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Physicochemical Characterization of PEG Hydrogel to Estimate Biocompatibility

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The most important feature of this study is the comprehension of the interfacial phenomenon between cells and substrate surface. It is well known that physiological processes of cell adhesions, movements, and eventual function can be controlled by various physicochemical signals. A poly (ethylene glycol)(PEG)-hydrogel was synthesized on the slide glass coated with poly-L-lysine(PLL) using PEG with multi arm. PEG-hydrogel is a nontoxic and has been approved biocompatible, thus it is used extensively in biomedical device. Although there are many reports for bulk physical properties of PEG-hydrogel, their surface properties are not still clearly understood. Special focus was shed light on the understanding of the surface properties appeared by changing arm length and numbers of the PEG monomer, correlating with the biocompatibility. Viscoelasticity, wetting character, and protein adsorption were measured by using atomic force microscope (AFM), contact angle, and quartz crystal microbalance (QCM) respectively, whose results were concluded to have the close relation with the biocompatibility.

Key words: PEG-hydrogel, biocompatibility

1. Introduction

Hydrogels are useful in biomedical and pharmaceutical applications such as drug delivery and tissue engineering, because of their biocompatibility, high water content, and rubbery state (1).

Because poly (ethylene glycol)(PEG) is a nontoxic, water-soluble polymer that resists recognition by immune system, and has been approved biocompatible, we selected hydrogels prepared from PEG with multi arm. Hydrogel prepared from PEG with multi arm produced a more highly cross-linked structure and a more structure stability. This will arrow to control the swelling and elastic modulus easily. PEG hydrgels have been widely used in the field of biomaterials. Although there are many reports investigating a relation between the hydrogel's bulk property (2, 3), viscoelasticity(4, 5), molecular mobility and permeation and biocompatibility, the interfacial phenomenon between cells and substrate surface is complicated and difficult to understand. In particular the surface properties of the PEG hydrogel including friction, adhesion, elasticity caused by changing the structure of monomer are not well understood.

The primary aim of the present study is to investigate the surface properties of PEG hydrogel and evaluate the relation with biocompatibility. Special focus of the surface characterization was the mechanical properties of hydrogel.

2. Materials and Methods

2.1 Gel Synthesis.

PEG hydrogel was synthesized by photocrosslinking of PEG monomers with multi arm (three, four and eight arms) having photoreactive function terminal group. Characteristics of the PEG monomer are shown in Table I. Toluene-dehydrated was used as the polymerization. PEG monomers were dissolved in toluene at concentrations of 1wt%. The solution was spin-coated on the slide glass coated with poly-L-lysine(PLL). The obtained film was irradiated with light using the low-pressure mercury vapor lamp.

Table I. PEG macromonomer used in this study

Monomer designation	MW	no. of arms	arm MW
3arm20k	20000	3	6667
4arm5k	5000	4	1250
4arm20k	20000	4	5000
8arm40k	40000	8	5000

2.2 Frictional Force Measurements

Frictional force measurements on the sample surface were performed with atomic force microscope (AFM) (E-sweep with SPI4000 probe station; SII NanoTechnology Inc., Tokyo, Japan). All experiments were carried out in aqueous solution. The amplitude and the speed of the lateral scan were 5μm and 5μ/s, respectively. The frictional force of the PEG-coated surface was evaluated in comparison with PLL surface.

2.3 Contact Angle Measurements

The wettability of the sample surfaces was estimated from the static contact angle measurements (CA-W

contact angle meter; Kyowa Interface Science Co., Ltd., Tokyo, Japan). The air-in-water system procedure followed the captive bubble technique, where the sample surface was immersed in water and a small bubble was placed on the sample surface from bottom using a curve needle.

