# SYNTHESIS OF POLYLACTIDE-GRAFTED DEXTRAN AND THEIR APPLICATION AS BIODEGRADABLE BIOMEDICAL MATERIALS

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## Introduction

Biodegradable, biocompatible polymers are of interest for use in several biomedical and pharmaceutical applications. Based on its biodegradability, biocompatibility, high mechanical strength, and good shaping and molding properties, polylactide (PLA) is frequently utilized as an implantable carrier for drug delivery systems, as well as a surgical repair material. However, its high crystallinity interferes with predictable degradation, reduces its compatibility with soft tissues and presents an obstacle to its application as a biodegradable soft plastic. One promising approach to overcoming these problems in PLA is the introduction of hydrophilic segments and branched structures. On the other hand, polysaccharides are natural, biodegradable and hydrophilic polymers that can be degraded enzymatically and possess relatively good biocompatibility, but are insoluble in common organic solvents. PLA is commonly synthesized by a ring-opening polymerization of lactic acid dimer (lactide, LA). The anionic ring-opening polymerization reaction of LA is performed in the presence of an alkali metal alkoxide to give PLA having the alkoxide used on the end. Our group has succeeded in obtaining graft copolymers consisting of PLA and polysaccharides using partially trimethylsilyl (TMS)-protected polysaccharides as initiators and subsequent removal of TMS groups.[1-3] Using dextran as a polysaccharide, the obtained PLA-grafted dextran (Dex-g-PLA) (Fig. 1) films were found to have lower values of $T_g$, $T_m$, crystallinity, and a higher viscosity compared with poly-L-lactide (PLLA) films due to the introduction of polysaccharide segments and branched structures into PLLA.[3-5] Properties of Dex-g-PLA can be varied by controlling of the molecular structure: length and number of graft chains, and proportion of hydrophilic to hydrophobic segments in the graft copolymers. In fact, degradation rates strongly depended on the content of polysaccharide.[4,6] In this presentation, to explore the possibility of Dex-g-PLA as a new biodegradable soft-biomaterial, preliminary investigations were carried out for the effect of molecular structure of Dex-g-PLA on the surface properties and cell attachment behavior of the films prepared from the copolymers.[5,6] Moreover, Dex-g-PLA has amphiphilic structure and should be useful drug release depot for hydrophilic macromolecules (proteins, nucleic acids). So we also report on the preparation of protein (BSA, bovine serum albumin)-loaded microspheres (MSs) from Dex-g-PLA.[7]

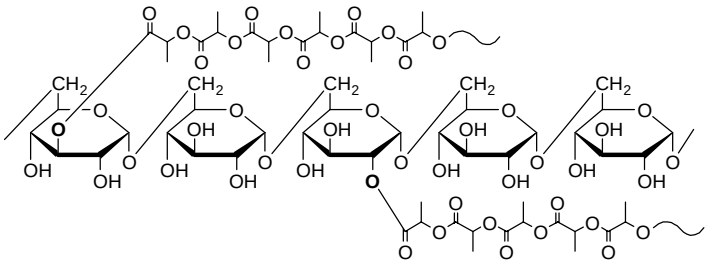

**Figure 1.** Structure of Dex-g-PLA.

## Experimental

***Synthesis of Dex-g-PLA***

Dex-g-PLA was synthesized through the graft-polymerization of L-LA on TMS-protected dextran in THF using *t*-BuOK as an initiator and the subsequent deprotection of the TMS groups according to the method reported previously.[1-3] The majority of the hydroxyl groups of dextrans were protected by TMS groups in order to achieve solubility in organic solvents and to control the number of reaction sites with the alkali metal initiator. All of the graft copolymers obtained were soluble in THF, chloroform, dimethylformamide, dimethylsulfoxide, and other organic solvents, but not soluble in water. We could obtain Dex-g-PLA with various length and numbers of graft chains and differential content of sugar unit. The obtained Dex-*g*-PLAs was expressed as a code number, G-x-y-z, where x means average degree of polymerization of L-LA of each graft chain, y means number of graft chains per molecule, and z means weight % of sugar unit content.

