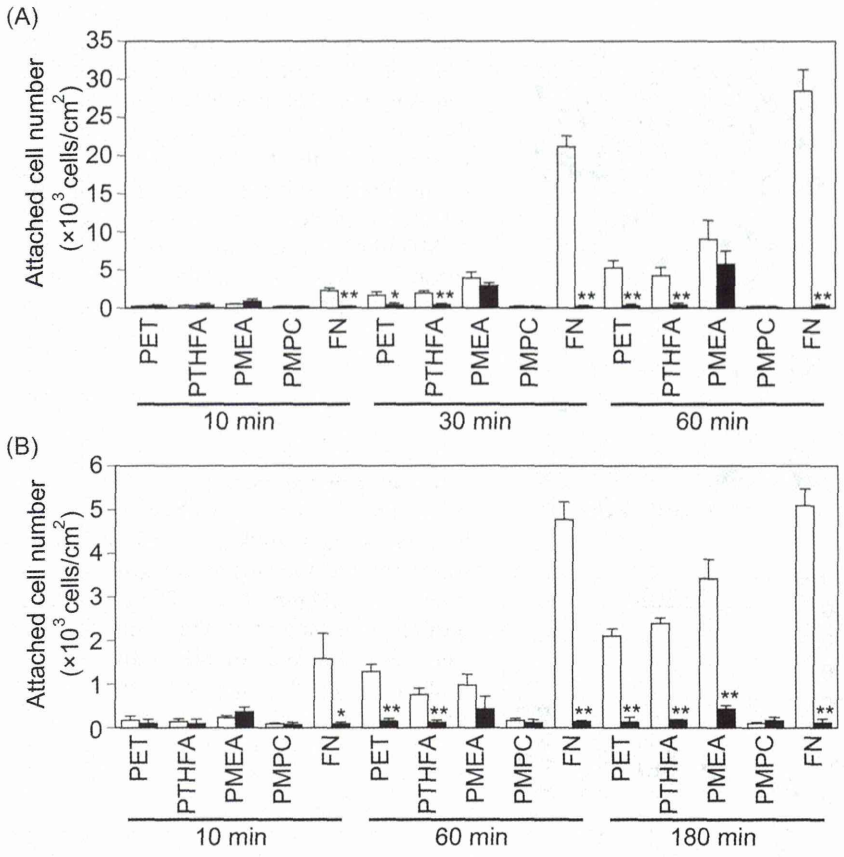

Figure 3. Integrin-dependent cell attachment. A) Inhibitory effect of EDTA on MDA-MB-231 cell attachment. B) Inhibitory effect of EDTA on MCF-10A cell attachment. The white and black bars represent the EDTA (–) and EDTA (+) data, respectively. The data represent the means ± SD ($n = 3$). *: $P < 0.05$, **: $P < 0.01$ vs EDTA (–).

inhibited. In contrast, the attachment of MCF-10A cells was almost completely inhibited on the PET, PTHFA, PMEA, and FN substrates in the presence of only EDTA. In the presence of only heparin, evident attachment inhibition of MDA-MB-231, MCF-7, and MCF-10A cells was not observed on all of the examined polymer substrates. Furthermore, in the presence of EDTA and heparin, a slight inhibitory effect of heparin on the attachment of these cells was evident. These results indicate that these cells do not attach to polymer substrates via syndecans.

2.4. Availability of Adsorbed Fibronectin on Polymer Substrates

The cells examined in this study partially or mainly attached to the blood-compatible PMEA and PTHFA substrates via integrin. For integrin-dependent attachment, ECM proteins, such as FN, should be adsorbed and expose their cell-attachment sites.[17–19] We thus compared the exposure degree of FN cell-attachment sites on the PTHFA, PMEA, and PMPC substrates through an enzyme-linked immunosorbent assay (ELISA) with an antibody that can detect the exposure of cell-attachment sites (**Figure 5**A). Additionally, the exposure degree of FN was examined on a polystyrene (PSt) substrate instead of the PET substrate. The cell-attachment sites were hardly exposed on the PMPC substrate and were exposed on the PSt substrate. The exposure degree of FN cell-attachment sites on the PMEA and PTHFA substrates was 1.9- and 2.1-folds, respectively, than that obtained with the PSt substrate, which indicates that the cells can attach to the blood-compatible PMEA and PTHFA substrates via integrin–FN interactions. Moreover, the exposure degree of FN cell-attachment sites on the PTHFA substrate was significantly greater than that obtained for the PMEA substrate.

2.5. Attachment Affinity of the Cells to Fibronectin and Vitronectin Substrates

Furthermore, we examined the ability of MDA-MB-231, MCF-7, and MCF-10A cells to attach to FN and vitronectin (VN), which is another major ECM protein in serum, through cell attachment assays (Figure 5B,C). This cell attachment assay was performed in serum-free medium to avoid the effect of adsorbed serum proteins. MDA-MB-231, MCF-7, and MCF-10A cells started to attach to FN and VN substrates within 30 min. The numbers of attached MCF-10A cells on the FN and VN substrates were 1.4- to 1.9-fold greater than those obtained with MDA-MB-231 and MCF-7 cells, which suggests that the affinities of MCF-10A cells to the FN and VN substrates are higher than those of MDA-MB-231 and MCF-7 cells. These results indicate that MDA-MB-231, MCF-7, and MCF-10A cells can attach to the blood-compatible PMEA and PTHFA substrates via interactions between integrin and the cell-attachment sites of FN/VN exposed on the PMEA and PTHFA substrates.

2.6. Cell Attachment on Polymer Substrates under Serum-Free Conditions

In the case of MDA-MB-231 and MCF-7 cell attachment, the cells attached to the PMEA substrate via integrin-dependent and -independent mechanisms. On the PMEA substrate, proteins are hardly adsorbed.[7,11–13,20,21] In fact, the amounts of bovine serum albumin (BSA) adsorbed to the PMEA, PTHFA, and PSt substrates were measured using quartz crystal microbalance (QCM) after 15 min and were found to be 13.7 ± 4.0 ng cm^{-2}, 374.2 ± 14.3 ng cm^{-2}, and 282.2 ± 27.8 ng cm^{-2}, respectively. Additionally, we quantified the amount of serum proteins adsorbed to PMEA, PTHFA, and tissue culture polystyrene (TCPS) from medium containing 10% serum using a micro-BCA assay. The amounts of serum proteins adsorbed on PMEA, PTHFA, and TCPS were found to be 200 ± 143 ng cm^{-2}, 475 ± 86 ng cm^{-2}, and 626 ± 133 ng cm^{-2}, respectively, after 1 h. On the PMPC substrate, protein adsorption could not be

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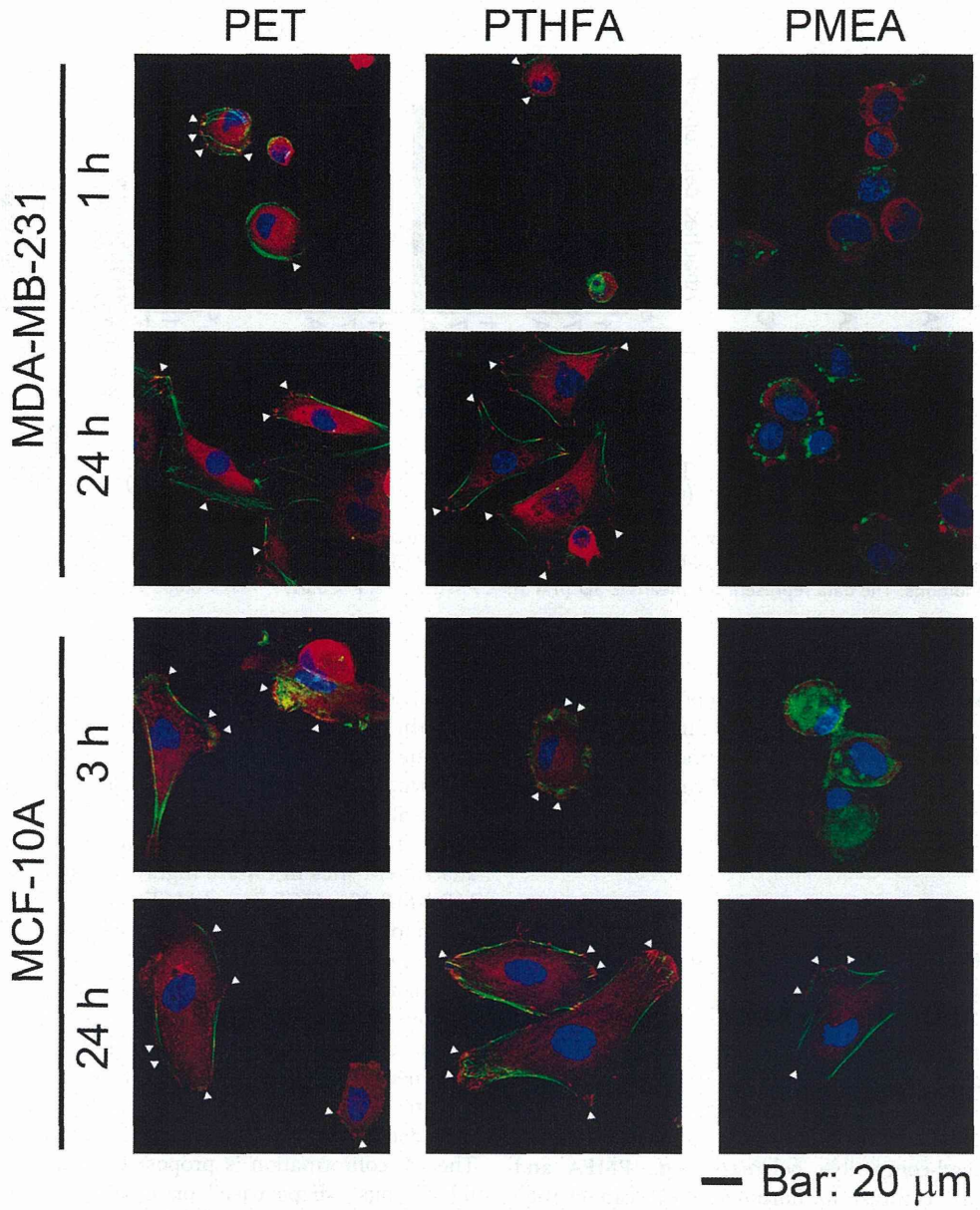

