Antibacterial Polypropylene via Surface-Initiated Atom Transfer Radical Polymerization

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Polypropylene (PP) coated by a non-leachable biocide was prepared by chemically attaching poly(quaternary ammonium) (PQA) to the surface of PP. The well-defined poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA), a precursor of PQA, was grown from the surface of PP via atom transfer radical polymerization (ATRP). The tertiary ammine groups in PDMAEMA were consequently converted to QA in the presence of ethyl bromide. Successful surface modification was confirmed by ATR-FTIR, contact angle measurement, and an antibacterial activity test against *Escherichia coli* (*E. coli*). The biocidal activity of the resultant surfaces depends on the amount of the grafted polymers (the number of available quaternary ammonium units). With the same grafting density, the surface grafted with relatively high MW polymers (M_n > 10,000 g/mol) showed almost 100% killing efficiency (killing all of the input *E. coli* (2.9×10^5) in the shaking test), whereas a low biocidal activity (85%) was observed for the surface grafted with shorter PQA chains (M_n = 1,500 g/mol).

Introduction

The use of biocidal compounds in synthetic polymers has steadily increased. This is because the polymeric materials that are widely used in food packaging, textiles, and biomedical applications such as medical devices can easily be colonized by bacteria or fungi, frequently causing severe infections and transmitting disease.

The prevention of microbial colonization of polymeric surfaces can be achieved by the release of antimicrobials physically incorporated into the polymers.[1−4] Although these leaching systems are useful, their practical applications are limited because they must by nature lose their capacity to kill over time. In addition, the release of biocides to the environment has the potential to increase bacterial resistance to biocides. Non-leaching antimicrobial polymeric surfaces, where the antimicrobial agent is permanently fixed to the surface through covalent bonding, is an attractive alternative strategy. There is now significant literature concerning the preparation of permanent antibacterial surfaces via the covalent coupling of poly(quaternary ammonium) (PQA) compounds to a variety of surfaces.[5−15] A particularly vexing challenge is the need to covalently couple biocidal polymers to the surface of plastics such as polypropylene (PP), which are common and inert materials.

In this communication we describe a convenient method to chemically attach biocidal PQA on PP surfaces and tune surface biocidal activity in a controlled manner. PP surfaces are notoriously difficult to modify without aggressive pre-activation with, for example, plasma[16] and ozone.[17,18] We are able to modify PP surfaces by combining a novel photochemical method[19,20] with a controlled/living radical polymerization technique, atom transfer radical polymerization (ATRP).[21−24]

Recently, Rühe[19] reported a novel approach to a polymeric network covalently attached onto a silicon surface coated with a monolayer of alkyl chains. The fabrication approach is based on the photochemistry of benzophenone. Triggered by UV light (λ = 365 nm), benzophenone has an n−π* transition, resulting in the formation of a biradical triplet excited state that then abstracts a hydrogen atom from neighboring aliphatic C−H groups to form new C−C bonds.[20] Herein we use the same photochemical technique to introduce benzophenonyl 2-bromoisobutyrate (BPBriBu, ATRP initiator) to the PP surface (Scheme 1a). Photoactive benzophenone functional groups in BPBriBu act as an anchor to promote the immobilization of the ATRP initiator by abstracting hydrogen from PP surfaces. We used ATRP to grow poly(2-dimethylaminoethyl methacrylate) (PDMAEMA)[13,25,26] from PP surfaces immobilized with ATRP initiators.[27,28] The surface tethered polymers were then converted to the corresponding PQA after a quaternization reaction using ethyl bromide. The chemical composition and wettability of the treated surface were determined by ATR-FTIR and water-contact angle measurement, respectively. Biocidal activity was confirmed by a biocidal test against *Escherichia coli* (*E. coli*).