***Preparation and Evaluation of Dex-g-PLA Films***

A chloroform solution of the graft copolymer (4 wt%, 150 μl) was cast on square glass plate (18 mm × 18 mm) and dried at 25 °C for 12 h in air and further dried at room temperature under vacuum for 48 h to give polymer films (0.15 mm thick). The dynamic contact angles of the air side of the graft copolymer films in water were measured by the sessile drop method at 25 °C with the help of a CCD camera to evaluate their wettabilities. The average values were determined from measurements at 15 different points on the films excluding the maximum and minimum values. The number of cells attached to the graft copolymer films was evaluated after 1–12 h by the following method. The suspension of mouse fibroblast L929 cells (2.0 ml, $2.5 \times 10^5$ cells/ml) in E-MEM containing 10% (v/v) FCS was distributed on the graft copolymer films in the glass dishes and cultured in a humidified atmosphere containing 5% $CO_2$ at 37°C. After incubation, the films on the glass dishes were rinsed 3 times with E-MEM to remove non-attached cells. The number of cells attached to the films was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. E-MEM (2.19 ml) and aqueous MTT solution (438 μl) were added to the glass dishes, and incubated at 37°C for 4 h. The formazan precipitate was then dissolved by the addition of 0.04 N HCl/isopropanol solution (2.19 ml) containing 10% Triton-X. Aliquots of the supernatant of the solution in the dishes were transferred into 96-well microplates. The absorbance at 630 nm was measured using a microplate reader (MTP-120 Corona Electric Co.). The number of cells attached was expressed as a percentage of the initial cell number ($5.0 \times 10^5$ cells). The morphology of the cells attached to the films was observed by scanning electron microscopy (SEM).

***Preparation and Evaluation of Protein-loaded Dex-g-PLA Microspheres***

BSA-loaded MSs were produced according to a W/O/W emulsion solvent evaporation/extraction method [7]. The aqueous solution of fluorescein isothiocyanate (FITC)-labeled BSA was added into an organic solution (mixture of methylene chloride and DMSO) containing PLLA or Dex-*g*-PLA and sonicated at 0 °C with stirring. This primary emulsion was poured into a 0.1% (w/v) external aqueous solution of polyvinyl alcohol (PVA) and stirred with a magnetic stirrer for 30 min at room temperature. Then, organic solvent was completely evaporated under reduced pressure. The suspension was centrifuged to obtain the precipitate of MSs. The obtained MSs were washed with ultra pure water and freeze-dried. The release behavior of FITC-BSA from MS(Dex-*g*-PLA) and MS(PLLA) was investigated in vitro. The MSs (20 mg) were suspended in 2.5 mL of 1/15 M phosphate buffer solution (pH 7.4) and incubated at 37 °C. The buffer solution was changed periodically to approximate sink

conditions. At various times the tubes were removed from the incubator, and the buffer solution collected and replaced with an equal volume of fresh buffer solution. Obtained samples were filtered using a 0.22 μm filter unit. Then, the solution was investigated by fluorescence spectrophotometer to determine the FITC-BSA concentration, operating at an excitation wavelength of 495 nm and an emission of 520 nm. All experiments were carried out under light protection. The release of FITC-BSA was investigated by dialysis method with monitoring fluorescence spectra. The localization of BSA in the MSs was evaluated by confocal laser scanning microscope (CLSM) observation. The stability of BSA entrapped in MSs was evaluated by measuring circular dichroism (CD) spectra. CD spectra for four samples (native BSA, denatured BSA, BSA released from MS(Dex-*g*-PLA) and BSA released from MS(PLLA)) were measured with a 1 mm cell. Native BSA and denatured BSA meant the BSAs incubated in 1/15 M PBS and 8 M urea PBS solution at 37 °C for 1 d, respectively.