Figure 4. Focal adhesion formation on polymer substrates. The cell nuclei, actin fibers, and vinculin were visualized with DAPI (blue), Alexa Fluor 488 (green), and Alexa Fluor 568 (red), respectively. The arrowheads indicate the focal adhesions. The bars represent 20 μm. The results for the FN substrates are shown in Figure S4 (Supporting Information).

detected by QCM and micro-BCA assay. These results suggest that the proteins did not completely cover the PMEA substrate. However, protein adsorption was suppressed on the PMEA substrate compared with the PTHFA and PSt/TCPS substrates. Therefore, it is possible that cells can access the PMEA substrate directly even in serum-containing medium. In fact, it has been reported that cells can attach to the substrate in serum-free medium via an integrin-independent mechanism.[22,23] We hypothesized that the cells examined in this study can directly attach to the PMEA substrate through an integrin-independent mechanism in the absence of adsorbed proteins.

To examine whether cells can attach to polymer substrates without protein adsorption, a cell attachment assay was performed in serum-free medium (**Figure 6** and Figure S6, Supporting Information). An increased number of MDA-MB-231 and MCF-7 cells attached to the PET, PTHFA, and PMEA substrates in serum-free medium compared with that found in serum-containing medium (Figure 6A and Figure S6, Supporting Information), which indicates that cells can attach to polymer substrates even in serum-free medium. In contrast, fewer MCF-10A cells attached to polymer substrates in serum-free medium compared with that in serum-containing medium (Figure 6B). In serum-free medium, the cell attachment to the PMEA, PTHFA, and PET substrates was not inhibited by EDTA, which suggests that cells attach to these substrates via integrin-independent mechanisms in serum-free medium.

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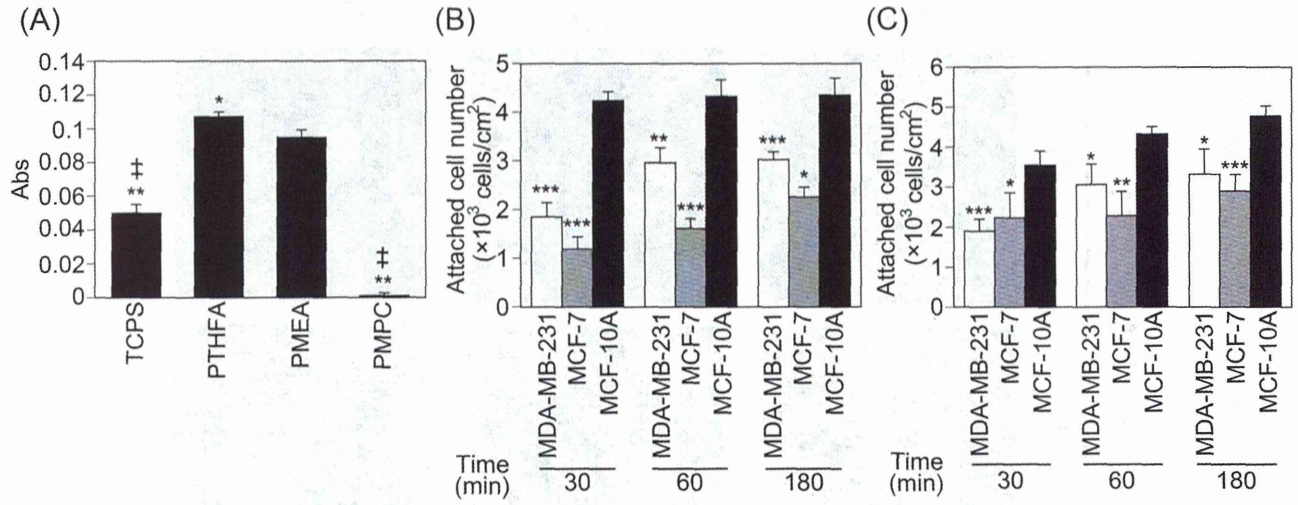

Figure 5. Attachment affinity of adsorbed ECM proteins. A) Degree of exposure of the cell-attachment sites of adsorbed fibronectin. The data represent the means ± SD ($n = 3$). *: $P < 0.05$, **: $P < 0.01$ vs PMEA; ‡: $P < 0.01$ vs. PTHFA. B,C) Attachment affinity of the cells to B) FN and C) VN substrates under serum-free conditions. The data represent the means ± SD ($n = 3$). *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.005$ vs MCF-10A.

This finding is consistent with the results reported by previous studies.[19] These results indicate that an integrin-dependent cell attachment mechanism in serum-free medium promotes the attachment of MDA-MB-231 and MCF-7 cells but not MCF-10A cells.

3. Discussion

3.1. Possible Mechanism of Cell Attachment to Blood-Compatible Polymer PMEA and PTHFA Substrates

In this study, we showed that MDA-MB-231, MCF-7, and MCF-10A cells attached to the blood-compatible PMEA and PTHFA substrates but not the PMPC substrate. Fibrinogen adsorption and its deformation are suppressed to prevent platelet attachment to blood-compatible polymers (e.g., PMEA and PTHFA).[7,12,13,24] In contrast to fibrinogen, FN can change its conformation to enable cell attachment even to the blood-compatible PMEA and PTHFA substrates. This conformational change of FN allows MDA-MB-231, MCF-7, and MCF-10A cell attachment to the PMEA and PTHFA substrates via FN–integrin interactions.

Platelets possess the FN receptor, integrin $\alpha5\beta1$. However, integrin $\alpha5\beta1$ is generally found at a resting state, in which it exhibits a low binding affinity to ligands.[25,26] To activate this integrin, glycoprotein VI on platelet surfaces has to interact with collagen, which localizes in the ECM in vivo.[25] As a result, platelets cannot attach to PMEA substrates even though the cell-attachment sites in FN are highly exposed, which explains why MDA-MB-231, MCF-7, and MCF-10A cells but not platelets can attach to the blood-compatible PMEA and PTHFA substrates.

We previously reported that intermediate water in hydrated polymers plays a key role in the suppression of protein deformation.[7,11–13,20] In fact, cell-attachment sites of FN were minimally detected on the PMPC substrate, which contains more intermediate water than the PMEA and PTHFA substrates (Figure 5A).[11,27] In contrast, the cell-attachment sites of FN were detected on the PMEA and PTHFA substrates (Figure 5A). The FN conformation is proposed to exhibit a "beads on flexible strings" shape based on electron microscopy and X-ray crystallography analyses.[17–19] Due to the high flexibility of FN, more intermediate water may be required to suppress FN deformation compared with the amount needed to suppress fibrinogen deformation (**Figure 7**A). Moreover, the degree of exposure of the cell-attachment sites of FN on the PMEA and

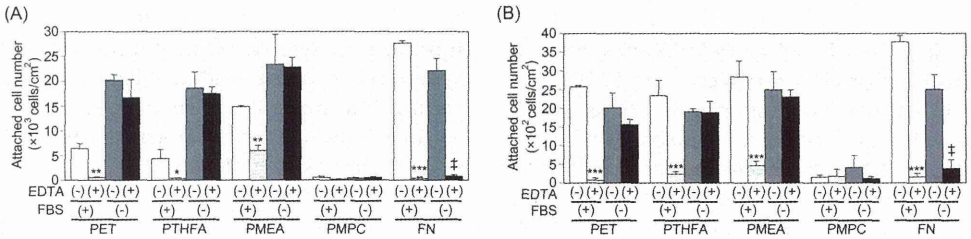

Figure 6. Cell attachment on polymer substrates in serum-free medium. A) Attachment of MDA-MB-231 cells in serum-free medium after 1 h. B) Attachment of MCF-10A cells in serum-free medium after 3 h. The data represent the means ± SD ($n = 3$). *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.005$ vs. FBS (+)/EDTA(–). ‡: $P < 0.01$ vs FBS (–)/EDTA (–).