Results and Discussion

Synthesis of Benzophenonyl 2-bromoisobutyrate (BPBriBu, ATRP Initiator) and Immobilization on PP Surfaces. BPBriBu was obtained by reacting 2-bromoisobutyryl bromide with 4-hydroxybenzophenone (Scheme 1a) in the presence of triethylamine. This reaction was very efficient with a yield of about 90%. The structure of the final product was confirmed

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Scheme 1. Synthesis of 2-Benzophenonyl bromoisobutyrate and Benzophenone Chemistry (a) and a Schematic Drawing of Surface-Initiated ATRP of DMAEMA (b)

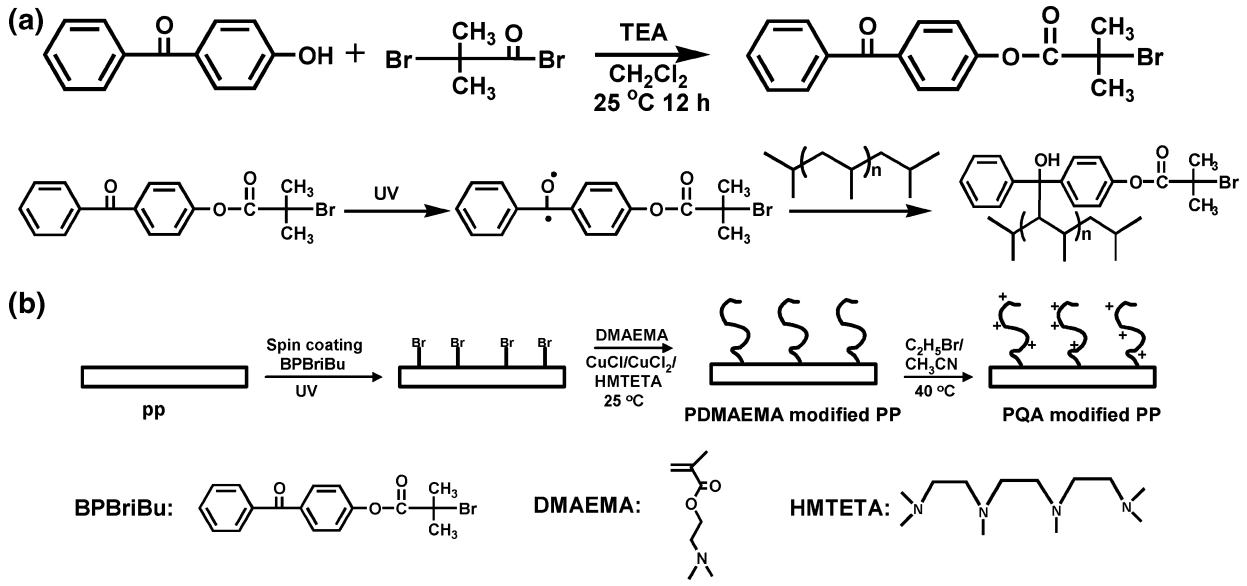

Table 1. Preparation of Antibacterial PP Surfaces and Surface Characterization[a]

entry	DMAEMA/ EBriBu/ CuCl/ $CuCl_2$/HMTETA	M_n (g/mol)	PDI	CA (PP)	CA (PP with PDMAEMA)	CA (PP with PQA)	grafting density (σ) (chain/nm^2) /(QA unit/nm^2)	killing percentage (%)
blank PP				100°			0	0
1	100:1:2:0.4:2.4	1,500	1.16	100°	99°	75°	0.23/2	85
2	200:1:2:0.4:2.4	9,800	1.18	110°	93°	66°	0.22/14	100
3	400:1:4:0.8:4.8	21,300	1.21	110°	83°	64°	0.20/31	100
4	400:1:4:0.8:4.8	35,100	1.22	110°	75°	60°	0.19/42	100

[a] The water-contact angle on the surface was measured using a VCA Optima system (AST products, Inc.) with a drop size of 1.5 μL of deionized water. Five measurements at different spots were taken with each substrate, and the average of these values was determined. The challenge: 2.9E+5 bacteria in 5 mL suspension; untreated PP was used as the blank.

by an ^{1}H NMR spectrum, where all of the characteristic peaks were identified (Supporting Information, Figure S1).

Immobilization of this initiator on PP surfaces was achieved through two sequential steps: (1) deposition of initiator (BPBriBu) on the PP surface via spin coating; and (2) UV irradiation ($\lambda = 365$ nm) of the initiator-deposited surface. The absorption of UV light at a wavelength of about 365 nm triggered the formation of a biradical triplet excited state. These biradicals then abstracted a hydrogen atom from the neighboring aliphatic PP chains on the surface and formed new C−C bonds.[19,20] As a result, the ATRP initiator, which was physically attached to the surface after spin coating, was covalently bound to the surface (Scheme 1b).

