



Cytocompatible Polymer Grafting from Individual Living Cells by Atom-Transfer Radical Polymerization

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Abstract: A cytocompatible method of surface-initiated, activator regenerated by electron transfer, atom transfer radical polymerization (SI-ARGET ATRP) is developed for engineering cell surfaces with synthetic polymers. Dopamine-based ATRP initiators are used for both introducing the ATRP initiator onto chemically complex cell surfaces uniformly (by the material-independent coating property of polydopamine) and protecting the cells from radical attack during polymerization (by the radical-scavenging property of polydopamine). Synthetic polymers are grafted onto the surface of individual yeast cells without significant loss of cell viability, and the uniform and dense grafting is confirmed by various characterization methods including agglutination assay and cell-division studies. This work will provide a strategic approach to the generation of living cell-polymer hybrid structures and open the door to their application in multitude of areas, such as sensor technology, catalysis, theranostics, and cell therapy.

Simple but highly versatile in the formation of polymer brushes on solid substrates is surface-initiated, atom-transfer radical polymerization (SI-ATRP), where polymers are grown, by controlled radical reactions, from the ATRP initiators that are introduced onto a substrate.^[1] During the last few decades, SI-ATRP has intensively been employed to provide planar substrates and micro/nanoparticles with diverse functionalities including non-biofouling properties, selective adhesiveness especially for cells, and stimuli-sensitivity (e.g., to light, temperature, and pH). For example, surfaces having both antimicrobial and non-biofouling properties were fabricated by SI-ATRP of zwitterionic polymers.^[2] The target materials of SI-ATRP have recently been expanded to functional biomaterials, such as self-assembled peptides,^[3] polysaccharide crystals,^[4] proteins,^[5] and viruses,^[6] for enhancing their physical stability, chemical activity, or therapeutic utility.

Although hybrid structures of living cells and synthetic polymers have a great deal of potential in cell-based applications, such as cell-based sensors, biomotors, biocatalysis, theranostics, cell therapy, and cells-on-a-chip,^[7] it is extremely challenging to perform SI-ATRP (and of course other polymerization protocols) on the surfaces of individual living cells, because the reaction conditions are lethal to chemically labile cells. However, the tight controllability of the characteristics of polymer brushes obtained by SI-ATRP, including length and surface density of the polymers and chemical compositions, would be greatly beneficial in the single cell-level manipulation of physicochemical and biological properties of cells and in the applications of cell-polymer hybrids aforementioned. We also envisioned that the functional and compositional diversity of polymers would advance chemical fabrication of artificial spores (cell-in-shell hybrid structures).^[8]

The cytotoxicity of (SI-)ATRP primarily originates from transition-metal catalysts (i.e., Cu^I), organic solvents, and the degassed conditions required for reducing the concentration of oxygen that deactivates both metal-based catalysts and reactive radical species during polymerization. These cytotoxic factors could be avoided, to some extent, by adopting a newly developed ATRP strategy, that is, atom-transfer radical polymerization using activators regenerated by electron transfer (ARGET ATRP).^[9] In the ARGET ATRP, a reducing agent (e.g., cytocompatible ascorbic acid, vitamin C) reactivates the catalysts from Cu^{II} to Cu^I, and, therefore, a low concentration of the metal-based catalyst is sufficient for the ATRP reaction. In addition, ARGET ATRP is performed in the aqueous solution under atmospheric conditions, which makes ARGET ATRP more cytocompatible than the conventional ATRP. However, ARGET ATRP still involves the radical chain reactions that consistently produce potentially cytotoxic radical species during polymerization. In this regard, it would be a legitimate strategy for forming cell-polymer hybrid structures without significant loss of viability to introduce a dually functional layer, which protects living cells from radical attack and also acts as an ATRP initiator simultaneously, onto cell surfaces. In this work, we took advantage of the radical-scavenging property of polydopamine (PD)^[10] to generate cell-polymer hybrids by surface-initiated ARGET ATRP (SI-ARGET ATRP). The radical species generated in SI-ARGET ATRP were effectively blocked by the PD-based layer that was primed onto cell surfaces, leading to the cytocompatible formation of cell-polymer hybrids.

