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A free-standing, sheet-shaped, "hydrophobic" biomaterial containing polymeric micelles formed from poly(ethylene glycol)-poly(lactic acid) block copolymer for possible incorporation/release of "hydrophilic" compounds

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ABSTRACT

Sheet-shaped materials with a large contact area relative to the drug targeting site lead to advantages over conventional particle-shaped drug carriers and have several advantages for their biomedical applications. The present study proposes a methodology for preparing a novel sheet-shaped "hydrophobic" and biocompatible biomaterial in which polymeric micelles are uniformly dispersed for the incorporation of "hydrophilic" compounds into the sheet. The methoxy-terminated poly(ethylene glycol)-*block*-poly(lactic acid) block copolymer (CH_3O-PEG-*b*-PLA) was successfully synthesized by means of the anionic ring-opening polymerization of both ethylene oxide and DL-lactide. CH_3O-PEG-*b*-PLA was self-assembled and formed stable micelle-like w/o emulsion with a hydrophilic inner core in organic solvents. A sheet-shaped material containing a hydrophilic inner space for incorporating hydrophilic compounds was obtained by spin-coating both the micelle solution and a sheet-forming polymer. Fluorescent images of the sheet proved that polymeric micelles providing hydrophilic spaces were uniformly dispersed in the hydrophobic sheet. The facile technique presented in this paper can be a tool for fabricating sheet-shaped biomaterials that have a hydrophilic inner core and, consequently, that are suitable for the sustained release of hydrophilic compounds.

1. Introduction

Tissue engineering is a technology serving to regenerate or create tissues that have lacked regular functions and morphology and hence to facilitate the therapeutic reconstruction of the human body by the deliberate and controlled stimulation of selected target cells through a systematic combination of molecular and mechanical signals [1]. A primary requirement of tissue engineering is to select the optimal cell types and scaffold properties for effective differentiation/growth of cells. However, even if the scaffolds show excellent properties, the incomplete cell growth and/or the deficiency of the factors to guide cell growth make a regeneration of tissues difficult. The regulation of the concentration of growth factors (GFs) on the targeted tissue surface is one method helping to guide effective regeneration of tissues as well as to promote wound healing. Here, the biomaterials that release proteins in a sustained manner are necessary.

A variety of materials for sustained protein release have been proposed including hydrogels prepared with natural and synthetic polymers [2–4], fibers prepared by electrospinning [5], and particles [6,7]. In particular, hydrogels that can incorporate and release GFs have been widely reported. In fact, hydrogels have various functional properties, such as the ability to absorb a significant amount of water and flexibility similar to a natural tissue. These properties have provided many potential applications particularly in biotechnological and medical fields [8–10]. Tissues are expected to be efficiently regenerated by the sustained release of GFs from hydrogels. However, it is difficult to control the sustained release properties of hydrogels because the release mechanism generally depends both on the diffusion of GFs through polymer networks in hydrogels and on the degradation of the hydrogel structure. In contrast, in aiming for the sustained release of hydrophobic compounds, we have developed a tissue-adhesive hydrogel covalently containing a reactive polymeric micelle formed from aldehyde-terminated poly(ethylene glycol)-*block*-poly(lactic acid) (CHO-PEG-*b*-PLA) block copolymers [11–13]. Interestingly, the tissue-adhesive hydrogels using polymeric micelles as crosslinks had an ability to incorporate hydrophobic drugs and release them in a sustained manner, because the polymeric micelles had a hydrophobic core providing a drug-loading site. The results prove that the combination of drug carriers and hydrogels can be a novel platform for the sustained release of drugs. However, since the polymeric micelles have a hydrophobic inner core, the methodology cannot be applicable to

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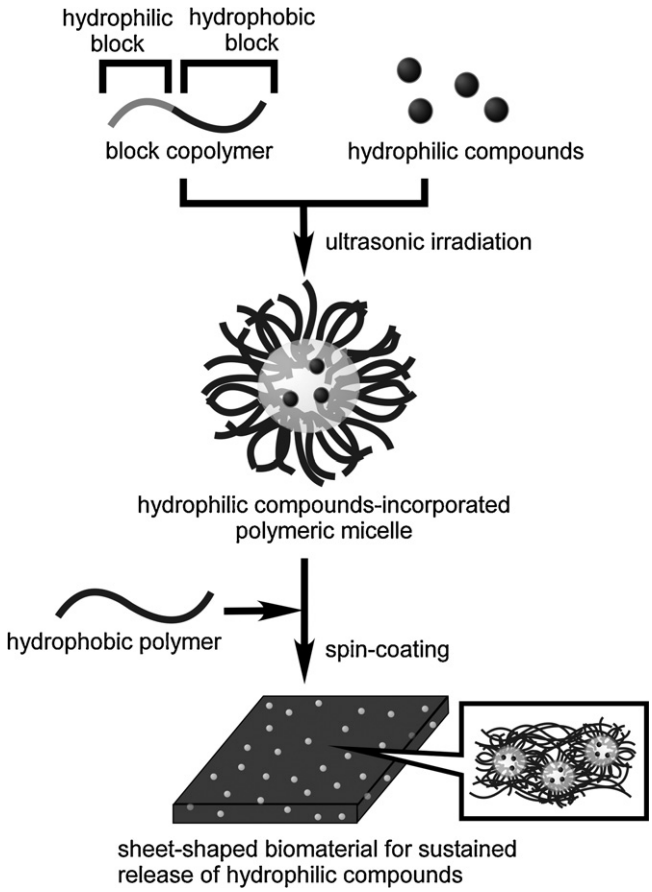

Fig. 1. Preparation of a novel sheet-shaped biomaterial in which polymeric micelles (spaces that can incorporate hydrophilic compounds) were uniformly dispersed.

the sustained release of hydrophilic compounds such as proteins including GFs.