ATRP of DMAEMA Initiated from the PP Surface. ATRP of DMAEMA from the initiator-attached PP surface was carried out in the presence of ethyl 2-bromoisobutyrate (EBriBu) and CuCl/$CuCl_2$/1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA) as the sacrificial initiator and catalyst system, respectively (Scheme 1b). The addition of the sacrificial initiator led to the formation of free polymer, which was used to estimate the molecular weight characteristics of the obtained polymer chains on the surface, given that the similar structure of the chains at the surface and those in solution has been clearly demonstrated.[29,30] These polymerizations were well-controlled (Table 1). Figure 1 represents the typical GPC profile of samples taken during the polymerization (entry 4, Table 1). The molecular weight of the free polymer progressively increased, and the polydispersity (PDI) remained relatively low during polymerization. The chain length of the polymer grafted on the surface

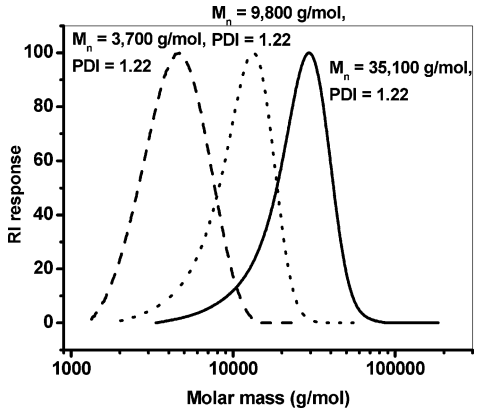

Figure 1. ATRP of DMAEMA initiated from the PP surface. [EBriBu]/[DMAEMA]/[CuCl]/[$CuCl_2$]/[HMTETA] = 1:400:4:0.8:4.8; $V_{monomer}/V_{acetone}$ = 4:1; T = 25 °C.

was controlled by varying the feeding ratio of the monomer to the sacrificial initiator and/or the polymerization time, assuming that the rate of propagation on grafted chains is equal to that on free chains. In this communication, a series of polymer-grafted surfaces were prepared, where the molecular weight of the grafted polymer chains ranged from 1,500 to 35, 000 g/mol.

After quaternization (Scheme 1b), the surface PDMAEMA was converted to PQA. The polymer chain density was then derived by dividing the amount of QA units by the degree of polymerization (DP) of PDMAEMA determined from GPC, assuming that the DP of the grafted polymer chain was equal to the DP of chains growing in solution derived from the

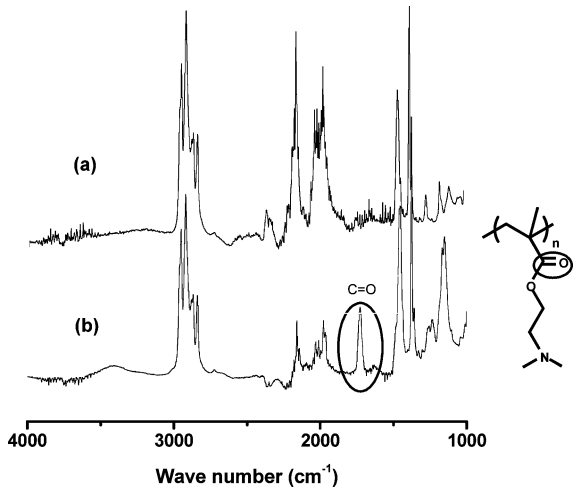

Figure 2. ATR-FTIR spectra of (a) untreated PP and (b) PP modified with PDMAEMA. (M_n = 35,100 g/mol).

sacrificial initiator. The amount of QA units on the surface was determined by measuring the amount of the complexing dye molecules using a colorimetric method based on fluorescent complexation and UV−vis spectroscopy (Supporting Information), and assuming that one QA unit complexes with one fluorescein dye molecule. The chain densities on all of these surfaces were similar (~0.2 chain/nm^2). Because the surface area was 2 cm^2, the amount of grafted chains corresponded to 3×10^{-11}mol. In a typical experiment, the concentration of sacrificial initiator was 10^{-2} mol/L in 26 mL of reaction solution, corresponding to 3×10^{-4} mol sacrificial initiator. Therefore, the amount of the sacrificial initiator in the solution was 10^7 times larger than the amount of grafted chains.

The presence of PDMAEMA on the surface was confirmed by ATR-FTIR spectroscopy. Compared with the spectrum of the untreated PP surface (Figure 2a), an additional peak at 1730 cm^{-1} was observed in the spectrum of the modified surface (Figure 2b), which was ascribed to the C═O stretching vibration in the ester groups of PDMAEMA. Figure 3 shows the digital images of the PP slides stained with fluorescein salt dye molecules that strongly bind to QA groups. It clearly shows an increase in color intensity with an increase in the MW of the grafted polymer.