We and others have previously developed PD-based ATRP macroinitiators (denoted as PDi herein) for SI-ATRP.^[11] We first investigated the polymerization efficiency of SI-ARGET ATRP, with gold substrates as a model, from

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the PDi layer. The PDi film was formed by mussel-inspired, material-independent coating of a dopamine derivative,^[12] which was synthesized by reacting dopamine with 2-bromoisobutyryl bromide (for the experimental details, see the Supporting Information).^[10a] A gold substrate was immersed in a TRIS-buffered solution (pH 8.5) of the dopamine derivative for 3 h, leading to the formation of the PDi film, the thickness of which was calculated to be 9.8 ± 0.9 nm based on ellipsometric measurements. For SI-ARGET ATRP, sodium methacrylate (SMA) was chosen as a monomer, because it is water-soluble and biocompatible (see Figure S1 in the Supporting Information). A PDi-coated gold substrate was placed in an aqueous solution (pH 8.5) of catalyst (CuBr_2), ligand (2,2'-bipyridyl), reducing agent (ascorbic acid), and monomer (SMA) under ambient conditions. After 4 h of SI-ARGET ATRP, the ellipsometric thickness increased to 25.0 ± 0.6 nm, indicating the successful polymerization of SMA. The Fourier-transform infrared (FT-IR) spectra confirmed the SI-ARGET ATRP of SMA (Figure S2). Further kinetic studies indicated that the polymerization rate was constant ($1.88 \text{ \AA min}^{-1}$) until 2 h, when all the reducing agents were consumed presumably, and the reaction stopped afterwards (Figure 1). In the conventional ATRP, the reaction rate rapidly decreases over time under aerobic conditions, because oxygen radicals rapidly oxidize Cu^{I} to catalytically inactive Cu^{II} . In contrast, the results showed that the polymerization rates in SI-ARGET ATRP did not decrease under aerobic conditions due to the presence of ascorbic acid that maintained the high percentage of catalytically active Cu^{I} . We believed that this characteristics would greatly contribute to cell-surface modification, because the polymerization rate did not decrease under respirable conditions until the reducing agents were consumed completely.

We applied the polymerization protocols to *Saccharomyces cerevisiae* (baker's yeast) cells. *S. cerevisiae* is a representative eukaryotic unicellular microorganism, and it is easy to identify characteristic changes of the cell after polymer grafting. In addition, much work has been done with *S. cerevisiae* in the field of cell-surface engineering, providing informative data for this study.^[13] For generation of cell-polymer hybrids, yeast cells were individually primed with PDi for 3 h (formation of yeast@PDi), and SI-ARGET ATRP

was performed from PDi-primed yeast with SMA as a monomer for a predetermined time (30, 60, or 120 minutes; generation of yeast@SMA; Figure 2a). The cell viability was investigated with fluorescein diacetate (FDA), which is hydrolyzed to fluorescein by esterases and becomes green-fluorescent in metabolically active cells. The viability of PDi-primed yeast was calculated to be 82.2%, which was higher than or similar to our previous results from the PD coating of yeast cells (70%).^[13a] To investigate the cytoprotective effect of the PDi layer, we used a control, where the ATRP-initiating, 2-bromoisobutyryl group was grafted to cell surfaces by directly reacting 2-bromoisobutanoic acid *N*-hydroxysuccinimide ester (BIB-NHS) with yeast cells. In other words, the control yeast cells presented the PD-absent ATRP initiators. The FDA assay showed that, in the case of the control, the survival ratio was 0.346 (from 91.5% to 31.7%) after 30 minutes of SI-ARGET ATRP (Figure 2b). In stark contrast, the survival ratio was 0.814 (from 82.2% to 66.9%) for the PDi-primed yeast, indicating a 2.35-fold enhancement in the viability. The value was similar after 1 h of polymerization (2.43-fold enhancement). These results implied that the PDi layer effectively protected the cell surfaces from peroxidation because of its radical scavenging

