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Synthesis of phosphorylcholine–oligoethylene glycol–alkane thiols and their suppressive effect on non-specific adsorption of proteins

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ABSTRACT

A series of phosphorylcholine–oligoethylene glycol–alkane thiols were synthesized, and their suppressive effect on the non-specific adsorption of proteins was evaluated by comparison with corresponding oligoethylene glycol–alkane thiols. It was found that phosphorylcholine–oligoethylene glycol–alkane thiols had a greater suppressive effect on the non-specific adsorption of proteins.

The complete deciphering of the human genome has made it important to analyze various biomolecules to reveal the functions of genes. The non-specific adsorption of proteins that occurs during biomolecule detection often has a serious detrimental effect on selectivity and the detection limit. Therefore, research on surface modification materials has attracted great interest with a view to avoiding the interference caused by the non-specific adsorption of proteins. The properties of polyethylene glycol (PEG) such as flexibility, chemical stability, water solubility, and low cytotoxicity make it a versatile surface modification material.[1] The most attractive property of PEG is its suppressive effect on the non-specific adsorption of proteins. While polymers consisting of the ethylene glycol moiety have been studied extensively, it has also been reported that self-assembled monolayers (SAMs) of oligoethylene glycol–alkane thiols suppress the non-specific adsorption of proteins in a similar way to surfaces modified with PEG.[2] This finding led to the examination of various SAMs of various alkane thiol derivatives for use in surface modification to suppress the non-specific adsorption of proteins with the goal of developing bioanalytical devices.[3] PEG and oligoethylene glycols are recognized as versatile components for surface modification. In our previous work,[4] we showed that SAMs of oligoethylene glycol–alkane thiols effectively suppressed the non-specific adsorption of proteins allowing us to realize the high performance detection of proteins.

On the other hand, phosphorylcholine derivatives could be other candidates for suppressing the non-specific adsorption of proteins because the phosphorylcholine group is one of the primary lipid components of plasma membranes. After polymers consisting of the phosphorylcholine moiety were reported to reduce the non-specific adsorption of proteins,[5] various polymers bearing the phosphorylcholine moiety have been synthesized and their properties examined.[6] Although SAMs of phosphorylcholine-alkane thiols were also reported to suppress the non-specific adsorption of proteins[3f], there have been relatively few studies related to SAMs of phosphorylcholine-alkane thiols compared with those on SAMs of oligoethylene glycol–alkane thiols. Therefore, we are interested in the properties of phosphorylcholine SAMs, especially, SAMs of phosphorylcholine-alkane thiols bearing the oligoethylene glycol moiety, namely phosphorylcholine–oligoethylene glycol–alkane thiol SAMs, with the expectation of achieving a superior suppressive effect on the non-specific adsorption of proteins. In this Letter, we report preliminary results on the synthesis of phosphorylcholine–oligoethylene glycol–alkane thiols and the nature of those phosphorylcholine SAMs by comparison with SAMs of corresponding oligoethylene glycol–alkane thiols.

The synthesis route is summarized in Scheme 1. Similar to our previous work, the synthesis of oligoethylene glycols[7], the starting materials and the procedures were chosen to make the synthesis as straightforward as possible. Therefore, Bittman's procedure[8] was adopted for the introduction of phosphorylcholine group in order to avoid using trimethyl amine, which is problematic in terms of handling because it vaporizes causing a serious odor. On the other hand, the trityl group was chosen as a protecting group for the mercapto group, as it is reported that deprotection of the trityl group was carried out without the hydrolysis of the phosphoryl group.[9]

The mono-introduction of the oligoethylene glycol moiety into 1,12-dibromododecane was achieved by using potassium *t*-butoxide as a base in THF. Oligoethylene glycol mono-12-bromododecyl ether **11b–e** was purified with silica-gel column chromatography. 12-Bromo-1-dodecanol **11a** was commercially available. Bromide was converted to thiol with a one-pot procedure, the reaction of bromide with thiourea and subsequent hydrolysis using ethylene diamine. The obtained crude thiols **12a–e** were used for a subsequent reaction without purification. To protect the mercapto group, trityl chloride was reacted with **12a–e**. The selective reaction of trityl chloride with the mercapto group was accomplished by using an equimolar amount of trityl chloride in the presence of potassium carbonate in acetonitrile. The products **13a–e** were