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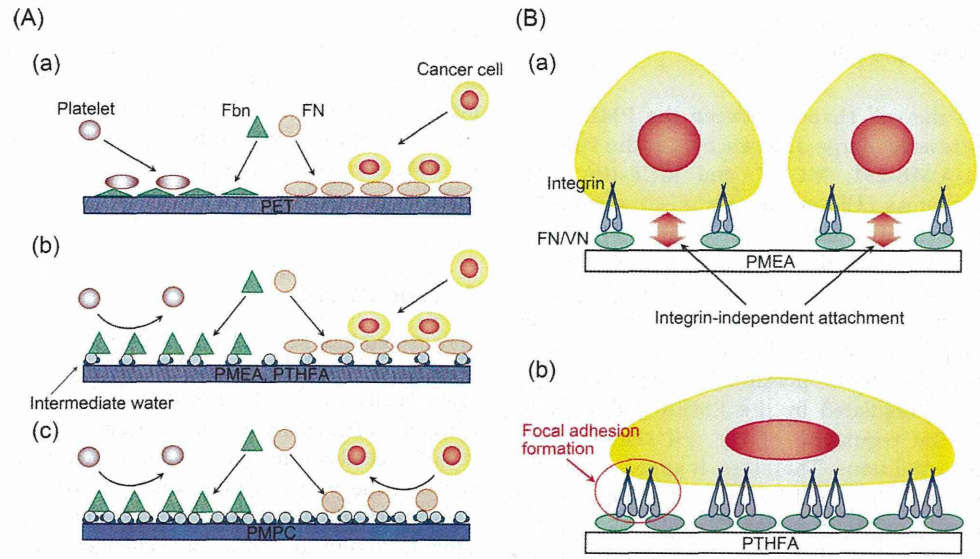

Figure 7. A) Models of cell attachment to the blood-compatible PMEA and PTHFA substrates. A-a) Both platelets and cancer cells can attach to the PET substrate with deformed fibrinogen and fibronectin (FN), respectively. A-b) Cancer cells can attach to the PMEA and PTHFA substrates with deformed FN. Platelets cannot attach to the PMEA and PTHFA substrates because fibrinogen deformation is suppressed by intermediate water. A-c) Cancer cells and platelets cannot attach to the PMPC substrate due to the absence of fibrinogen and FN deformation. FN and Fbn indicate FN and fibrinogen, respectively. B) Differences in the attachment mechanisms between the PMEA and PTHFA substrates. B-a) On the PMEA substrate, the MDA-MB-231 and MCF-7 cells interact with the adsorbed FN and VN. Moreover, MDA-MB-231 and MCF-7 cells directly interact with the PMEA substrates through an integrin-independent attachment mechanism. The integrin-independent attachment of MCF-10A cells is weak and does not appear to affect the total cell attachment. B-b) On the PTHFA and PET substrates, the cells are attached through an integrin-dependent mechanism that forms focal adhesions. More proteins are adsorbed on the PTHFA and PET substrates compared with the PMEA substrate, which suppresses integrin-independent attachment.

PTHFA substrates was greater than that obtained with the PSt substrate, although the protein adsorption on the PMEA and PTHFA substrates was lower than that obtained with the PSt substrate. The cell-attachment sites of FN contain two sites to promote cell attachment, namely an RGD site and a synergistic site. These two sites are strictly regulated to face the same surface.[18] It appears easy that a strong interaction between substrates and FN can break the spatial alignment between these two cell-attachment sites of FN. Therefore, it is possible that a major non-physiological deformation occurred and obscured these cell-attachment sites on the PSt substrate.

3.2. Differences in the Cell-Attachment Mechanisms of MDA-MB-231, MCF-7, and MCF-10A Cells Between Different Polymer Substrates

Although the degree of exposure the cell-attachment sites of FN was greater on the PTHFA substrate compared with the PMEA substrate, more MDA-MB-231 and MCF-7 cells attached to the PMEA substrate than to the PTHFA substrate. Our results showed that the attachment of MDA-MB-231 and MCF-7 cells to the FN and VN substrates was lower than that found for MCF-10A cells (Figure 5B,C). Therefore, the minor effects of FN and VN adsorbed on the PTHFA substrate were apparent in the attachment of MDA-MB-231 and MCF-7 cells.

Compared with their attachment to the PTHFA substrate, MDA-MB-231 and MCF-7 cells attached through both integrin-dependent and -independent mechanisms to the PMEA substrate (Figure 3A and Figure S3, Supporting Information), which suggests that an integrin-independent mechanism enhanced the MDA-MB-231 and MCF-7 cell attachment. Fewer proteins were adsorbed to the PMEA substrate compared with the PTHFA substrate, which results in the exposure of the PMEA substrate to allow direct interactions with the cells. This finding is similar to the interactions observed in serum-free medium. The MDA-MB-231 and MCF-7 cell attachments in serum-free medium were greater than those obtained with serum-containing medium. This cell attachment mechanism in the absence of serum proteins may increase the attachment of MDA-MB-231 and MCF-7 cells to the PMEA substrate (Figure 6A and Figure S6, Supporting Information). Therefore, the difference in the attachment of MCF-10A cells to the PMEA and PTHFA substrates was smaller than that obtained with the MDA-MB-231 and MCF-7 cells.

In contrast, the attachment of MCF-10A cells in serum-free medium was weak, and increased attachment was observed on the FN and VN substrates compared with the MDA-MB-231 and MCF-7 cells (Figure 7B). This finding shows that the minor effects of integrin-independent attachment are apparent in MCF-10A cell attachment to the PMEA substrate. It has been reported that integrin-independent attachment prevents focal adhesion formation and suppresses integrin signaling for cell spreading.[28] In addition to these weak interactions between the MDA-MB-231 and MCF-7 cells and the FN/VN substrates, integrin-independent attachment appears to influence the suppression of focal adhesion formation and cell spreading on the PMEA substrate, despite the exposure of many cell-attachment sites of FN on the PMEA substrate.

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3.3. Possible Biomedical Application of the Novel Blood-Compatible PMEA and PTHFA Polymers

The comparison of the cell shapes between the PMEA and PTHFA substrates revealed large differences. On the PTHFA substrate, the MDA-MB-231, MCF-7, and MCF-10A cells were well-spread, whereas cell spreading was suppressed on the PMEA substrate. Many studies have demonstrated that the cell shape affects its functions, including cell survival, growth, differentiation, and the expression of tissue-specific functions.[29–31] For the development of artificial tissues and organs, both the regulation of cell functions and the regulation of cell attachment are important. It is expected that cell functions can be regulated by the PMEA and PTHFA substrates through the regulation of the cell shapes, even under blood-contact conditions. Therefore, it appears that the PMEA and PTHFA substrates will be suitable substrates for the regulation of cell functions under blood-contact conditions, although this possibility should be further examined in the future.

In the clinic, the detection and isolation of CTCs from blood are important for the diagnosis and prognosis of metastatic cancer. Currently, CTCs are detected and isolated using an antibody against EpCAM, which is a cell membrane protein.[32] Although this antibody-based CTC detection and isolation system has been applied in the clinic, this system misses the detection of EpCAM-negative cancer cells undergoing the epithelial-mesenchymal transition. Moreover, the avoidance of artifact generation during CTC isolation appears important for CTC-based drug testing in personalized medicine.[32] To overcome these problems, many approaches, including size-dependent isolation of CTCs, have been developed.[33] Newly categorized blood-compatible polymers, such as PMEA and PTHFA, represent a novel approach for cell attachment-based CTC detection and isolation.

In addition to CTCs, the isolation of stem cells and somatic cells is also important for regenerative medicine. Recently, Reves and García[34] showed that it is possible to select between human pluripotent stem cells and differentiated cells based on the difference between their cell-attachment strengths. In our study, we showed that cancer cells attach to the PMEA substrate via integrin-dependent and -independent mechanisms. We also showed that the focal adhesion formation in the cells on the PMEA substrate was delayed compared with the PTHFA and PET substrates. It has been reported that FN-dependent attachment increases the strength of the resulting cell-substrate attachment.[35] It appears that the strengths of the cell attachment to the PMEA and PTHFA substrates are different. Therefore, it is possible that different cell types can be selected by the PMEA and PTHFA substrates based on differences in the cell-attachment strength.