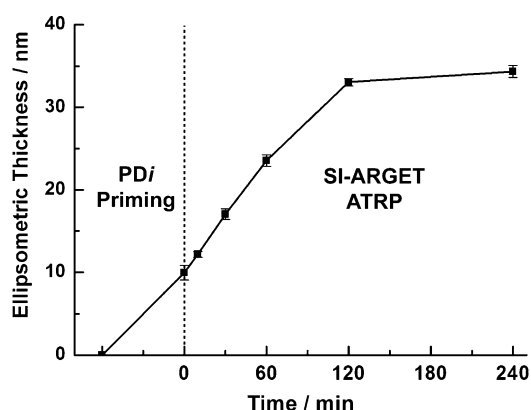


Figure 1. A graph of ellipsometric thickness versus polymerization time. The film thickness after PDi priming is also indicated in the graph.

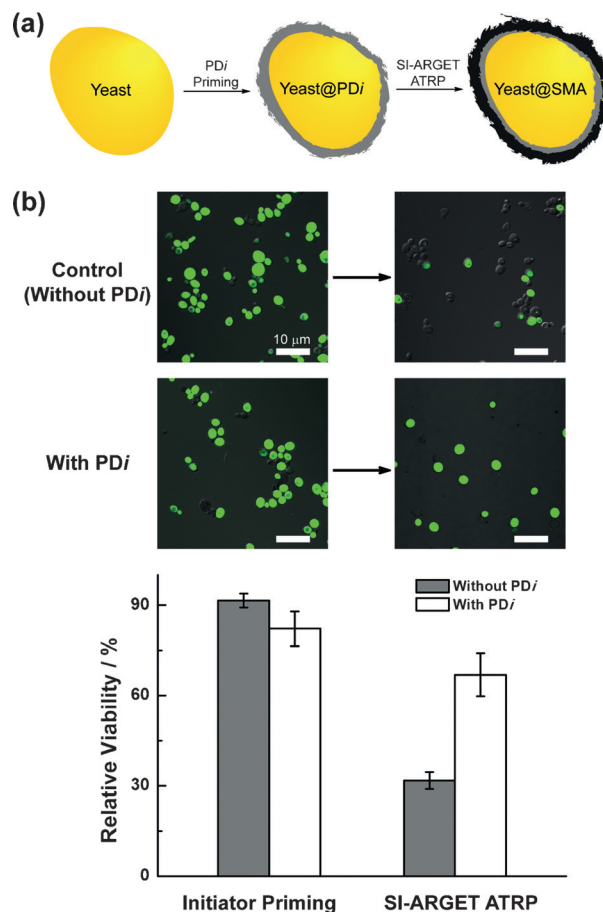


Figure 2. a) Schematic representation for cyto-compatible polymer grafting from individual yeast cells, composed of priming of the PDi-based ATRP initiator and SI-ARGET ATRP of SMA. b) Relative viability of PDi-primed yeast and SMA-coated yeast with the viability of native yeast as a reference. The control (without PDi) was performed by direct reaction with BIB-NHS and subsequent SI-ARGET ATRP.

capability. We also noticed that the viability decreased over time during polymerization, and a sharp drop in viability was observed for the control set (Figure S3). The development of fast-polymerizing reactions, therefore, would further contribute to cytocompatible polymer grafting. In some cases that do not require dense and uniform coating, the early termination of the polymerization could be used for cytocompatible cell-surface modification.

