



## Gradation of proteins and cells attached to the surface of bio-inert zwitterionic polymer brush



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### ABSTRACT

A self-assembled monolayer (SAM) of a 2-bromoisobutryl end group-carrying initiator for atom transfer radical polymerization (ATRP) was constructed on the surface of silicon wafer or glass substrates via a silane-coupling reaction. When the initiator SAM was irradiated with UV light at 254 nm, the surface density of bromine atoms was reduced by the scission of C–Br bonds as observed by XPS. With the surface-initiated ATRP of the zwitterionic vinyl monomer, carboxymethyl betaine (CMB), the surface density of PCMB brushes could be easily varied by changing the irradiation period of UV light prior to the polymerization. Furthermore, by using a UV-cut shutter sliding above the initiator SAM-modified substrate at a constant speed, the degree of bromine atom removal could be linearly varied along the direction of movement of the shutter. Consequently, the amount of both proteins adsorbed and cells adhered to the PCMB brush-covered substrate could easily be controlled by the gradation of the surface density of PCMB brushes, which suppressed protein adsorption and cell adhesion. Such a technique is very simple and useful for the regulation of the surface density of adsorbed proteins and adhered cells on an originally bio-inert surface.

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### 1. Introduction

For the modification of solid surfaces, so-called “polymer brushes” have been widely used. In the construction of polymer brushes, “grafting-from” and “grafting-to” procedures can be used. To obtain condensed polymer brushes, the former method has been preferentially adopted, whereas the latter can be easily pursued [1–5]. For the preparation of polymer brushes by the grafting-from method, controlled radical polymerization methods such as atom transfer radical polymerization (ATRP) [6–9], reversible addition-fragmentation chain transfer polymerization [10,11], and nitroxide-mediated polymerization [12,13] have been widely used.

Zwitterionic polymers have been extensively used for the construction of biocompatible surfaces [14–17]. By the vibrational

spectroscopic analyses of the hydrogen-bonded network structure of vicinal water, it has been pointed out that charge-neutralized polymers including zwitterionic and amphoteric polymers are inert to vicinal water, which provides biocompatible properties to the polymer surface [18–21]. It has previously been reported that the surface of zwitterionic brushes exhibit interesting properties with respect to friction and lubricity [22–24].

Recently, the construction of surfaces with additional values, including the modification of solid surfaces with polymeric materials and micro-fabrication technology, is highly sought after [25–27].

Many research groups, including our group, have reported that surfaces modified with zwitterionic polymers can efficiently suppress protein adsorption and cell adhesion [28–32]. Furthermore, patterned surfaces of proteins and cells could be constructed using UV light or high-energy beams such as an ArF-excimer laser and focused ion beams [33–35]. Ahmad et al., for example, reported poly[oligo(ethylene glycol)methacrylate] brushes grown from photo-patterned halogen initiators using a unique technol-

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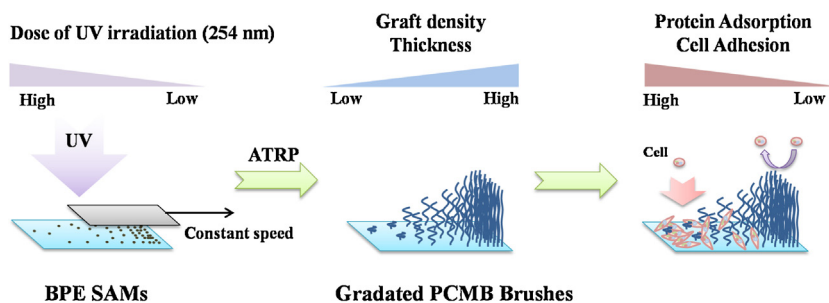


Fig. 1. Schematic illustration of protein adsorption and cell adhesion to the gradated polymer brushes prepared after UV irradiation of BPE SAMs.

ogy, the selective decomposition of the C–Br bond of the initiator for ATRP by using UV light at 244 nm [36].

It is very difficult to understand cellular behaviors *in vivo* because various events occur simultaneously. In the literature, gradated materials have been quite useful for the construction of surfaces with a concentration gradient of cells and proteins [37–40]. The applicability of gradated materials is quite promising because a wide range of information on the interaction of biological materials can be obtained on the same surface. Until now, various methods have been reported for constructing a gradation surface by using SAMs and polymers [41–43]. Moreover, a method, which adjusts a dose of irradiation to cause decomposition or photo-polymerization while moving the light shutter, has also been adopted to prepare the gradation surfaces [44–47]. In particular, based on the inherent property of a polymer, the wettability and charge of the surface can be controlled by the gradation of the polymer; these properties are attracting significant attention in advanced research fields [48–52]. The length and graft density of the polymer chains could be easily controlled for the concentrated polymer brush constructed via SI-ATRP. Therefore, it can be expected that microfabricated surfaces can be prepared more precisely by this method compared to other methods [53,54].