## Results and Discussions

***Degradation, Surface Properties, and Cell Attachment Behavior of the Copolymer Films***

The water contact angles of the graft copolymer films and control PLLA films are showed in Fig. 2. The annealing of films was performed in water at 37ºC over $T_g$ of Dex-g-PLA (16–19ºC) for certain period. Generally, the surface of amphiphilic polymer film is covered with hydrophobic domains in air, while it tends to change to the hydrophilic surface by annealing in water. The dynamic contact angle decreased with increasing the content of sugar units. Namely, the wettability of surface of Dex-g-PLA films increased with sugar content. This result suggested that the hydrophilic dextran segment were moved onto the film surface through micro-Brownian motion by water annealing.

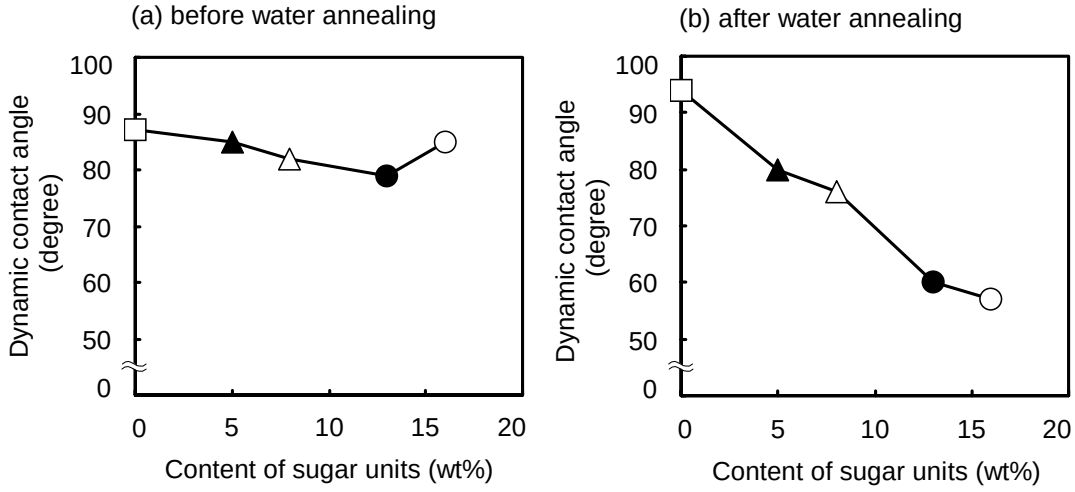


**Figure 2.** Dynamic contact angle for the film surfaces as a function of the content of sugar units. (a) Before annealing in water, (b) After annealing in water.[5]

The cell attachment behavior on the copolymer films were shown in Fig. 3. All of the Dex-g-PLA films prepared exhibited lower cell attachment ability than PLLA film. The number of attached cells decreased with increase of the contents of sugar unit. The cell attachment was effectively suppressed by the introduction of sugar units. The morphology of the cells on the copolymer films were shown in Fig.

3B. The cells on PLLA film or the graft copolymer film having lowest content of sugar unit exhibited a well-spread morphology, while the cells on graft copolymer having highest content of sugar unit exhibited almost round shapes. These results are in harmony with the results of the cell attachment test. Dex-g-PLA films annealed in water gain high hydrophilic surface by the hydrophilic dextran segments were moved onto the film surface through micro-Brown motion. Consequently, serum proteins and cells were difficult to adhere to the Dex-g-PLA films.