We characterized PDi-primed yeast and SMA-coated yeast (after 1 h of grafting) with various characterization methods including zeta (ζ) potentiometry, scanning electron microscopy (SEM), and confocal laser-scanning microscopy (CLSM). The ζ -potential measurements showed the formation of PDi-primed yeast and SMA-coated yeast (Table S1): The ζ -potential of native yeast was about -32 mV, which was in a good agreement with a reported value,^[14] but the value was changed to -25 and -20 mV after PDi priming and polymer grafting, respectively. Conversion in the surface charge was also observed with 2-(dimethylamino)ethyl methacrylate (DMAEMA) as a monomer. The SI-ARGET ATRP of positively charged DMAEMA changed the ζ -potential value of yeast cells from negative (-32 mV) to positive ($+16$ mV), confirming the successful grafting of polymers. The SEM images showed dramatic morphological changes after SI-ARGET ATRP (Figure 3a and Figure S4). Compared with native yeast (and PDi-primed yeast), the surface of SMA-coated yeast was rougher, composed of nanometer-scaled polymer particles, indicating the presence of poly(SMA) on the cell surface. 3-Azido-2-hydroxypropyl methacrylate (AzHPMA) was used as a co-monomer with SMA (SMA:AzHPMA = 100:1), and the polymer layer was visualized, after azide-alkyne cycloaddition of alkyne-linked Alexa Fluor[®] 594, by CLSM (Figure 3b; for the bright-field image, see Figure S5). Red rings were observed only for azide-functionalized SMA-coated yeast, and native yeast and SMA-coated yeast did not show any red-fluorescence signals. The z-stack CLSM images confirmed that the red fluorescence originated from the cell surface (Figure S6). We also used azide-bearing poly(ethylene glycol) methacrylate (PEGMA- N_3) for another visualization: a mixture of PEGMA and PEGMA- N_3 (9:1) was polymerized, and the resulting PEGMA- N_3 -coated yeast was coupled with alkyne-linked Alexa Fluor[®] 594 (Figure S5). The CLSM images further supported the uniform polymer grafting on individual cells.

The uniform poly(SMA) grafting was further confirmed by an agglutination assay (Figure 4a and Figure S7). Yeast cells form aggregates, when they are mixed with *Escherichia coli*, because *E. coli* has multiple copies of α -D-mannose-binding proteins on the cilium.^[15] Figure 4a showed that native yeast, when mixed with *E. coli*, rapidly aggregated into a cell cluster within 3 minutes (left panel). However, the dense polymer layer of SMA-coated yeast physically blocked the binding of *E. coli* and prevented the agglutination. The formation of dense (and durable) polymer layers was also supported by the retarded cell division, which is one of the characteristics of artificial spores (Figure 4a, right panel).^[8,16] The growth curves of native yeast, PDi-primed yeast, and SMA-coated yeast, obtained by measuring the optical densities at 600 nm (OD_{600}), showed that the polymer grafting suppressed the

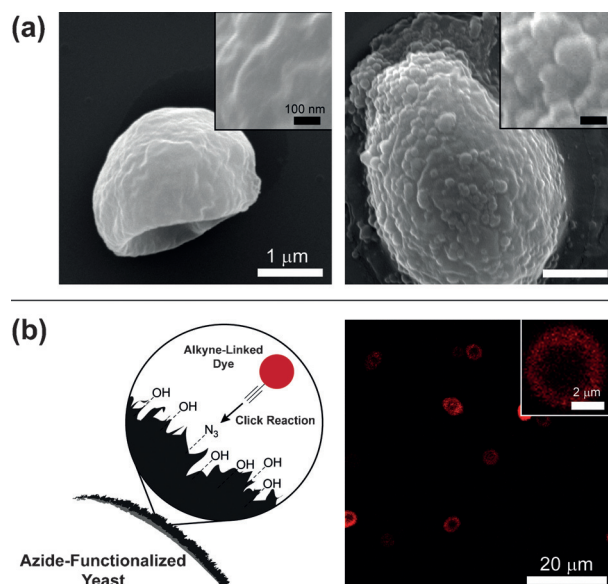


Figure 3. Characterizations of SMA-coated yeast. a) SEM images of (left) native yeast and (right) SMA-coated yeast. b) CLSM images of azide-functionalized SMA-coated yeast after coupling with alkyne-linked Alexa Fluor[®] 594.

