Smart Materials

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Controlled Cell Adhesion on PEG-Based Switchable Surfaces**

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Surfaces coated with poly(ethylene glycol) (PEG) or oligo(ethylene glycol) are generally regarded as the materials of choice for preventing bioadhesion.[1] For instance, numerous publications described the protein-repellency of PEG-modified substrates.[2] Such anti-fouling behavior is mainly due to the steric repulsion between hydrated neutral PEG chains and proteins.[3] Furthermore, as cell-adhesion mechanisms are generally protein-mediated, PEG-modified surfaces are also cell-repellent.[4] Thus, PEG-coated materials have been extensively studied in various bio-applications, such as blood-compatible materials, implants, and stealth carriers for either drug- or gene- delivery.

Yet, although very useful, bio-repellent PEG surfaces remain, on the whole, passive materials. Several emerging areas of biosciences and biotechnology certainly require "smarter" surfaces with more sophisticated properties. For instance, switchable surfaces capable of performing reversible bio-interactions are highly relevant for modern applications, such as bioseparation, biosensors, bio-assays and cell engineering. Such smart surfaces can be, for example, constructed with stimuli-responsive polymers.[5] For instance, the thermoresponsive polymer poly(*N*-isopropylacrylamide) (PNIPAM) and closely related polyacrylamides have been widely investigated for preparing biorelevant switchable surfaces.[6] With a lower critical solution temperature (LCST) of 32 °C, PNIPAM is the currently most studied material for inducing surface changes between room and body temperature. However, strictly speaking, PNIPAM is not a bio-inert polymer. Indeed, the presence of multiple secondary amide functions in the molecular structure of PNIPAM may lead to the formation of cooperative hydrogen-bonding interactions with other amide polymers, in particular with proteins.[7] In this context, the development of efficient switchable surfaces based on polymers of other chemical structures is certainly a topical matter. For instance, surfaces exhibiting thermoresponsive properties comparable to those of PNIPAM and the bio-repellent behavior of hydrated PEG would be of interest for numerous applications in modern biosciences.

We and others recently highlighted that macromolecules constructed with short oligo(ethylene glycol) methacrylates constitute an interesting new class of thermoresponsive polymers.[8] For instance, random copolymers of 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA) and oligo(ethylene glycol) methacrylate (OEGMA) have a LCST in water, which can be precisely adjusted by varying the comonomer composition. For example, cloud points of either 32 °C, 37 °C, or 39 °C were observed in pure water for copolymers having on average 5, 8 or 10 %, respectively, of OEGMA units per chain.[9] Moreover, the phase transitions of copolymers poly(OEGMA-*co*-MEO_2MA) are reversible and relatively insensitive to important parameters such as concentration, ionic strength, chain-length, and polydispersity. Hence, copolymers poly(OEGMA-*co*-MEO_2MA) appear to be promising candidates for bio-applications and more generally for building any kind of thermoresponsive materials.

Polymer brushes of oligo(ethylene glycol) methacrylates can be easily prepared on flat substrates by either surface-initiated atom transfer radical polymerization (ATRP) or surface adsorption of well-defined polymers with anchor moieties.[10] Yet, brushes prepared with long PEG methacrylates (i.e. side-chains of five ethylene oxide units or longer) behave somewhat like standard bio-repellent PEG coatings.[11] However, thermoresponsive polymers with shorter oligo(ethylene glycol) side-chains might behave differently. For instance, Jonas et al. recently demonstrated that surface-initiated poly(OEGMA-*co*-MEO_2MA) brushes exhibit LCST values, which coincide with those observed for free copolymers in aqueous solution.[12] The partial dehydration and the change of conformation of these surface brushes above LCST may be of practical interest for tuning bio-adhesion.[13]

Herein, poly(OEGMA-*co*-MEO_2MA)-modified gold surfaces were evaluated for their ability to control cell adhesion. In such an application, temperature variations should be relatively mild as mammalian cells may be damaged by extreme temperature fluctuations. Thus, the surfaces should ideally mediate cellular adhesion at physiological temper-

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ature and be cell repellent at room temperature. To attain such properties, a copolymer poly(OEGMA-*co*-MEO_2MA) containing in average 10 mol% of OEGMA units per chain was selected. This copolymer exhibits a LCST of about 39°C in pure water, whereas in phosphate buffered saline solution, as a result of a weak salting-out effect, the LCST is around 35°C (Figure 1a), rendering it ideal for the present application.