Recently, several approaches have been proposed for fabricating "sheet"-shaped materials having a large surface area, including a layer-by-layer (LbL) sheet of polyelectrolytes [14–16], a self-assembled monolayeres (SAMS) sheet [17] and a nanoparticle-fused sheet [18–21]. Sheet-shaped materials that are formed from hydrophobic polymers such as PLA and poly(lactide-*co*-glycolide) (PLGA) are expected to have several advantages for their biomedical applications. They are easy to handle in surgery and exhibit gentle adhesion to body tissue by interactions between molecules such as van der Waals forces. The sheet has a large contact area relative to the drug-targeting site, leading to advantages over conventional particle-shaped drug carriers. However, the incorporation of hydrophilic compounds into hydrophobic sheet-shaped biomaterials was generally difficult to be achieved. Actually, there have been reports on only a few sheet-shaped hydrophobic biomaterials internally containing the structure for incorporating hydrophilic compounds. These materials include a PLA porous sheet that was prepared from an organic solvent containing both Span80 (sorbitan monooleate)-based water-in-oil (w/o) emulsion and sheet-forming PLA, followed by the evaporation of the organic solvent [19]. In this case, the sustained release of hydrophilic compounds from the sheet was difficult to achieve, because Span80 is a low-molecular-weight surfactant and forms relatively unstable emulsions.

The present study proposes a methodology for fabricating a novel sheet-shaped hydrophobic and biocompatible biomaterial in which polymeric micelles (i.e., spaces that can incorporate hydrophilic compounds) are uniformly dispersed, as shown in Fig. 1. Amphiphilic block copolymers are self-assembled and are

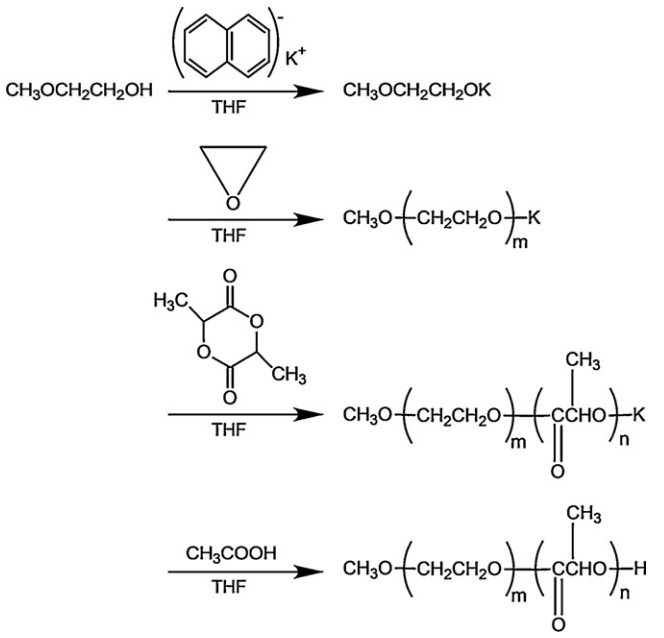

Fig. 2. Synthesis of methoxy-terminated poly(ethylene glycol)-*b*-poly(lactic acid) block copolymer (CH_3O-PEG-*b*-PLA).

expected to lead to stable micelle-like w/o emulsion with a hydrophilic inner core in organic solvents. By spin-coating this micelle solution, we obtained a sheet-shaped material containing a hydrophilic inner space for incorporating hydrophilic compounds. The material should release hydrophilic compounds in a sustained manner, because the stability of the micelle is easily regulated by the molecular properties of the block copolymers.

2. Materials and methods

2.1. Materials

Ethylene oxide (Sumitomo Seika Chemicals Co., Ltd., Osaka, Japan) was purified through distillation with CaH_2. DL-Lactide (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was recrystallized twice from ethyl acetate. 2-Methoxy ethanol was distilled with sodium under reduced pressure. Potassium naphthalene was obtained from potassium and naphthalene in anhydrous tetrahydrofuran (THF) for 18 h. Fluorescein isothiocyanate-dextran (FITC-dex, average molecular weight: 20,000) and rhodamine B isothiocyanate-dextran (RITC-dex, average molecular weight: 20,000) were purchased from Sigma–Aldrich. Poly(vinyl alcohol) (PVA, the degree of polymerization: 500, saponification degree: 86–90 mol%) and Span80 were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). PLA (average molecular weight: 300,000) was purchased from Polysciences Inc. (Warrington, PA). All other reagents were of analytical grade and were used without further purification.

2.2. Synthesis of methoxy-terminated poly(ethylene glycol)-block-poly(lactic acid) block copolymer (CH_3O-PEG-b-PLA)

Methoxy-terminated poly(ethylene glycol)-*block*-poly(lactic acid) block copolymer (CH_3O-PEG-*b*-PLA) was synthesized by anionic ring-opening polymerization of both ethylene oxide and DL-lactide in anhydrous THF according to the previously reported method (Fig. 2). The following is the typical experimental condition for the synthesis of CH_3O-PEG-*b*-PLA (code 2 in Table 1, as