4. Conclusions

We showed that MDA-MB-231, MCF-7, and MCF-10A cells can attach to the blood-compatible PMEA and PTHFA substrates. Additionally, we showed that the balance of protein deformation between fibrinogen and FN is important for the attachment of cells to blood-compatible polymers. A summary of the differences in the protein adsorption, deformation, and cell-attachment mechanisms on blood-compatible polymers is shown in **Table 1**. Protein conformational changes affect various cell functions, including cell differentiation.[36,37] Intermediate water in hydrated polymers plays an important role in the suppression of protein deformation.[7,11–13,20] Therefore, it may be possible to regulate cell functions using blood-compatible polymers by controlling the intermediate water content. These polymers can be used to isolate CTCs and stem cells from blood and for the development of blood-contact biomedical applications, such as endothelial cell-covered artificial blood vessels and bioartificial livers using liver cells.

5. Experimental Section

Cell Culture: The MDA-MB-231 and MCF-10A cells were obtained from American Type Culture Collection (ATCC, Manassas, VA). The MCF-7 cells were obtained from Health Science Research Resources Bank (Osaka, Japan). All of the cells were maintained in Dulbecco's Modified Eagle/Nutrient Mixture F-12 (DMEM/F-12, Gibco, Carlsbad, CA) containing 10% fetal bovine serum (FBS, Equitech-Bio, Kerrville, TX). Prior to the experiments, the cells were detached from the tissue culture polystyrene (TCPS) dish (IWAKI, Chiba, Japan) with a 0.25% trypsin/EDTA solution (Gibco).

Preparation of Polymer Substrates: PMEA and PTHFA were synthesized according to previous reports.[7,11] The copolymer of MPC and butyl methacrylate (30:70 mol%, PMPC) was kindly gifted by the NOF Corporation (Tokyo, Japan). The chemical structures of the polymers are shown in Figure S7 (Supporting Information). The polymer substrates

Table 1. Summary of cell attachment to blood-compatible polymers.

Cell type	MDA-MB-231/MCF-7				MCF-10A			
Attachment to FN/VN substrates	Weak				Strong			
Attachment in serum-free medium	Strong				Weak			
Types of substrates	PMEA	PTHFA	PET (PSt)	PMPC	PMEA	PTHFA	PET (PSt)	PMPC
Protein adsorption	Small	Large	Large	Small	Small	Large	Large	Small
Exposure of cell-attachment site	Large	Large	Large	Small	Large	Large	Large	Small
Attachment mechanism	Integrin- + non-integrin-dependent	Integrin-dependent	Integrin-dependent	N. D.	Integrin-dependent	Integrin-dependent	Integrin-dependent	N.D.

N.D.: Not determined

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were prepared on PET discs (Ø = 14 mm, thickness = 125 μm, Mitsubishi Plastics, Tokyo, Japan) using a spin-coating method. Briefly, PMEA and PMPC were dissolved in methanol at concentrations of 0.2 wt%. PTHFA was dissolved in methanol/chloroform (5:1) at a concentration of 0.2 wt%. Forty milliliters of the polymer solution was cast on the PET disc and spin-coated twice under the following conditions: 500 rpm for 5 s, 1500 rpm for 10 s, 1500 to 4000 rpm (slope) for 5 s, 4000 rpm for 10 s, and 4000 to 0 rpm (slope) for 5 s. The polymer substrates were sterilized by UV exposure for 2 h and stored at 4 °C until use. The FN, which was derived from bovine plasma (FN, Calbiochem, Darmstadt, Germany), and vitronectin, which was derived from bovine plasma (VN, Sigma, St Louis, MO), substrates were prepared on PET discs. Sterilized PET discs were immersed in FN (10 μg ml^{-1}) or VN (2 μg mL^{-1}) solutions for 4 h at 37 °C for protein coating. After coating, the discs were washed with water and air-dried for 1 h. The FN and VN substrates were stored at 4 °C until use.

Cell Attachment Assay: Prior to cell culture, the polymer substrates were immersed in 10% FBS containing DMEM/F-12 or FBS-free DMEM/F-12 for 1 h at 37 °C. The MDA-MB-231 and MCF-7 cells were seeded on the polymer substrates at a density of 5×10^4 cells cm^{-2}. The MCF-10A cells were seeded on the substrates at a density of 1×10^4 cells cm^{-2}. The cells were allowed to attach to the substrates in 10% FBS containing DMEM/F-12 or serum-free DMEM/F-12 for the indicated time periods. The non-attached cells were removed from the culture by washing twice with PBS. The attached cells were fixed with 0.1% glutaraldehyde overnight at room temperature. The cells were stained with a 0.2% crystal violet (Wako, Osaka, Japan) solution for 15 min for visualization. After staining, the attached cells in three randomly selected fields were counted using an optical microscope.

For the inhibition assay, the cells were treated with 5×10^{-3} M EDTA or 500 μg mL^{-1} heparin (Sigma) for 10 min at 37 °C before cell seeding. After treatment, the cell attachment assay was performed as described above.

To compare the cell-attachment affinity to the FN and VN substrates, all of the cell types were seeded in serum-free medium at a density of 1×10^4 cells cm^{-2}. A summary of the seeding cell density used in each cell attachment assay is shown in Table S1 (Supporting Information).

Evaluation of Cell Shape: The MDA-MB-231 and MCF-7 cells were seeded on the polymer substrates at a density of 5×10^4 cells cm^{-2}. The MCF-10A cells were seeded on the substrates at a density of 1×10^4 cells cm^{-2}. The cells were cultured in 10% FBS containing DMEM/F-12 for 1 d. After culture, the cells were fixed with 0.1% glutaraldehyde and stained with crystal violet as mentioned above. Each cell shape was traced using a graphics tablet (Bamboo Fun, Wacom, Saitama, Japan). After tracing, the projected cell area was measured using the Adobe Photoshop CS4 and imageJ software programs.

Immunocytochemical Analysis: The cells were cultured on the substrates for the indicated time periods. After culture, the cells were fixed with 4% paraformaldehyde containing phosphate-buffered saline (PBS) (Wako) for 10 min at 37 °C and treated three times with 1% Triton X-100 containing PBS for 10 min at room temperature. After permeabilization, the samples were incubated with anti-vinculin antibody (Ab) (Millipore, Billerica, MA) for 2 h at 37 °C and then treated with Alexa Fluor 488-conjugated phalloidin (Invitrogen, Carlsbad, CA) and Alexa Fluor 568-conjugated anti-mouse IgG Ab (Invitrogen) for 1 h at 37 °C. For the immunocytochemical analysis, the Can Get Signal (ToYoBo, Osaka, Japan) was used. For the counterstaining of the cell nuclei, the ProLong Gold Antifade Reagent with DAPI was mounted. The observations were performed using a confocal laser scanning microscope (Olympus, Tokyo, Japan).

Enzyme-Linked Immunosorbent Assay (ELISA): For ELISA, the polymer substrates were prepared using the polymer casting method. Briefly, 11.2 μL of each polymer solution (0.2 w/v%) was added to the 96-well plate, and the plate was air-dried for 1 week. After incubation with PBS at 37 °C for 1 h, human FN (5 μg mL^{-1}, 50 μL $well^{-1}$, Sigma) was added to the plate, and the plate was incubated for 1 h at 37 °C. After the adsorption of human FN, the samples were incubated with Blocking-One (Nacalai Tesque, Kyoto, Japan) for 30 min at room temperature to prevent non-specific reactions. After blocking, the samples were incubated with HFN7.1 Ab (Abcam, Cambridge, UK) for 2 h at room temperature and then with peroxidase-conjugated anti-mouse IgG Ab for 1 h at room temperature. After incubation with the Abs, the samples were incubated with 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid ammonium salt) (ABTS) substrate (Roche Diagnostics). The absorbance was measured at a wavelength of 405 nm.

Measurement of Protein Amount Adsorbed on the Substrates: QCM analysis and micro-BCA assay were performed to measure the amount of proteins adsorbed on the polymer substrates. QCM was used to measure the amount of adsorbed BSA. Affinix Q N Pro was used as a 27-MHz QCM instrument (Initium, Tokyo, Japan). The 27-MHz QCM was calibrated to detect at a frequency of 1 Hz, which corresponds to a mass increase of 0.62 ng cm^{-2}. Quartz plates with an Au electrode coated with PSt were also obtained from Initium. The plates were coated with the polymers as mentioned above. The polymer-coated plates were equipped with sensor cells, and the plates were then immersed in PBS to stabilize the quartz crystal frequency. After stabilization, the plates were immersed in 1 mg mL^{-1} bovine serum albumin (Sigma) containing PBS for 15 min at 37 °C, and the change in frequency was measured.