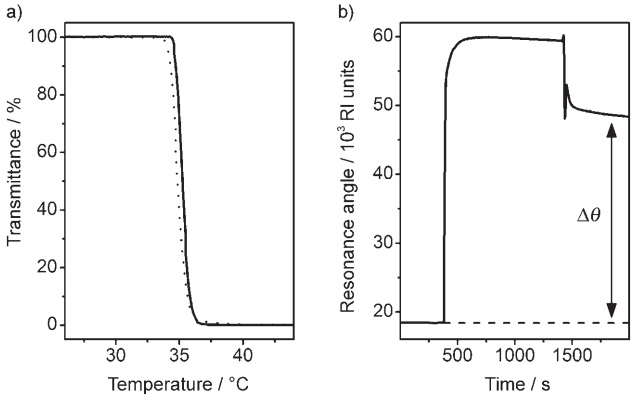
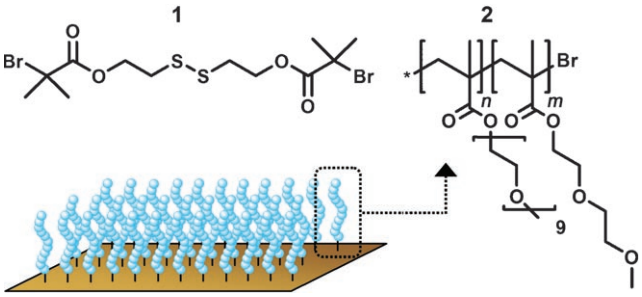


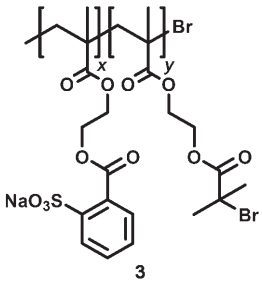
Figure 1. a) Plots of transmittance as a function of temperature measured for a phosphate buffered saline solution of a poly(OEGMA-*co*-MEO_2MA) **2** (3 mg mL^{-1}) prepared by ATRP in the presence of initiator **1**. (——) heating, (•••••) cooling. b) Functionalization of a gold surface with poly(OEGMA-*co*-MEO_2MA) **2** by the "grafting-onto" approach (strategy A), as followed by SPR. θ = resonance angle, RI = Refractive index.

To explore the potential of these thermoresponsive copolymers, three different strategies were investigated for modifying the surfaces. In strategy A, well-defined copolymers poly(OEGMA-*co*-MEO_2MA) were prepared by solution ATRP with the low-molecular-weight disulfide initiator **1** (Scheme 1), purified, and subsequently adsorbed on clean



Scheme 1. Molecular structure of the disulfide ATRP initiator **1** and the thermoresponsive copolymers poly(OEGMA-*co*-MEO_2MA) **2** used as a smart surface coating.

gold substrates ("grafting-onto" approach). In strategy B, **1** was first adsorbed on gold surfaces, which were used to initiate the atom transfer radical copolymerization of OEGMA and MEO_2MA in ethanol/H_2O mixtures ("grafting-from" approach). In strategy C, pristine gold surfaces were first modified by layer-by-layer polyelectrolyte deposition and subsequently functionalized by the polyanionic ATRP macroinitiator **3** (macroinitiator "grafting-from" approach).[14] Poly(OEGMA-*co*-MEO_2MA) brushes were then grown from the surfaces using either standard-ATRP or AGET-ATRP methods.[15]