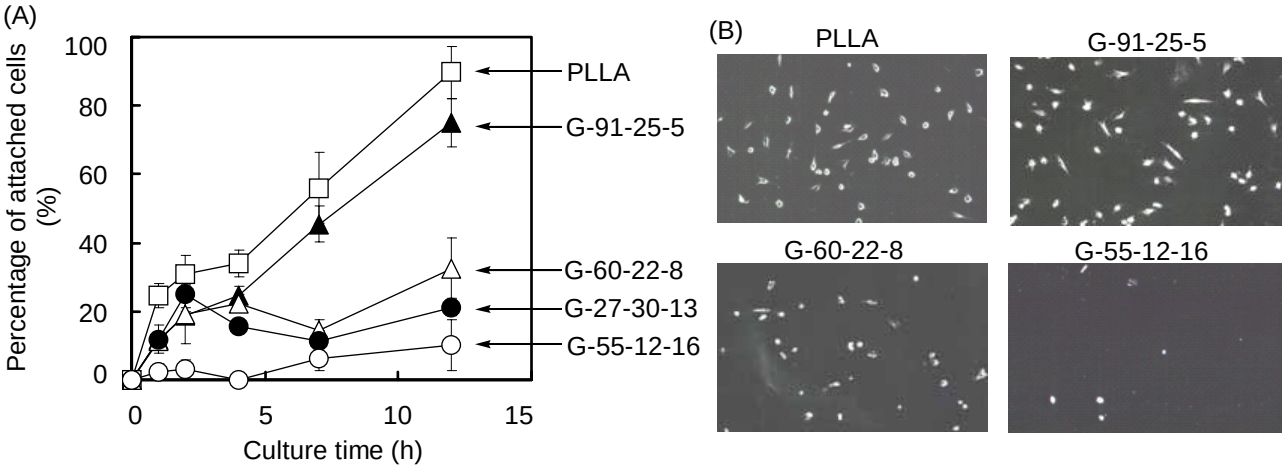


**Figure 3.** (A) Percentage of L929 cells attached on the film surfaces in a medium containing serum after 1–12 h incubation. (B) SEM views of L929 cells attached on Dex-g-PLA and PLLA film surfaces after 12 h incubation. [5]

***Entrapment and release behavior of BSA for the Copolymer MS***

The results of entrapment of BSA as a hydrophilic model protein drug into MS(Dex-*g*-PLA) and MS(PLLA) by W/O/W emulsion solvent evaporation/extraction method are summarized in Table 1. The entrapment efficiency of MS(Dex-*g*-PLA)s was higher than that of MS(PLLA) and depended on the content of sugar unit. These results could be explained by the reason that amphiphilic Dex-*g*-PLA stabilized the interface of primary W/O emulsion as a surfactant. Dex-*g*-PLA protects from aggregation of inner water layer including BSA and prevent from escape of BSA to outer water layer. Such a procedure must derive the high load of BSA within the domains consisting of hydrophilic dextran segments in MS(Dex-*g*-PLA)s.

CLSM was employed to observe how was the localization of entrapped FITC-BSA in MSs. Fig. 4 shows the confocal fluorescence images of MS(G-123-23-5) and MS(PLLA). Although, slight aggregation of FITC-BSA in some places of MS(PLLA) was observed, good dispersion of FITC-BSA was observed in MS(G-123-23-5) compared with MS(PLLA). Such good dispersion was recognized in the other MS(Dex-*g*-PLA)s. These results mean amphiphilic Dex-*g*-PLA exhibited surfactant ability in the process of MS preparation.

The results of release behavior of FITC-BSA from these MSs in PBS (pH 7.4) at 37 °C are shown in Fig. 5. The release rate of FITC-BSA from MS(Dex-*g*-PLA) was faster compared with that from MS(PLLA). Sustained release of FITC-BSA without initial burst was observed for MS(Dex-*g*-PLA), and the release rate depend on the content of sugar unit of Dex-*g*-PLA. These results can be explained by the fact that biodegradation rate of Dex-*g*-PLA is faster than that of PLLA [4].

CD was used to determine whether BSA molecules were denatured through the MS formation procedure. The ellipticity of spectrum of BSA released from MS(Dex-*g*-PLA) showed small value compared with the spectrum of native BSA. However, the spectrum of BSA released from MS(Dex-*g*-PLA) was nearer than the spectrum of BSA released from MS(PLLA) to the spectrum of native BSA. That is MS(Dex-*g*-PLA) could load BSA more stably than MS(PLLA).