2.4 Protein adsorption Measurements

Protein adsorption measurements, which are an important reference for cell attachment, were performed with quartz crystal microbalance (QCM) measurements using an AT-cut gold-sputtered quartz crystal with resonance frequency of 27 MHz (Initium Inc., Tokyo, Japan). The PEG hydrogels were fixed on the crystals. The frequency was recorded after immersing the crystals in water. After baseline stabilization, bovine serum albumin (BSA) solution was injected at a concentration of 10mg/mL. The amount of adsorbed BSA was calculated by:

$$\Delta F = -\frac{2 \cdot F_0^2}{A\sqrt{\mu \cdot p}} \Delta m \qquad (1)$$

where ΔF is the magnitude of the frequency change, F_0 is the initial frequency, A is electrode surface area, μ is shear stress of the crystals, p is the crystals density, and Δm is the amount of mass change.

3. Results and Discussion

The frictional force measurements on PEG hydrogels surface were shown in Fig.1. The frictional force on the surface of PEG hydrogel is different within each PEG hydrogel compared with the PLL surface. The observations of the present study suggested that the surface of PEG 4arm5k hydrogel was the largest friction and the surface of PEG 3arm20k hydrogel was the smallest. When the chain length of the each arm was short, the frictional force was greatly detected. In contrast, when the chain length of the arm was long, the small frictional force was detected. The distance between each crosslinking point was influenced by the chain length of monomer. The PEG 4arm20k monomer and the PEG 8arm40k monomer have the same chain length, whereas these structures are different. These frictional forces indicated almost the same signal. Thus, the frictional force of PEG hydrogel surfaces has the close relation with the distance between crosslinking points. Thus, it was indicated surface property of friction of PEG hydrogel is affected by the size of chain length.

The static wettability of the PEG hydrogel surfaces was estimated by contact angle measurements (Fig.2). The difference of the wettability was observed on each PEG hydrogel surfaces. Note that the increase in contact angle corresponds to an increase in wettability for the air-in-water system. The observations of the present study suggested that the surface of the PEG 3arm20k hydrogel was most hydrophilic. This hydrogel was synthesized from the longest chain of PEG monomer. In contrast, the PEG 4arm5k hydrogel that was synthesized from the shortest chain lengh of PEG monomer was most hydrophobic. It was suggested that the distance between crosslinking points was highly correlated with wettability of the PEG hydrogel surface. Considering from the results above obtained, crosslinking density may have the close relation with the gel surface properties. The

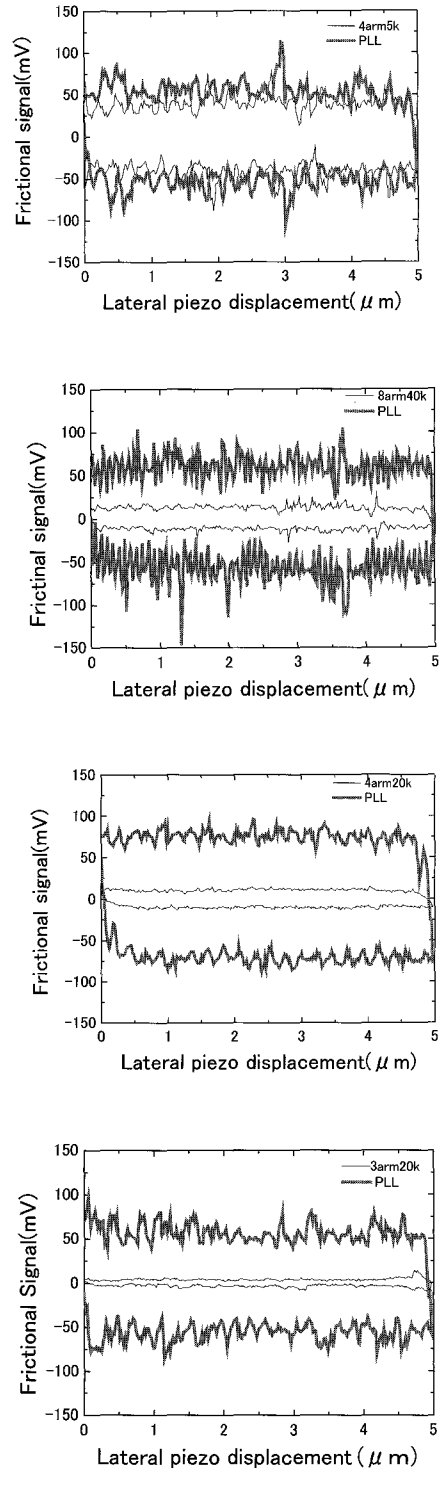

Fig.1. Raw friction date: (upper) PEG 4arm5k; (middle upper) PEG 4arm20k; (middle bottom) PEG 8arm40k; (bottom) PEG 3arm20k