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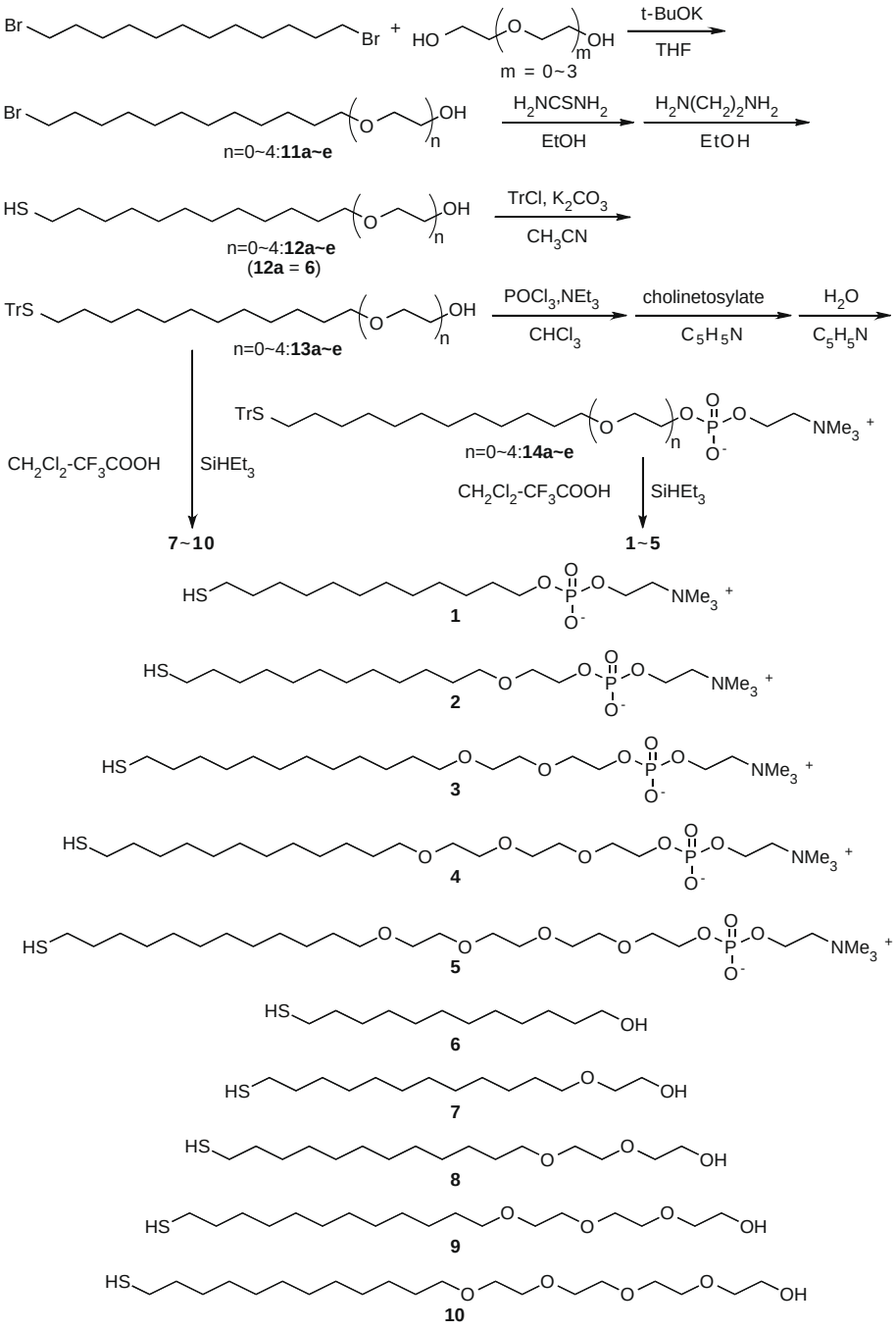

Scheme 1. Synthesis of phosphorylcholine–oligoethylene glycol–alkane thiols and corresponding oligoethylene glycol–alkane thiols.

