Novel sheet-shaped materials in which polymeric micelles are dispersed for the sustained controlled release of proteins

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ABSTRACT SUMMARY
The sheet-shaped materials in which polymeric micelles are dispersed were developed by spin-coating the mixed solutions of water-in-oil (w/o) emulsion containing an amphiphilic block copolymer and a hydrophobic polymer as a base material for the sheets. It was clear that the sustained controlled release of the proteins depends on the composition of amphiphilic block copolymers forming the polymeric micelles in the sheets.

INTRODUCTION
Regenerative medicine is a method to regenerate unusual tissues and cells. The method can be carried out by restoring the defect sites of biological tissues and internal organs. In this approach, the stem cells which have high proliferating and differentiating ability to regenerate both biological tissues and internal organs are gathered and cultured in the scaffolds, and subsequently inducing the mature cells to proliferate. These regenerations are performed more efficiently by incorporating growth factors into the scaffolds. In general, growth factors are unstable, and show almost no sufficient effects for regenerating cells by means of administration to the target region in a solution state. It is necessary to release growth factors to defect sites in the long term, by keeping the concentration of the factors over effective levels in which the damage sites can be regenerated.

From these points of view, we have developed a novel sheet-shaped material for controlled sustained release of hydrophilic compounds (1). Sheet-shaped materials have several advantages. The sheet is easy to adjust to various shapes and size by cutting the sheet, and have a large contact area relative to the targeted site. Moreover, because the sheet shows gentle adhesion to body tissue by van der Waals forces, an inflammatory reaction does not occur at the surface of the tissue. In the present study, we propose the fabrication method of the sheet-shaped material containing polymeric micelle formed from block polymer for controlled sustained release of proteins by using a bovine serum albumin (BSA) as a model protein. This sheet-shaped material has a promise to control the sustained release of proteins by changing compositions of amphiphilic copolymers.

EXPERIMENTAL METHODS
The sheet-shaped materials were prepared by the method as shown in Fig. 1. First, methoxy-terminated poly(ethylene glycol)-block-poly (caprolactone) block copolymer (CH3O-PEG-b-
PCL) was synthesized by ring-opening polymerization of both ethylene oxide and ε-caprolactone in anhydrous THF. Next, w/o emulsion were prepared by sonicating organic solvents dissolving CH₃O-PEG-b-PCL and PVA aqueous solution containing BSA. Then, the obtained w/o emulsion was added to the organic solvents containing hydrophobic polymers (PCL) as a base material for the sheets, and we prepared a thin sheet by spin-coating the mixed solutions.

RESULTS AND DISCUSSION

The sheet-shaped materials in which polymeric micelles containing CH₃O-PEG-b-PCL are dispersed were obtained by spin-coating (Fig. 2).

![Fig. 2. The appearance of a thin PCL sheet in which polymeric micelles are dispersed.](image)

In order to evaluate the dispersity of polymeric micelles in the sheet, the sheet-shaped materials containing fluorescein isothiocyanate-dextran (FITC-dextran) instead of BSA were prepared, and observed with a fluorescent microscope (λ_ex=470 nm, λ_em=535 nm for FITC-dextran). Figure 3 shows the fluorescent images of the sheets in which polymeric micelles containing FITC-dextran were dispersed. It was found that the dispersity of polymeric micelles in the sheet depended on the stability of the w/o emulsion. When the stable w/o emulsion (formed of PEG3360-PCL2800, where numbers showed M_n of the units) was used to prepare the sheet, the polymeric micelles were uniformly dispersed (Fig. 3(A), whereas an unstable emulsion that was formed of PEG3470-PCL1020 was used to form the sheet in Fig. 3(B)).

![Fig. 3. The fluorescent images of the sheets in which polymeric micelles containing FITC-dextran formed of (A) stable emulsion or (B) unstable emulsion.](image)

Finally, the sustained release of proteins from the sheet was performed. Figure 4 shows the release of BSA from the sheets. The sustained release of BSA from the sheets was found for approximately 20 days, and the release behavior of BSA changed by changing of compositions of CH₃O-PEG-b-PCL. However, it is still unclear that proteins were released either the degradation of block polymers or the collapse of polymeric micelles formed from block polymers.

![Fig. 4. The release behavior of proteins from the sheet-shaped materials](image)

CONCLUSION

The sheet-shaped materials which had the ability of the sustained controlled release of proteins were successfully prepared. It was clear that the release behavior of proteins from the sheets depended on the composition of CH₃O-PEG-b-PCL forming polymeric micelles.

REFERENCES