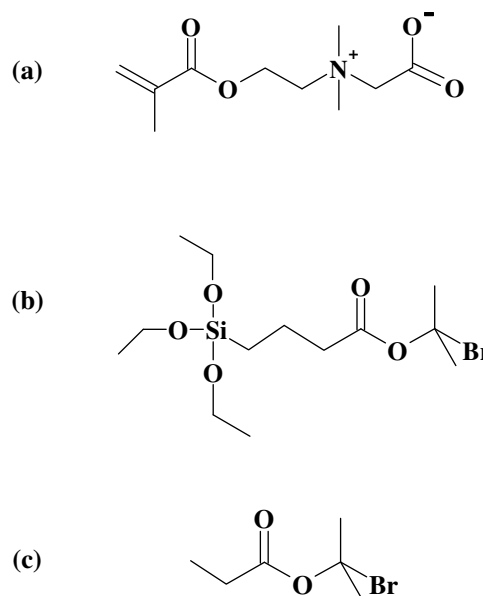
In this study, a zwitterionic polymer brush was prepared by surface-initiated atom transfer radical polymerization (SI-ATRP) from the self-assembled monolayer (SAM) of the ATRP initiator having a 2-bromoisobutyryl end group. With UV irradiation at 254 nm, the bromine atom essential for the initiation of ATRP could be selectively cleaved and therefore, by varying the irradiation time, the surface density of the polymer brush could be easily controlled. Since the zwitterionic polymer brush strongly suppressed protein adsorption and cell adhesion, we expected that we could manipulate the protein adsorption and cell adhesiveness onto the originally bio-inert polymer brush introduced to the surface of glass, silicon wafers, and various metal oxides.

Furthermore, by gradation of the irradiation period along the ATRP initiator-modified SAM, the gradation of the zwitterionic polymer brush can be easily realized (Fig. 1). Such a technique will be highly useful for bio-related applications.

## 2. Experimental

### 2.1. Materials

1-Carboxy-*N,N*-dimethyl-*N*-(2-methacryloyloxyethyl)methanaminium hydroxide inner salt (CMB, GLBT<sup>®</sup>) (Scheme 1(a)) was kindly donated by Osaka Organic Chemical Industries, Osaka, Japan. 3-(2-Bromo-2-isobutyryloxy)propyltriethoxysilane (BPE, Scheme 1(b)) was prepared as described elsewhere [55]. 2,2'-Bipyridine (Bpy, 99.5%) and copper(I) bromide (Cu(I)Br, 99.999%) were purchased from Wako Pure Chemicals Osaka, Japan. Cu(I)Br was purified by stirring in acetic acid overnight and dried after filtration at low pressure. Ethyl 2-bromoisobutyrate (EBiB, 98%,



Scheme 1. Chemical structures of (a) 1-carboxy-*N,N*-dimethyl-*N*-(2-methacryloyloxyethyl)methanaminium hydroxide inner salt (CMB, GLBT<sup>®</sup>), (b) 3-(2-bromo-2-isobutyryloxy)propyltriethoxysilane (BPE), and (c) ethyl 2-bromoisobutyrate (EBiB).

Scheme 1(c)) and bovine serum albumin (BSA) were purchased from Nacalai Tesque (Kyoto, Japan) and Sigma-Aldrich (Milwaukee, WI, USA), respectively. Toluene (99.5%, Wako Pure Chemicals) was stirred with concentrated sulfuric acid, followed by washing with saturated aqueous sodium carbonate solution and water. The purified toluene was finally obtained by distillation. Other reagents were commercially available. Milli-Q grade water (<18 M $\Omega$  cm) was used for preparation of sample solutions.

### 2.2. Construction of polymer brush

#### 2.2.1. Introduction of ATRP initiator (BPE) (Scheme 2(a))

A glass substrate (20 × 26 mm<sup>2</sup>) was washed by ultrasonication in methanol for 10 min and rinsed with water before immersing in a piranha solution (sulfuric acid:hydrogen peroxide solution = 7:3) for 1 h. The glass substrate was further washed with deionized water more than ten times, rinsed with acetone, and dried by N<sub>2</sub> gas. The pristine glass substrate was incubated in a toluene solution of BPE (2 mM) overnight in the dark. The BPE-modified substrate was washed with toluene, ultrasonicated in toluene, repeatedly rinsed with methanol and acetone, and finally dried by N<sub>2</sub> gas.

#### 2.2.2. Construction of PCMB brush via SI-ATRP (Scheme 2(b))

CMB (3.50 g, 15.0 mmol) and ethyl 2-bromoisobutyrate (EBiB, ATRP initiator, 22.2  $\mu$ L, 0.15 mmol) were dissolved in methanol

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