purified with HPLC, gel permeation chromatography. The introduction of the phosphorylcholine moiety is the key step in this synthesis. The adopted Bittman's procedure is a one-pot introduction of the phosphorylcholine group which consists of three steps, namely the reaction of alcohols with phosphoryl chloride, the introduction of the choline moiety, and hydrolysis.[8] Based on the results of control experiments, pyridine was used as a solvent instead of chloroform, and the second step of the reaction was carried out at 0 °C. Purification was conducted with silica-gel column chromatography. In the final step, deprotection of the trityl group was achieved using triethyl silane in a mixture of dichloromethane and trifluoroacetic acid.[9] The products **1–5** were purified with silica-gel column chromatography. Oligoethylene glycol–alkane thiols **7–10** were obtained by deprotection of the trityl group for **13b–e** because the purification of **12** afforded only **6** with sufficient purity.

Gold surfaces were modified with **1–10**, and the nature of those surfaces was explored. First, a single crystal gold surface was modified with a water–ethanol solution of **1–10**, and then the molecular concentrations on the surfaces were determined with the electrochemical method.[10] The results are given in Table 1. As shown in Table 1, the molecular concentration of the surface modified with **6** was 7.2×10^{-10} mol/cm^2. As the theoretically calculated molecular concentration for alkane thiol SAMs is known to be 7.7×10^{-10} mol/cm^2, terminally hydroxylated alkane thiol **6** seems to form SAMs with a high density in a similar way. The introduction of oligoethylene glycol moieties at the terminals of alkane thiols reduced the molecular concentrations from 7.2×10^{-10} mol/cm^2 to 6.4×10^{-10} mol/cm^2 in the increase of the oligoethylene glycol chain length for **6–10**. The additional introduction of the phosphorylcholine moiety to oligoethylene glycol–alkane thi-

Table 1
Properties of SAMs of phosphorylcholine–oligoethylene glycol–alkane thiols **1–5** and oligoethylene glycol–alkane thiols **6–10**[a]

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	Bare gold
Surface concentration	5.2 ± 0.2	5.3 ± 0.4	5.3 ± 0.3	5.2 ± 0.4	5.1 ± 0.5	7.2 ± 0.2	7.1 ± 0.3	7.2 ± 0.4	7.0 ± 0.4	6.4 ± 0.8	—
Fibrinogen adsorption	0.91 ± 0.20	1.09 ± 0.12	0.82 ± 0.32	1.12 ± 0.53	0.97 ± 0.38	3.51 ± 0.39	2.09 ± 0.30	1.68 ± 0.06	2.40 ± 0.24	2.60 ± 0.45	20.6
Concanavalin A adsorption	0.60 ± 0.06	0.85 ± 0.03	0.25 ± 0.21	0.37 ± 0.07	0.12 ± 0.05	2.48 ± 0.31	0.92 ± 0.20	1.05 ± 0.32	0.78 ± 0.52	0.86 ± 0.09	26.8

[a] The units for surface concentrations and protein adsorption amounts are $\times 10^{-10}$ mol/cm^2 and ng/cm^2, respectively. The adsorption measurements were conducted in the presence of a surfactant, Tween 20.

ols resulted in a more significant reduction in the molecular concentrations being 5.1–5.3 $\times$ 10^{-10} mol/cm^2 for **1–5**. However, the molecular concentrations were almost the same regardless of the length of the oligoethylene glycol chain. These results suggest that alkane thiols **1**–**10** form SAMs on gold surfaces and the molecular concentrations of SAMs reflect the bulkiness of the moieties introduced into the terminals of the alkane thiols, where the phosphorylcholine moiety is bulkier than the oligoethylene glycol moiety.

There have been several reports on SAMs of a phosphorylcholine-alkane thiol, 11-mercaptoundecyl-phosphorylcholine.[11,12] According to the literature providing a detailed characterization of SAMs,[11] it has been concluded that with respect to the structure of phosphorylcholine-alkane thiols in SAMs the orientation of the phosphorylcholine head groups is nearly parallel to the gold surface. Therefore, the reported SAM thickness is only 13.7 Å, and the molecular concentration is thought to be less than half of that of 11-mercaptoundecanol. However, our results for the molecular concentrations of 12-mercaptododecylphosphorylcholine **1** and 12-mercapto-dodecanol **6** were 5.2 $\times$ 10^{-10} mol/cm^2 and 7.2 $\times$ 10^{-10} mol/cm^2, respectively, and the observed difference between their molecular concentrations was far smaller than that suggested in the literature. At this stage, it is not clear why the difference of only one methylene-chain-length in alkyl groups induces such a difference in the molecular concentration of SAMs as regards phosphorylcholine-alkane thiols.