The micro-BCA assay was performed to measure the amount of serum proteins adsorbed on the polymer substrates. Polymer-casted 96-well TCPS plates were immersed in PBS for 1 h at 37 °C. Then, 100 mL of 10% FBS containing DMEM/F-12 medium was added to each well. After a 1-h incubation at 37 °C, the incubated wells were washed seven times with PBS. The adsorbed proteins were extracted by incubation with a solution of 5% sodium dodecyl sulfate (SDS) and 0.1 N NaOH for 10 min at room temperature. The extracted proteins were assessed by a micro-BCA assay (Thermo Scientific, Rockford, IL) according to manufacturer's instructions. The protein amount was calculated using the BSA standard curve.

Statistical Analysis: All of the data are expressed as the means ± SD. The significance of the differences between two samples was determined through an unpaired Student's t test using Microsoft Excel 2010. The statistical analyses used to analyze the differences between three or more samples were performed using R, which is a language and environment for statistical computing. The significance of the differences between three or more samples was determined using analysis of variance (ANOVA). Tukey's multiple comparison test was applied as a post-hoc test. Differences with P values less than 0.05 were considered statistically significant.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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FOCUS REVIEW

Design of biocompatible and biodegradable polymers based on intermediate water concept

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Polymeric biomaterials have significant impact in the aged society. Biocompatible and biodegradable polymers have emerged during the past decades to promise extraordinary breakthroughs in a wide range of diagnostic and therapeutic medical devices. Understanding and controlling the interfacial interactions of the polymeric biomaterials with biological elements, such as water, ions, proteins, bacteria, fungai and cells, are essential toward their successful implementation in biomedical applications. Here we highlight the recent developments of biocompatible and biodegradable fusion polymeric biomaterials for medical devices and provide an overview of the recent progress of the design of the multi-functional biomedical polymers by controlling bio-interfacial water structure through precision polymer synthesis and supramolecular chemistry.
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INTRODUCTION

In biomedical applications, there are continuous efforts to enhance methods, materials and devices. The recent development of novel biomaterials and their applications to biomedical problems have dramatically improved the treatment of many diseases and injuries.[1–3] Although a various types of materials in biomedicine have been used widely, most biomaterials lack the desired functional properties to interface with biological systems and have not been engineered for optimum performance. Therefore there is an increasing demand to develop novel materials to address such problems in biomedicine arena. Biocompatible and biodegradable fusion polymers are a class of new generation of biomaterials that have demonstrated great potential for medical devices, tissue engineering scaffolds, drug delivery and biomedical-healthcare sensors.

There are numerous parameters of polymeric biomaterials that can affect the cellular behavior in a controlled manner. The underlying mechanisms for the biocompatibility of polymers at the molecular level are complex and have not been clearly demonstrated, although many theoretical and experimental efforts have been made to understand these mechanisms.[4,5] Water and proteins interactions have been recognized as fundamental for the biological response upon contact with polymers. We have proposed the 'Intermediate Water' concept[6–8] on the basis of results on the water sorption process into polymeric biomaterials. The water exhibited clearly defined peaks for cold crystallization in the differential scanning calorimetry (DSC) chart, a strong peak at 3400 cm^{-1} in a time-resolved infrared spectrum and higher mobility of water in a ^2H-nuclear magnetic resonance.[6–8] As a result, the biocompatibility of polymers was ascribed to the predominant population of intermediate water in the hydrated polymers. Intermediate water interacts with polymer chains in a intermediate way, that is, stronger than free water but weaker than tightly bound non-freezing water. We hypothesized that intermediate water, which prevents the proteins and blood cells from directly contacting the polymer surface on the polymer surface, has an important role in the biocompatibility of polymers.

In this focus review, we describe the recent design of biocompatible and biodegradable polymeric biomaterials for various applications in medical devices. Here we present various synthetic strategies for the preparation of the biomaterials, which include characteristic properties of the biocompatibility, biodegradability and anti-microbial activity of polymer-based biomaterials in a self-organization manner. In addition, we describe the applications of polymer-based biomaterials in tissue engineering and medical devices and provide an overview of the recent experimental progress of the screening of multi-functional biocompatible polymers based on bio-interfacial water structure.

BIOCOMPATIBLE POLYMERIC BIOMATERIALS

Polymeric materials for the medical devices that may come in contact with human blood should have capacity to resist protein adsorption and blood cell adhesion and thus triggering the organism's defense systems.[1] Some biocompatible polymer surfaces have been developed, and they fall into the following three categories:[1,6] (a) hydrophilic surfaces, (b) surfaces with micro-phase-separated domains, and (c) biomembrane-like surfaces, including zwitterionic groups. Physicochemical properties, including wettability, surface free energy, surface charge, stiffness, topography and the presence of specific

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chemical functionalities, surface bound water appears to bear an instrumental role in the biological response induced by the synthetic polymers.[1,9] New-generation polymer poly(2-methoxyethyl acrylate) (PMEA) shows excellent blood compatibility and biocompatibility and has been approved for medical use by the Food and Drug Administration.[6–8] For instance, PMEA-coated circuits and tubes exhibit significantly reduced blood cell activation when used in cardiopulmonary bypass and catheters for central veins of human blood vessels. It has been maintained that PMEA's compatibility with platelets, white and red blood cells (RBCs), complement and coagulation systems has been dictated by the presence of the intermediate water.[6–8]

It should be noted that the word 'biocompatibility' is used in general as the term evaluating properties of materials that do not cause adverse effect when the materials come into contact with living organisms, such as proteins, biological cells and tissues.[5] This review primarily deals with 'biocompatibility' of polymer materials against various biological elements in human blood flow system.

PRINCIPLE OF CELL ATTACHMENT ON POLYMERS

Cells can attach in serum-containing medium even on polymers, such as polystyrene and polyethylene terephthalate, which do not possess any specific cell attachment ligands.[10,11] On these polymers, serum proteins (for example, fibrinogen and fibronectin) generally adsorb and change their conformation to allow the cells to attach or to function as cell attachment ligands (Figure 1a).[10,11] Protein adsorption and its conformational change are thus critical for cell attachment on polymers, and the regulation of protein adsorption leads to the control of cell attachment on polymers. We have suggested that intermediate water can influence protein adsorption on polymers.[12,13] Therefore the attachment behavior of the cells will be different between PMEA and conventional polymers such as polystyrene due to the difference of protein adsorption and its conformational change.

Different attachment of human platelets and non-blood cells on PMEA and its analogous polymers

Cell attachment ligands are different among the cells. It has been reported that platelets require the adsorption of fibrinogen and adsorption-induced conformational change, which exposes cell attachment sites for their attachment.[11] Therefore, it is necessary to prevent the adsorption and conformational change of fibrinogen on the polymers for the acquirement of blood compatibility. In contrast to platelet attachment, non-blood cells require the adsorption and the conformational change of fibronectin rather than fibrinogen for their attachment. Previously reported blood compatible polymers such as polyethylene glycol and the polymers containing 2-methacryloyloxyethyl phosphorylcholine (PMPC) have been reported to prevent the adsorption and the conformational changes of any proteins, including both fibrinogen and fibronectin, and thus any types of cells cannot attach on the substrates coated with them (Figure 1c).[14,15]

PMEA and its analogous polymer, poly(tetrahydrofurfuryl acrylate) (PTHFA), have been reported as blood compatible polymers.[16,17] These polymers suppress the adsorption and conformational change of fibrinogen to prevent platelet attachment.[12,16] Recently, we have reported that PMEA and PTHFA do not suppress the conformational change of fibronectin, and the fibronectin can expose their cell attachment sites on the polymers.[13] Non-blood cells can attach on PMEA and PTHFA due to such fibronectin (Figure 1b).[13] PMEA and PTHFA are thus newly categorized as blood compatible polymers, which allow the attachment of non-blood cells but not platelets.[13,18]

Adsorption-induced conformational change is determined by protein flexibility. The difference of conformational change between fibronectin and fibrinogen observed on PMEA and PTHFA might be due to the difference of flexibility of these proteins. Fibronectin shapes 'beads on strings' and shows high flexibility.[19,20] Fibronectin can change its conformation even on the polymers that prevent conformational change of adsorbed fibrinogen. Intermediate water keeps proteins away from non-freezing water, which induces conformational change of protein.[12,16] It appears that necessary amounts of intermediate water to prevent conformational change are different between fibronectin and fibrinogen due to the difference of flexibility. Therefore cell attachment can be regulated by the regulation of intermediate water content through the regulation of protein conformational change (Table 1).