The adsorbed proteins are responsible for subsequent cellular adhesion. Thus, the evaluation of the adsorbed protein is important. Nonspecific protein adsorption was estimated on each PEG hydrogels surface by QCM. The

amount of adsorbed BSA was calculated by the magnitude of frequency change using eq.1. On bare gold

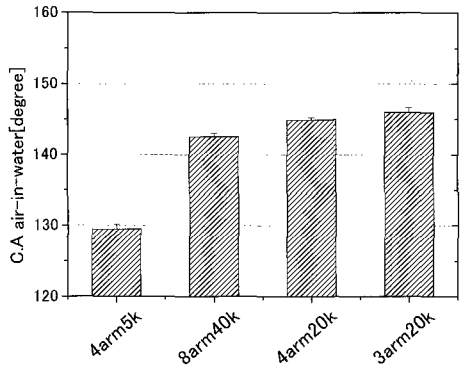

Fig.2. Static contact angles on PEG hydrogels surface by Air-in-water system.

surface as a control, the amount of adsorbed BSA was 0.42μg/cm^2. In contrast, PEG hydrogel surfaces clearly reduced protein adsorption (Fig.3). Fig.3 shows a comparison of the amount of adsorbed BSA on each PEG hydrogel surface. The amount of adsorbed BSA was 0.096μg/cm^2,0.035μg/cm^2, 0.020μg/cm^2, 0.061μg/cm^2 on PEG 4arm5k, PEG 8arm40k, PEG 4arm20k, and PEG 3arm 20k hydrogel surfaces, respectively. The PEG 4arm20k hydrogel surface showed the greatest degree of inhibition of BSA adsorption. It seemed that the property of inhibition of BSA adsorption is different in each PEG hydrogel surface. In fact, this property depends on the chain length or the structure of PEG monomer synthesizing a hydrgel. Based on these results, it was concluded that the property of inhibition of BSA adsorption was correlated with the Surface property of PEG hydrogel.

4. Conclusions

The PEG hydrogels were synthesized by using PEG macromonomer with multi arm, and these surface properties were measured by AFM, contact angle, QCM. It was indicated that the surface property of hydrogel characterized by frictional force was affected by the change of monomer structure. In particular, the chain length of arm had a large effect on the frictional property. It was insisted that the frictional force of PEG hydrogel surfaces had the close relation with the crosslinking density. The chain length of arm had a similar effect on the wettability of PEG hydrogel surface. When the chain length was long, the PEG hydrogel surface was more hydrophilic. Therefore, the crosslinking density of hydrogels is highly correlated with the frictional force and the wettability of PEG hydrogel surface. The amount of adsorbed BSA on each PEG hydrogel surface was significantly different. The PEG 4arm20k hydrogel surface showed the greatest degree of inhibition of BSA adsorption.

The results presented in this paper suggested that the PEG hydrogel surface property was controlled by chain length of PEG monomer. The PEG hydrogel surface property has the proper conditions to inhibit a protein adsorption, and the close relation with the biocompatibility.

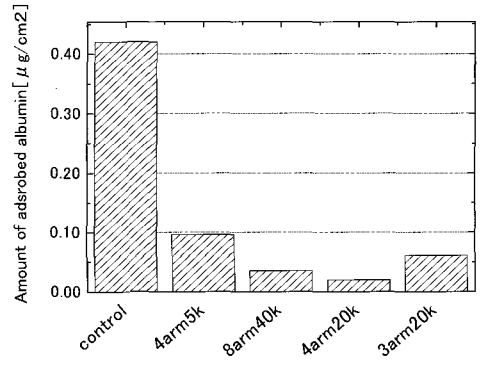

Fig.3. Amount of adsorbed albumin on PEG hydrogel surfaces by QCM. Control was bare gold surface.

5. References

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