To evaluate the suppressive function of the modified surfaces for the non-specific adsorption of proteins, we examined the amounts of fibrinogen and concanavalin A adsorbed on the modified surfaces using the BIACORE® system (T100, GE healthcare). Fibrinogen is a well-known blood coagulation factor and is used to evaluate antithrombogenic surfaces. The modified surfaces in this work consist of the phosphorylcholine moiety, which is reported to show antithrombogenicity.[13] In addition, concanavalin A is a commonly used protein in various studies. Therefore, these two proteins were used to evaluate the suppressive function of the modified surfaces for the non-specific adsorption of proteins. The adsorption amounts of both proteins are summarized in Table 1 together with the molecular concentrations. Compared with a bare gold surface, the adsorption amounts of both proteins were greatly reduced for all modified surfaces, even for a surface modified with terminally hydroxylated alkane thiol **6**. While the introduction of oligoethylene glycol moieties into alkane thiols did not enhance the suppressive effect on the adsorption amounts of fibrinogen, the adsorption amounts of concanavalin A were reduced by half, from 2.48 ng/cm^2 for **6** to 0.78–1.05 ng/cm^2 for **7**–**10** by the incorporation of the oligoethylene glycol moieties. In this case, the oligoethylene glycol chains exhibited no length dependence although chain-length dependence was reported for the SAMs of some oligoethylene glycol derivatives.[14] Surfaces modified with phosphorylcholine-alkane thiol **1** and phosphorylcholine–oligoethylene glycol–alkane thiols **2–5** suppressed fibrinogen adsorption more effectively, where the adsorption amounts were about half of those for **6–10**. This tendency supports the view that modification with SAMs of phosphorylcholine derivatives produces antithrombogenic surfaces possessing the potential for medical applications. Furthermore, similar to the cases of **6**–**10**, the incorporation of oligoethylene glycol moieties in phosphorylcholine-alkane thiol **1** did not enhance the suppressive effect on the adsorption of fibrinogen. In contrast, the adsorption amounts of concanavalin A were reduced from 0.60–0.85 ng/cm^2 for **1** and **2** to 0.12–0.37 ng/cm^2 for **3**–**5** again by the incorporation of oligoethylene glycol moieties. These results indicate that surfaces modified with SAMs consisting of phosphorylcholine moiety exhibit a more suppressive effect on fibrinogen adsorption than those with SAMs of oligoethylene glycol–alkane thiols, and the incorporation of oligoethylene glycol moieties in phosphorylcholine-alkane thiols does not enhance the suppressive effect on fibrinogen adsorption. By contrast, the incorporation of oligoethylene glycol moieties in alkane thiols enhances the suppressive effect on concanavalin A adsorption even in the presence of the phosphorylcholine moiety. In other words, it is implied that fibrinogen interacts with the phosphorylcholine moiety, while concanavalin A tends to interact with both the phosphorylcholine and oligoethylene glycol moieties.

The adsorption amounts of fibrinogen were reported to be 3–400 ng/cm^2 for SAMs of 11-mercaptoundecyl-phosphorylcholine.[11,12] These amounts are relatively high compared with our result of 0.91 ng/cm^2 in Table 1. This difference might be caused by the presence of a surfactant Tween 20® in the BIACORE® system, as the adsorption amount of fibrinogen on bare gold in our experiments was also very low at about 20 ng/cm^2 compared with the reported values of about 1000 ng/cm^2.[12] Surfaces modified with co-polymers consisting of phosphorylcholine and oligoethylene glycol moieties have also been examined.[15,16] It was reported that the co-polymers effectively reduced the adhesion of platelets to the modified surface[15], but the adsorption amounts of fibrinogen unexpectedly increased from about 50 ng/cm^2 to 250 ng/cm^2 as the oligoethylene glycol chain length increased from 0 to 9-mer.[16] In our results, oligoethylene glycol moieties had no influence on fibrinogen adsorption, however some effect derived from oligoethylene glycol moieties was observed with concanavalin A adsorption. These results imply that the influence of oligoethylene glycol moieties on the non-specific adsorption of proteins depends on not only the length of the oligoethylene glycol chains but also the nature of proteins, and might be reduced under packed conditions caused by SAM formation.