Recent advances in biology and medicine require blood-contact biomedical applications, including cell isolation from blood and endothelial cell-covered artificial blood vessels and stents. Newly categorized blood-compatible polymers, such as PMEA and PTHFA, are useful for these applications. Therefore the techniques to control intermediate water contents will strongly progress blood-contact biomedical applications through the regulation of protein adsorption and the following cell attachment.

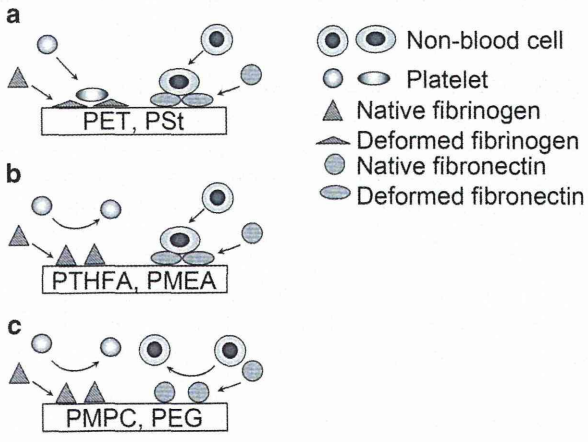


Figure 1 Schematic illustration of cell attachment on polymers, (**a**) conventional polymers (polyethylene terephthalate (PET) and polystyrene (PSt)), (**b**) PTFHA, PMEA and PMEA analogous polymers. (**c**) PMPC and polyethylene glycol (PEG).

Table 1 Relationship among intermediate water contents, protein conformational change, and cell attachment

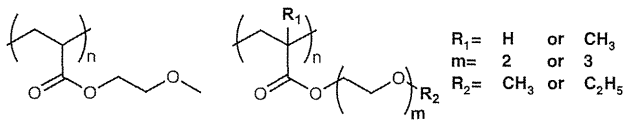
Intermediate water contents	None	Low	High
Fibrinogen conformation	Change	Remain	Remain
Fibronectin conformation	Change	Change	Remain
Platelet attachment	Capable	Incapable	Incapable
Non-blood cell attachment	Capable	Capable	Incapable
Polymers	For example, PSt, PET	For example, PMEA, PTHFA	For example, PMPC, PEG

Abbreviations: PEG, polyethylene glycol; PET, polyethylene terephthalate; PMEA, poly(2-methoxyethyl acrylate); PMPC, poly(2-methacryloyloxyethyl phosphorylcholine); PTHFA, poly (tetrahydrofurfuryl acrylate); PSt, polystyrene.

CONTROLS ON WATER STRUCTURE AT THE BIOINTERFACE THROUGH PRECISION POLYMER SYNTHESIS

Precision control over the polymers' biocompatibility is a longstanding drawback in the arena of biocompatible polymeric materials, and the synthesis of well-defined polymers having precisely controlled molecular architecture is a powerful approach for the manipulation of polymer properties. This is particularly true in the development of biocompatible polymeric materials where the primary structure of polymers, for example, molecular weight, molecular weight distribution, monomer sequence distribution, stereoregularity, side-chain functionality, chain-end structures and long-chain branching, can greatly affect the biocompatibility of polymeric materials. To clarify the fundamental relationship between the biocompatible property of polymers and the chemical structure of polymeric biomaterials, we have started a study to elucidate the structure-property relationships in blood-compatible polymers by means of precision polymer synthesis.

Thus far, we have been investigating the relationship between the polymer primary structures and their blood compatibility by utilizing vinyl polymers having hydrophilic functional groups. In our previous studies, we have reported that PMEA, which has a quite simple chemical structure, exhibits superior blood compatibility;[21–23] and PMEA possesses the unique hydration water structure, intermediate water in the hydrated state.[24–29] We have further investigated the blood compatibility of PMEA analogous polymers (Figure 2) having differences in the backbone structure (acrylate or methacrylate), oligo (ethylene glycol) (EG) side-chain lengths (number of units = 1 to 3) and side-chain terminal groups (methyl or ethyl).[30]



PMEA
poly(2-methoxyethyl acrylate)

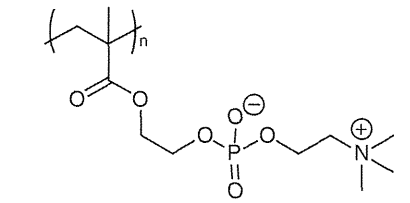
POEG(M)A
poly[oligo(ethylene glycol) (meth)acrylate]

Figure 2 Chemical structures of poly(2-methoxyethyl acrylate) (PMEA) and PMEA analogous polymers (POEG(M)A).

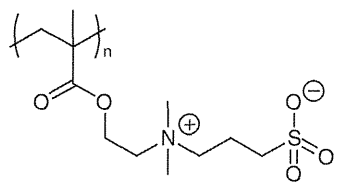
Side-chain modification

The modification with oligoEGs is a well-established methodology to tune the hydrophilicity of polymeric materials.[31–39] Poly[oligo(ethylene glycol)(meth)acrylate]s (POEG(M)As) consist of poly(meth) acrylate backbones and oligo(ethylene glycol) side-chains, consequently, the EG functionalized poly(meth)acrylate is one of the most readily accessible hydrophilic polymers. Although POEG(M)As have simple chemical structures and numerous research studies have been conducted to date, there is still plenty of room for controlling hydrophilicity/hydrophobicity by modifying the chemical structure of side-chains. The basic way to modify the side-chain structure is by tuning the number of EG units and chain-end terminal group. Hydrophilicity of the polymer increases with the number of EG units, as the polymers have longer side-chains, the polymers become soluble in water and typically show lower critical solution temperature (LCST) in aqueous solutions.[33] The number of carbon atoms in terminal alkoxy group also affects the water solubility and some of the polymers having longer alkyl terminal group show LCST below 37 °C.[40] The DSC measurement revealed that intermediate water content was increased by tuning the chemical structure of polymer to be more hydrophilic (much EG units with less terminal carbons), and a decrement trend was observed in the number of adhered platelets with increasing the intermediate water content.

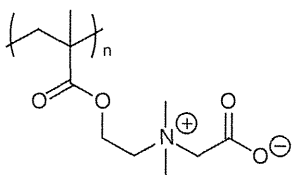
Zwitterionic polymers are known as the promising biocompatible materials for medical devices.[40] For example, poly(2-methacryloyloxyethyl phosphorylcholine) (PPBMA: poly(phosphobetaine methacrylate), generally known as PMPC)) is a biomimetic material containing phosphorylcholine group for resisting nonspecific protein adsorption and platelet adhesion.[41,42] Recently, synthetic polymers containing zwitterionic structures similar to PPBMA, such as poly{[2-(methacryloyloxy)ethyl]dimethyl-(3-sulfopropyl)ammonium hydroxide} (poly (sulfobetaine methacrylate)),[43,44] and poly(1-carboxy-*N*,*N*-dimethyl-*N*-(2'-methacryloyloxyethyl)methanaminium) (poly(carboxybetaine methacrylate)),[45–47] bearing sulfo- and carboxy- betaine group, respectively, are also reported as blood-compatible polymers, which show good plasma protein-fouling resistance. Most recently, poly (serine methacrylate) was reported as a new family of a zwitterionic polymer having an amino acid, L-serine, as the side-chain group.[48] (Figure 3)



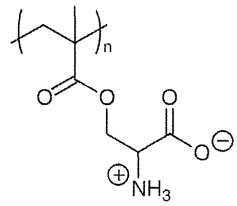
PPBMA: poly(phosphobetaine methacrylate)



PSBMA: poly(sulfobetaine methacrylate)



PCBMA: (poly(carboxybetaine methacrylate)



PSrMA: poly(serine methacrylate)

Figure 3 Chemical structures of zwitterionic polymers (phospho-betaine, sulfo-betaine and calboxy-betaine), which possess the intermediate water in their hydrated state.