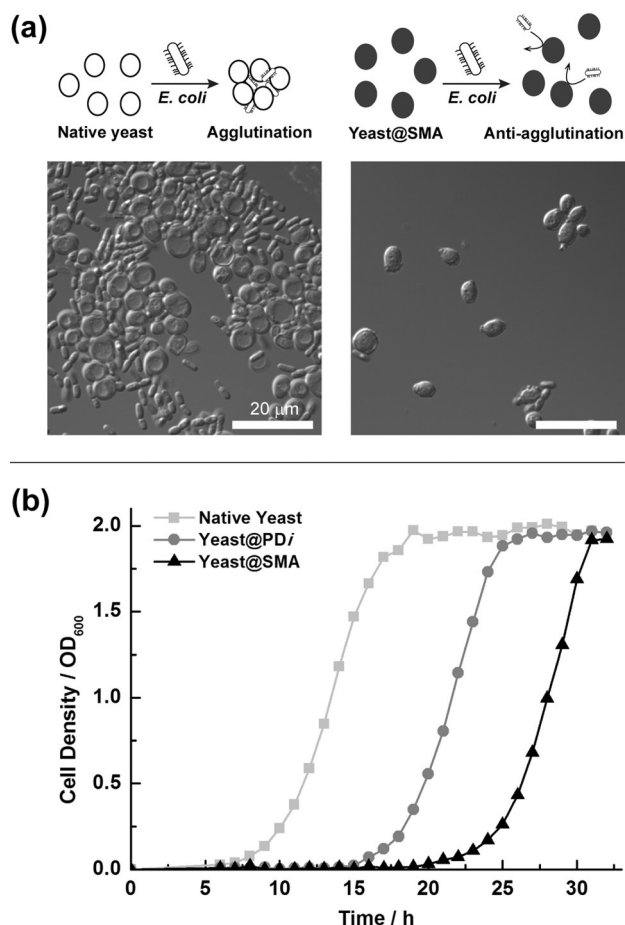


Figure 4. a) Agglutination assay of yeast: (left) native yeast and (right) SMA-coated yeast. b) Growth curves of native yeast (■), PDi-primed yeast (●), and SMA-coated yeast (▲).

cell-division activities and prolonged the lag phase (Figure 4b and Figure S8). Taken all together, the characterizations clearly confirmed the cytocompatible formation of highly dense polymers on individual living cells.

Polymer synthesis has so far never come along with living cells, while several cytocompatible bioorthogonal reactions have been developed for chemical manipulation of cells with small molecules.^[17] In this work, we developed a cytocompatible SI-ARGET ATRP method for grafting polymers from living cells with use of polydopamine priming. Modulation of cell-surface properties (i.e., charges and recognition capability) and cellular activities (i.e., cell division) was demonstrated, along with the ability of post-functionalization of polymer coats. Considering a plethora of functional and structural variations in synthetic polymers, we believe that grafting polymers onto cell surfaces would generate multifunctional cellular hybrids for many biotechnological and biomedical applications, not to mention providing an advanced tool for chemical manipulation of cells.

Experimental Section

Polymer grafting from *Saccharomyces cerevisiae*: The dopamine-based ATRP initiator was synthesized by following the previous report^[11a] and dissolved in a TRIS-buffered solution (pH 8.5). *S. cerevisiae* (baker's yeast) was cultured in YPD broth media for 36 h in 33 °C and washed with deionized (DI) water. The yeast cells were incubated for 3 h in the initiator solution for PDI priming, washed with DI water, and transferred to the aqueous ATRP solution that contained SMA (1M), CuBr₂ (1 mM), 2,2'-bipyridyl (2 mM), and ascorbic acid (10 mM). After 1 h of polymer grafting, the resulting SMA-coated yeast was concentrated by centrifugation and washed with DI water.

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