**Table 1.** Results of preparation of microspheres entrapping BSA from Dex-*g*-PLA.[7]

| Microsphere | Yield[a)] | Entrapment Efficiency[b)] | Average diameter |
|---|---|---|---|
| | % | % | μm |
| MS(G-112-23-5) | 73.4 | 52.9 | 52.0 |
| MS(G-55-12-16) | 76.5 | 36.1 | 82.9 |
| MS(G-26-17-25) | 74.9 | 17.5 | 78.6 |
| MS(PLLA) | 77.5 | 10.2 | 30.6 |

[a)] [MS obtained/(BSA + polymer) in feed in g] × 100.
[b)] (BSA/polymer found in g)/(BSA/polymer in feed in g) × 100.

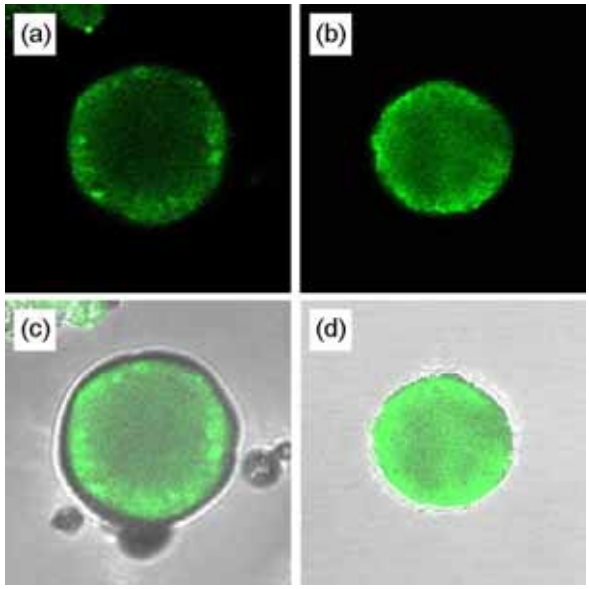


**Figure 4.** Conforcal fluorescence images and superpositions of the differential interference micrographs over the fluorescence images for MSs entrapping FITC-BSA. (a) and (b): fluorescence images of MS(PLLA) and MS(G-112-23-5), respectively; (c) and (d): superpositions of the differential interference micrographs over the fluorescence images of MS(PLLA) and MS(G-112-23-5), respectively.[7]

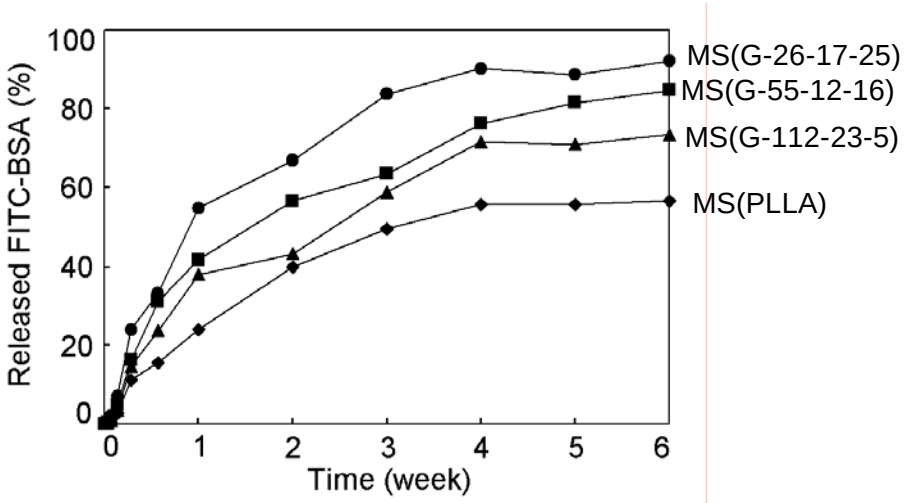


**Figure 5.** Release behavior of FITC-BSA from MS(Dex-*g*-PLA) and MS(PLLA).[7]

***References***

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