The presence of PDMAEMA on the PP surface led to a dramatic change in surface wettability. As shown in Table 1, the untreated PP surface was very hydrophobic with a static contact angle (CA) of about 106°. After surfaces were grafted with hydrophilic PDMAEMA, they became less hydrophobic, as illustrated by a dramatic decrease in the contact angle (75−99°). In addition, a clear correlation was observed between the surface-contact angle and the molecular weight of the grafted polymer. The water-contact angle decreased with the increase in the molecular weight of the PDMAEMA on the surface. This was probably due to the fact that the higher molecular weight polymer on the surface led to a higher degree of coverage of the hydrophobic substrate. After quaternization, the water-contact angle of the surface decreased further (60−75°) because PDMAEMA was converted to the more hydrophilic PQA.

Biocidal Activity. The dynamic shake flask method[31] has been used to assess the ability of the PP surface to kill bacteria. Modified and blank PP slides (1 × 1 cm) were shaken with 5 mL of a bacterial suspension containing 2.9×10^5 *E. coli* for 1 h at 37 °C. Then the suspension was taken, diluted appropriately, and plated on L-agar plates. After overnight incubation, the number of viable cells was then determined as colony-forming units (CFU). Ideally, each surviving cell develops into a distinct colony after incubation, thus providing a direct measure of bacterial viability. Surface biocidal activity was represented by a kill percentage that was calculated on the basis of eq 1, where $N_{control}$ and N_{sample} correspond to the colonies on the L-agar plates of the control and the sample, respectively, whereas $F_{control}$ and $F_{experiment}$ represent the dilution factor of the control and the sample, respectively.

$$\text{Killing percentage} = (F_{control}N_{control} - F_{sample}N_{sample})/F_{control}N_{control} \times 100$$

The density of QA played an import role in the biocidal activity. The surface grafted with low molecular weight PQA or the low density of QA (1,500 g/mol, σ (QA) = 2 unit/nm^2, entry 1, Table 1) exhibited relatively low biocidal activity, killing only 85% of the bacteria. However, when the surfaces were grafted with higher MW PQA (>9,800 g/mol, σ(QA) > 14 unit/nm^2, entry 2−4, Table 1), essentially all of the bacteria were killed. These experimental results agreed with those from the literature that demonstrated that the higher density of QA led to higher biocidal activity in a certain concentration range of QA.[7,32] In the above cases, the chain density was constant, and the difference in the density of QA is due to the difference in the chain length. The essentially complete killing by the surface in entry 2−4 does not mean that all of these surfaces had the same biocidal activity. These results only demonstrate that the challenge in these experiments is below their killing capability. Further studies on the correlation of surface biocidal activity with the density of QA and the effects of the chain density and structure of polymers on biocidal activity are underway. Figure 4 shows the digital photographs of L-agar plates incubated with the bacteria from the suspensions treated with the modified surface (entry 3, Table 1) and the unmodified surfaces. No colonies formed on the plate incubated with the bacteria suspension treated by the modified surface (Figure 4a). The control plate, however, was covered with bacterial colonies (Figure 4b).

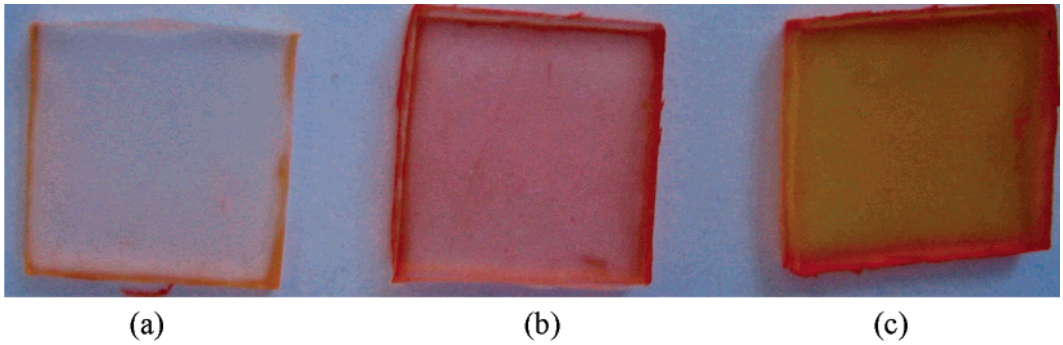

Figure 3. Digital pictures of the PP plates stained with fluorescein salt dye. (a) PP modified with PDMAMEA (M_n = 1,500 g/mol); (b) PP modified with PDMAMEA (M_n = 9,800 g/mol); (c) PP modified with PDMAMEA (M_n = 21,300 g/mol).