All surface-modification strategies efficiently produced poly(OEGMA-*co*-MEO_2MA) brushes on gold substrates. For example, the adsorption of polymer **2** ("grafting-onto" approach; strategy A) on gold could be monitored in real time by surface plasmon resonance (SPR; Figure 1b). The contact angle of water on the gold surfaces changed from 70° (pristine surface) to 50° (polymer-modified surface) at 25°C. These values are in good agreement with literature data.[12] At 37°C, contact angles were found to be about 5° higher, indicating a change in surface properties. Successful "grafting-from" (strategy B) modifications were confirmed by SPR, X-ray photoelectron spectroscopy (XPS) and ellipsometry measurements (Figure S1 and S2, in the Supporting Information). Both standard- and AGET-ATRP approaches allowed efficient synthesis of polymer brushes.

The three different types of poly(OEGMA-*co*-MEO_2MA)-modified gold surfaces were used for cultivating L929 mouse fibroblasts at 37°C. The fibroblasts adhered efficiently and spread well on all types of substrates (Figure 2a), indicating that the poly(OEGMA-*co*-MEO_2MA)-modified surfaces are bio-adherent at physiological temperature. The maximum adhesion was typically observed after 40 h of cultivation (Figure S3, in the Supporting Information). Such kinetics of adhesion are roughly comparable to those generally observed on PNIPAM-modified surfaces.[16]

However, when the temperature of the cultivation medium is decreased to 25°C, a rapid cell rounding is observed within approximately 30 min (Figure 2b), allowing their facile detachment and harvesting by gentle rinsing at room temperature. No cell rounding of spread fibroblasts occurs on plain gold surfaces upon a temperature decrease from 37°C to 25°C. Thus, the poly(OEGMA-*co*-MEO_2MA)-modified gold substrates can be switch from cell-attractive to cell-repellent (i.e. standard PEG repellency). Importantly, this behavior is reversible and successive cycles of spreading/rounding can be triggered by temperature switches (data not shown).

In summary, thermoresponsive oligo(ethylene glycol)-based gold surfaces allow efficient control over cell-adhesion within a convenient and applicable temperature range (25–

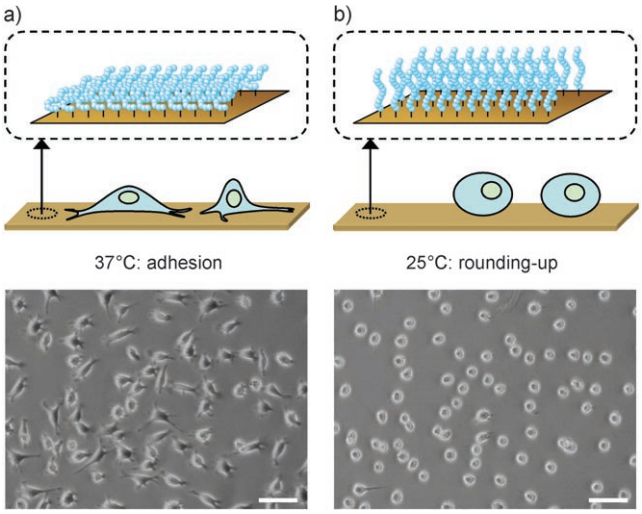


Figure 2. Phase-contrast microscopy images of L929 mouse fibroblasts on poly(OEGMA-*co*-MEO_2MA)-modified gold substrates after 44 h of incubation at 37 °C (a) and 30 min after cooling the sample to 25 °C (b). The surface presented was prepared using the macroinitiator "grafting-from" approach (strategy C). Scale bars correspond to 100 μm. The top panel shows a schematic view of the polymer coatings at 37 (a) and 25 °C (b).

37 °C). Thus, these novel smart substrates advantageously combine some features of PNIPAM surfaces (i.e. switchability) and PEG surfaces (i.e. bio-repellency at room temperature). These findings open new avenues for the design of advanced functional surfaces for cell culture engineering, bioseparation, and diagnostics applications.

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