Backbone modification

Changing the polymer backbone structure is also an effective approach to tune the polymer properties.[49,50] Poly[oligo(ethylene glycol) vinyl ether] is an analog of POEG(M)A. The structural difference between the two polymers is only in the side-chain linkage that the former OEG side-chains were connected to polymer backbone through ether bonds instead of ester connections (Figure 4). However, the difference engenders large differences in the molecular mobility and the hydrophilicity of the polymers. For instance, most of poly(vinyl ether)s having OEG side-chains show quite low glass transition temperature ($T_g < -60\,°C$) and are soluble in water or exhibit an LCST in aqueous media. Some of the poly(vinyl ether)s (for example, poly(2-ethoxyethyl vinyl ether), LCST = 21 °C) are insoluble at body temperature, and the human platelets adhesion test could be performed at 37 °C. Accordingly, we have analyzed the hydration water structure in POEG(M)A and their poly(vinyl ether) analogs by DSC, and the poly(vinyl ether)s showed a cold crystallization of water at around −40 °C and exhibited low platelet adhesion as well as the case of POEG(M)A.[51]

Modification on side-chain branch spacing

As mentioned above, the structural control over the macromolecular chemical structure is an effective approach to modify/control the hydration water structure and the blood compatibility of polymeric materials. There remains ample scope for further modification in the chemical structure of polymers, for example, tacticity, side-chain linkage and side-chain branch placement. The structural control over the side-chain placement along the polymer backbone is one of the most challenging topics in vinyl polymer synthesis. Fortunately, an effective pathway to achieve the model sequence-regulated vinyl polymers was reported, and the methodology utilizing the regio- and stereo-selective ring-opening metathesis polymerization (ROMP) of allyl-substituted cycloalkenamers[52] opened a new window to precisely control the side-chain branch placement.[53–58] Based on the works, we have started a study to elucidate the structure–property relationships in biocompatible polymeric materials by means of precision polymers synthesized through regio- and stereo-selective ROMP (Figure 5).

The single substitution of a functional group at the allyl-position of *cis*-cyclooctene (COE) allows to achieve the regioregular polymers by means of ROMP with the second-generation Grubbs catalyst (***G2***).[59] Thus we have synthesized COEs having hydrophilic functional groups at allyl-position,[60] for example, polymerized the COEs with ***G2*** in $CHCl_3$. ROMP of the allyl-substituted COEs proceeded in a regio- and stereo-selective manner to afford polymers exhibiting remarkably high head-to-tail regioregularity and high *trans*- stereo-regularity as we previously reported. Figure 6 shows olefinic region of ^1H nuclear magnetic resonance and ^1H-^1H correlated spectra of 3-methoxy-substituted COE. The coupling constant for the two olefinic signals is $J_{ab} = 15.5$ Hz, indicating that the double bond has *trans*- configuration. The *dd* and *dt* multiplicities for H^a and H^b, respectively, and the correlation between H^a and H^b reveal the near-perfect *trans*- head-to-tail regularity.

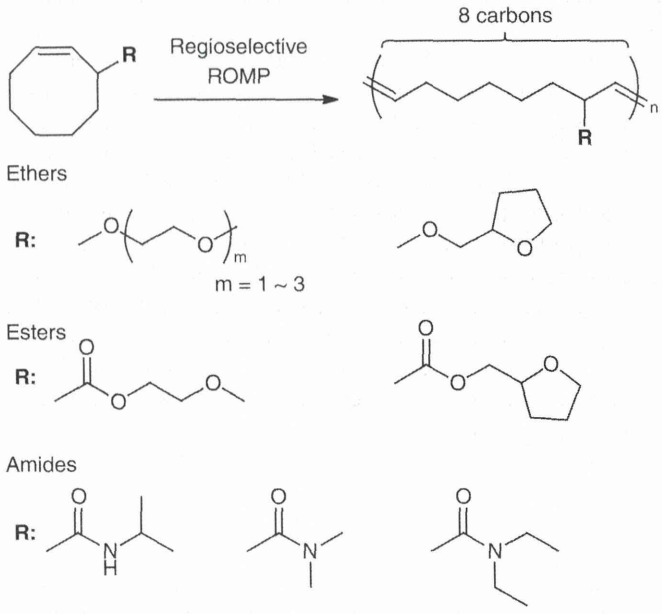
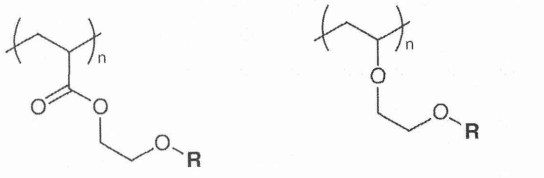


Figure 5 Synthesis of polymers having precisely placed side-chain branches by regio- and stereo-selective ROMP of allyl-substituted *cis*-cyclooctenes.



R	LCST (H_2O)
CH_3	insoluble
C_2H_5	insoluble

R	LCST (H_2O)
CH_3	69 °C
C_2H_5	21 °C

Figure 4 Poly(2-alkoxyethyl acrylate)s and their poly(vinyl ether) analogs. Poly(2-ethoxyethyl vinyl ether) (**R** = C_2H_5) exhibits LCST at 21 °C.

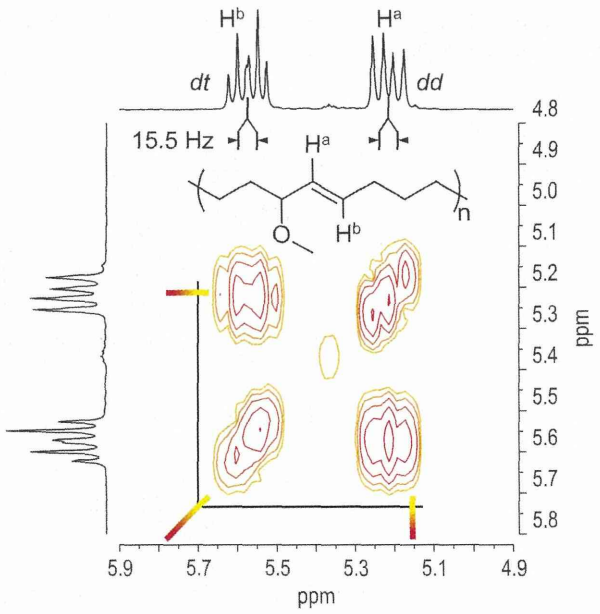


Figure 6 Olefinic region of ^1H NMR spectra and ^1H-$_1H$ correlated spectra of poly(3-methoxy-1-cyclooctene).

Polymers having precisely placed branches on every eighth backbone carbons were obtained upon hydrogenation. Water contact angle measurement confirmed the presence of hydrophilic surface for all polymers. The water structure in hydrated polymers was determined by DSC, cold crystallization of water and/or low melting of ice in hydrated polymers were observed on heating process. Cold crystallization of water is the clear evidence for the presence of intermediate water, and the content was able to be varied by changing the polymer structure. A human platelet adhesion test was employed to assess the blood compatibility of regioselective ROMP-produced polymers. The number of adhered platelets was also varied by changing the polymer structure, and we found out that the number was suppressed by introducing the longer EG side-chains. The platelets adhesion number was decreased with increasing the content of intermediate water regardless of the polymer structure. This result suggests that our hypothesis could be true that the presence of intermediate water is the key to provide the polymer materials with antithrombotic character, and the blood compatibility of polymers should be controlled by tuning the water structure at the bio-interface through precision polymer synthesis.[60]

BIODEGRADABLE SYNTHETIC POLYMERS USED/STUDIED IN MEDICAL APPLICATIONS

Some biomedical devices, especially for temporary use or disposable purpose, such as surgical suture, bone-fixation materials and drug-eluting stents comprise biodegradable synthetic polymers, including polylactides, polyglycolide, poly(ε-caprolactone), poly(trimethylene carbonate) (PTMC) and poly(p-dioxanone), as shown in Figure 7.[61,62] These polymers are degraded by hydrolysis with/without enzyme and absorbed in the body through metabolic pathway, although the duration of these existing biodegradable polymers in the body varies.[63] They have drawn keen attention as alternatives to biopolymers such as peptides, nucleic acids and polysaccharides that cost high to produce and purify and potentially possess the risk of antigenicity and infection.

Such implantable medical devices need to be compatible with host cells to reduce adverse effects. As it has been confirmed that the aforementioned polymers exhibit minimal or acceptable cytotoxicity,[64] most of those polymers are approved for medical application. Few reports have ever described the relationship between the biocompatibility and structural features of those polymers. In the case of PMEA, the ester and ether groups on the side-chains contribute to the hydration and generation of intermediate water.[29] The hydration generally occurs through hydrogen bonding between polar moieties in the polymer and water molecules. This concept may be extended to the aforementioned polymers comprising ester or carbonate linkage and alkyl- or alkyloxy-chains of varying lengths. The detailed study for hydration and intermediate water in those polymers is now in progress by our group. Next examples also imply that the intermediate water concept should be employed to explain the observed biocompatibility.