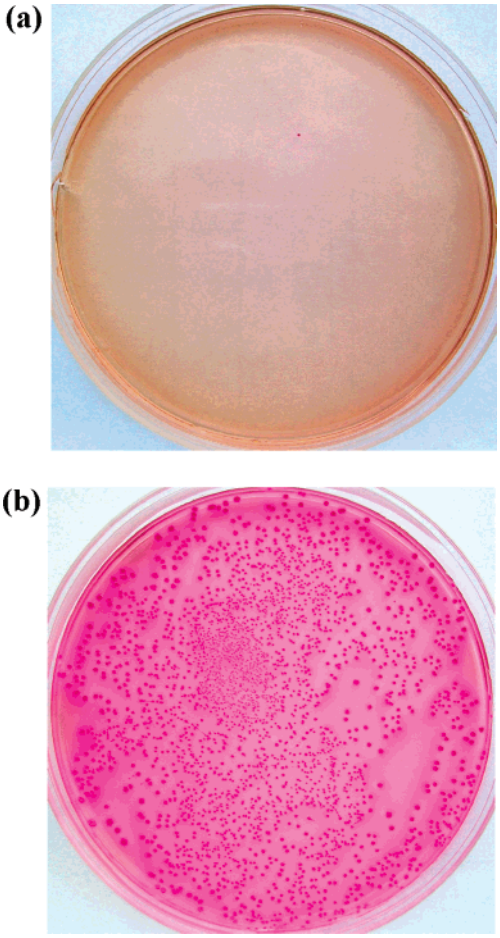

Figure 4. Photographs of L-agar plates onto which the *E. coli* suspension of distilled water treated with the PP slide (entry 3, Table 1) grafted with PQA (a) and the untreated PP slide (b) were deposited and incubated for 24 h. The PP slides were shaken with 5 mL of bacterial suspension for 1 h at 37 °C. The solution was taken, diluted appropriately, and plated on L-agar plates. After overnight incubation, pictures were taken.

Conclusions. Antibacterial PP was prepared by introducing PQA on the surface via surface-initiated ATRP of DMAEMA followed by quaternization. The molecular weight of the polymer or the density of QA on the surface was well controlled. The surface biocidal activity against *E. coli* was assessed and found to increase with an increase in the density of surface QA units. The surface (1 × 1 cm) with σ (QA) = 2 unit/nm^2 killed 85% of the contacted bacteria (challenge was 2.9 × 10^5 bacteria). This corresponds to one bacteria killed by 7 × 10^8 surface QA units. The surfaces with σ >14 QA unit/nm^2 killed essentially all of the bacteria.

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Supporting Information Available. Experimental details and 1H NMR spectrum of BPBriBu. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Oyane, A.; Yokoyama, Y.; Uchida, M.; Ito, A. *Biomaterials* **2006**, *27*, 3295−3303.
(2) Golubovich, V. N.; Rabotnova, I. L. *Mikrobiologiya* **1974**, *43*, 1115−1117.
(3) Francolini, I.; Ruggeri, V.; Martinelli, A.; D'Ilario, L.; Piozzi, A. *Macromol. Rapid Commun.* **2006**, *27*, 233−237.
(4) Sun, Y.; Sun, G. *J. Appl. Polym. Sci.* **2003**, *88*, 1032−1039.
(5) Thome, J.; Hollander, A.; Jaeger, W.; Trick, I.; Oehr, C. *Surf. Coat. Technol.* **2003**, *174*−*175*, 584−587.
(6) Sauvet, G.; Fortuniak, W.; Kazmierski, K.; Chojnowski, J. *J. Polym. Sci., Part A: Polym. Chem.* **2003**, *41*, 2939−2948.
(7) Tiller, J. C.; Lee, S. B.; Lewis, K.; Klibanov, A. M. *Biotechnol. Bioeng.* **2002**, *79*, 465−471.
(8) Lin, J.; Murthy, S. K.; Olsen, B. D.; Gleason, K. K.; Klibanov, A. M. *Biotechnol. Lett.* **2003**, *25*, 1661−1665.
(9) Lin, J.; Qiu, S.; Lewis, K.; Klibanov, A. M. *Biotechnol. Bioeng.* **2003**, *83*, 168−172.
(10) Lin, J.; Tiller, J. C.; Lee, S. B.; Lewis, K.; Klibanov, A. M. *Biotechnol. Lett.* **2002**, *24*, 801−805.
(11) Hu, F. X.; Neoh, K. G.; Cen, L.; Kang, E. T. *Biotechnol. Bioeng.* **2005**, *89*, 474−484.
(12) Hollander, A.; Thome, J.; Keusgen, M.; Degener, I.; Klein, W. *Appl. Surf. Sci.* **2004**, *235*, 145−150.
(13) Lee, S. B.; Koepsel, R. R.; Morley, S. W.; Matyjaszewski, K.; Sun, Y.; Russell, A. J. *Biomacromolecules* **2004**, *5*, 877−882.
(14) Cen, L.; Neoh, K. G.; Kang, E. T. *Langmuir* **2003**, *19*, 10295−10303.
(15) Chen, K.-S.; Ku, Y.-A.; Lin, H.-R.; Yan, T.-R.; Sheu, D.-C.; Chen, T.-M. *J. Appl. Polym. Sci.* **2006**, *100*, 803−809.
(16) Choi, H. S.; Rybkin, V. V.; Titov, V. A.; Shikova, T. G.; Ageeva, T. A. *Surf. Coat. Technol.* **2006**, *200*, 4479−4488.
(17) Fujimoto, K.; Takebayashi, Y.; Inoue, H.; Ikada, Y. *J. Polym. Sci., Part A: Polym. Chem.* **1993**, *31*, 1035−1043.
(18) Nie, H. Y.; Walzak, M. J.; Berno, B.; McIntyre, N. S. *Appl. Surf. Sci.* **1999**, *144*−*145*, 627−632.
(19) Toomey, R.; Freidank, D.; Ruehe, J. *Macromolecules* **2004**, *37*, 882−887.
(20) Turro, N. J. *Modern Molecular Photochemistry*; Benjamin/Cummings Pub. Co.: Menlo Park, CA, 1978.
(21) Matyjaszewski, K.; Xia, J. *Chem. Rev.* **2001**, *101*, 2921−2990.
(22) Matyjaszewski, K.; Davis, T. P. *Handbook of Radical Polymerization*; Wiley-Interscience: Hoboken, NJ, 2002.
(23) Matyjaszewski, K. *Prog. Polym. Sci.* **2005**, *30*, 858−875.
(24) Wang, J.-S.; Matyjaszewski, K. *J. Am. Chem. Soc.* **1995**, *117*, 5614−5615.
(25) Zhang, X.; Xia, J.; Matyjaszewski, K. *Macromolecules* **1998**, *31*, 5167−5169.
(26) Lee, S. B.; Russell, A. J.; Matyjaszewski, K. *Biomacromolecules* **2003**, *4*, 1386−1393.
(27) Pyun, J.; Kowalewski, T.; Matyjaszewski, K. *Macromol. Rapid Commun.* **2003**, *24*, 1043−1059.
(28) Matyjaszewski, K.; Miller, P. J.; Shukla, N.; Immaraporn, B.; Gelman, A.; Luokala, B. B.; Siclovan, T. M.; Kickelbick, G.; Vallant, T.; Hoffmann, H.; Pakula, T. *Macromolecules* **1999**, *32*, 8716−8724.
(29) Ejaz, M.; Tsujii, Y.; Fukuda, T. *Polymer* **2001**, *42*, 6811−6815.
(30) Husseman, M.; Malmstroem, E. E.; McNamara, M.; Mate, M.; Mecerreyes, D.; Benoit, D. G.; Hedrick, J. L.; Mansky, P.; Huang, E.; Russell, T. P.; Hawker, C. J. *Macromolecules* **1999**, *32*, 1424−1431.
(31) Isquith, A. J., A. E. A.; Walters, P. A. *Appl. Microbiol.* **1972**, *24*, 859−863.
(32) Kugler, R.; Bouloussa, O.; Rondelez, F. *Microbiology* **2005**, *151*, 1341−1348.
(33) Tiller, J. C.; Liao, C.-J.; Lewis, K.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5981−5985.

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