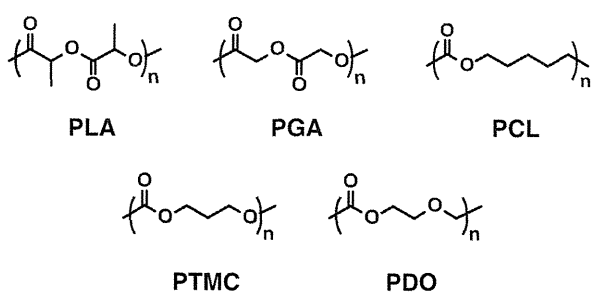


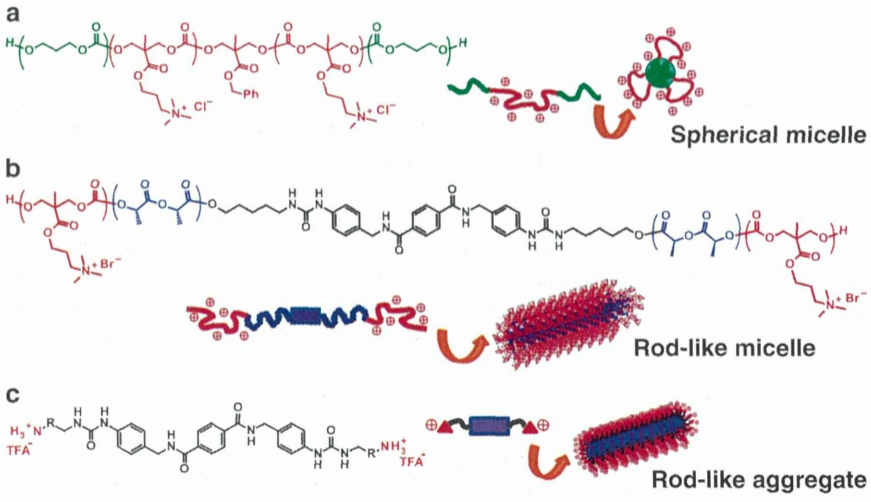
Figure 7 Biodegradable polymers used/studied in medical applications.

Biodegradable antimicrobial polymers with low hemolytic property

In recent years, a PTMC analog bearing a side group of quaternary ammonium salt have demonstrated potent antimicrobial activities but showed minimal hemolytic properties (Figure 8).[65,66] In contrast, most of the cationic polymers are well known to interact with negatively charged bacterial cell membranes, subsequently inducing the membrane disruption.[67] As the cationic polymers physically destroy cells, drug resistance is hard to develop differently from the use of conventional antibodies. However, this electrostatic interaction often influences mammalian cells resulting in cytotoxicity, which is a serious issue to be solved in developing antimicrobial polymers with positive charges. The first antimicrobial polycarbonate reported in 2011 shows efficient antimicrobial activity but displays no hemolytic property.[65] This polymer has amphiphilic triblock nature to form nano-sized micelles by conjugating hydrophobic PTMC as peripheral blocks (Figure 8a). Accumulation of positive charges on micelle surface might contribute to differentiating bacteria and mammalian cells. In similar speculation proposed by Kuroda and colleagues, localization of charges and segregation of the hydrophobic part by micellization suppress the interaction with mammalian cell membrane with less negative charges than those of bacterial cell membrane.[68,69] Considering that the other PTMC analogs bearing different side-chains have also exhibited little cytotoxicity;[70–72] however, this low hemolytic property may also be supported by the contribution of hydration involving the carbonate linkages in the main chain. In particular, as both RBCs and platelets are blood cells, the inactive behavior of the polymer to RBCs is likely to occur in a similar way that PMEA shows excellent compatibility to platelets.[16] IBM propounds to call a series of these antimicrobial biodegradable polycarbonates 'Ninja Polymer' describing the function to work behind the scenes and eventually disappear.

Supramolecularly bolstered antimicrobial activity and blood compatibility

Multiple activities against several types of bacteria present another challenge for the design of antimicrobial materials. The first antimicrobial polycarbonate described above shows the efficacy only against Gram-positive bacteria and their drug-resistant strains such as *Bacillus subtilis* and (methicillin-resistant) *Staphylococcus aureus*, respectively.[65] Because Gram-negative bacteria and fungi are not as negatively charged as Gram-positive bacteria are,[73] other artifices should be integrated into the macromolecular architecture. Lipophilicity and hydrophobicity are generally required for valid antimicrobial activity against Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, owing to affinity to superficial lipopolysaccharide on the cell wall. However, increased hydrophobicity of the cationic polymers often develops hemolytic property. Fukushima *et al.*[66] introduced a rigid hydrogen bonding motif in the middle of a center hydrophobic segment of a triblock copolymer composed of poly(L-lactide) (PLLA) and the cationic polycarbonate, forming fibrous micelles by orientation of self-assembly (Figure 8b). Interestingly, this polymer shows antimicrobial activity against a wide range of bacteria covering Gram-positive/negative bacteria and fungi but induced no hemolysis. In all cases, minimum inhibitory concentration of this polymer was higher than critical micelle concentration, supporting that the polymer serves as aggregates. It turns out that the fibrous shape is somewhat responsible for the improved antimicrobial activity.



	MIC (mg/L)				
Antimicrobial	S. aureus	MRSA	E. Coli	C. neoformans	C. albicans
A	60	65	N.A.	100	N.D.
B	75	40	75	20	75
C	N.A.	N.A.	N.A.	31	31

Figure 8 Synthetic biodegradable antimicrobials in different active forms. (**a**) spherical micelles (critical micelle concentration (CMC) = 28 μg ml^{-1}), (**b**) rod-like micelles (CMC = 25 μg ml^{-1}), (**c**) rod-like aggregate (CMC = 6 μg ml^{-1}).

Later, Fukushima *et al.*[74] have also reported supramolecular antifungals where the same rigid hydrogen bond motif is used directly to attach low molecular cationic primary ammonium at the both ends instead of the cationic polycarbonate (Figure 8c). The antifungal becomes active against fungi such as *Candida albicans* and *Cryptococcus neoformans* only in the form of nanofiber that indicates glass transition at 120 °C similar to molecular glass.[75]

In these above two cases, the hemolytic activity of the polymer also remained minimal. The cationic moiety obviously affects bacterial cell membranes, but the interaction with RBCs is mitigated even though the assembly form varies. At the latter case, especially, the interaction of molecules with cells, such as cytotoxicity, antimicrobial activity and biocompatibility, is managed by cooperation of primary structure of the peripheral functional groups to tune the chemical functions and higher-order structure forming specific shape to restrict or expand the chemical function as biological system generally adopts. The cationic moiety usually forms hydration layer, including strongly oriented water molecules that are categorized as non-freezing water, often causing adverse effects. If the intermediate water is responsible for the low hemolytic property of the cationic fibrous assemblies, the following hypothesis would be supposed: By assembling to such fibrous form, the surface hydration layer is disorganized with electrostatic repulsion of condensed cationic groups, which may trigger forming intermediate water from non-freezing water. In fact, a similar insight has been reported for generation of intermediate water by disorganization of hydration layer of non-freezing water in a copolymer of poly[*N*-methyl-*N*-(4-vinylphenethyl)ethylenediamine] with a small amount of additional poly(2-hydroxyethyl methacrylate).[76]

Control over the geometry of polymeric aggregates often entails non-covalent interactions, including hydrogen bond, π-π stacking, charge transfer complex and ion complex. These interactions also differentiate nano-rheology and dynamics of the aggregates. According to Stupp and colleagues, strength of the interaction at the internal domain of cationic supramolecular aggregates affects accumulation on and disaggregation of mammalian cell membrane.[77] The aggregates with strong 'internal bond strength' interact with the cell membrane resulting in membrane disruption, while those with weak internal bond strength remain dynamic nature to release unimers upon approaching cells leading to no damage of the cells. It turns out that the selection of types and direction of bond (covalent vs non-covalent) significantly involves regulation of biocompatibility and cytotoxicity. In consequence, design of high-performance biomaterials in future should actively employ supramolecular chemistry in terms of geometry control, subsequent development of secondary function and dynamic behavior of the material besides primary chemical functions.

The research on clarification of relationship between biocompatibility of these polymer systems and water structure is ongoing.[78] The intermediate water was only found in hydrated biopolymers (proteins, polysaccharides and nucleic acid; DNA and RNA) and hydrated biocompatible synthetic polymers but not in hydrated non-biocompatible synthetic polymers.[79–82] Therefore we propose intermediate water concept for directional design of functional polymeric biomaterials, but it is needed for the quantitative and precise description of biocompatibility driven by novel interface-sensitive approaches, such as spectroscopic (including sum-frequency generation and dielectric spectroscopy), X-ray and neutron scattering, and force curve measurements combined with computer simulations under the physiological condition.[83–94]

CONCLUSION

Surfaces made of biocompatible and biodegradable polymers profoundly influence cell behavior at all hierarchical levels. The interaction of polymers with cells is managed by cooperation of primary structure including backbone and functional groups at the side-chain

to tune the chemical functions and higher-order structure forming specific shape to restrict or expand the chemical function as biological system generally adopts. Using principles of intermediate water, which is common in hydrated biopolymers and in only biocompatible synthetic polymers, the synthetic and supramolecular methodology to create novel biocompatible polymers moves toward a more high-throughput way. Such well-defined polymeric biomaterials could find application in the age of personalized medicine.

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