As a typical SAM surface feature, a surface modified with **3** was observed with an electrochemical scanning tunneling microscope (EC-STM) [17] as shown in Figure 1. In Figure 1a, the surface roughness was less than 0.3 nm indicating that the molecules were closely packed with some dark spots indicating dents. As shown in Figure 1b, in some areas a double-stripe shaped structure was also observed, which resulted from the molecular arrangements of the SAMs. It has been reported that these dark spots are dents on the SAMs derived from 2.4 Å deep etching pits on the gold surface and the formation of such etching pits during SAMs fabrication is known to be a typical phenomenon that occurs during gold surface modification with alkane thiols.[18] Furthermore, these etching pits are reportedly covered with SAMs similar to other areas.[18] It has also been suggested that such dents on the surface of SAMs could significantly influence the suppressive function for the non-specific adsorption of proteins.[19] However, no significant difference could

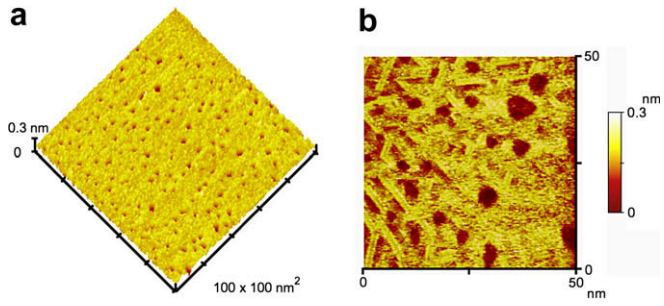

Figure 1. EC-STM images of SAM surface of **3** obtained in 0.1 M of aqueous perchloric acid solution at room temperature. (a) 100×100 nm^2. (b) 50×50 nm^2. Electrode potential and tunneling current were 0.7 V versus SCE and 0.6 nA, respectively.

be found in the numbers or shapes of the dents in our experiments. A detailed analysis of the relationship between the surface structure and the function suppressing the non-specific adsorption of proteins is now in progress.

In conclusion, it was found that phosphorylcholine–oligoethylene glycol–alkane thiols formed SAMs on gold surfaces and suppressed fibrinogen adsorption more effectively than the corresponding oligoethylene glycol–alkane thiols. The incorporation of oligoethylene glycol moieties in phosphorylcholine-alkane thiols enhanced the suppressive effect on concanavalin A adsorption but not on fibrinogen adsorption.

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Supplementary data

Supplementary data (synthesis procedures and evaluation for SAMs) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.04.091.

References and notes

1. *Poly(ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications*; Harris, J. M., Ed.; Plenum Press: New York, 1992.
2. (a) Prime, K. L.; Whitesides, G. M. *Science* **1991**, *252*, 1164–1167; (b) Pale-Grosdemagne, C.; Simon, E. S.; Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 12–20.
3. (a) Deng, L.; Mrksich, M.; Whitesides, G. M. *J. Am. Chem. Soc.* **1996**, *118*, 5136–5137; (b) Houseman, B. T.; Mrksich, M. *Angew. Chem., Int. Ed.* **1999**, *38*, 782–785; (c) Kane, R. S.; Deschatelets, P.; Whitesides, G. M. *Langmuir* **2003**, *19*, 2388–2391; (d) He, L.; Robertson, J. W. F.; Li, J.; Kärcher, I.; Schiller, S. M.; Knoll, W.; Naumann, R. *Langmuir* **2005**, *21*, 11666–11672; (e) Ostuni, E.; Chapman, R. G.; Holmlin, R. E.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605–5620; (f) Holmlin, R. E.; Chen, X.; Chapman, R. G.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 2841–2850; (g) Ostuni, E.; Chapman, R. G.; Liang, M. N.; Meluleni, G.; Pier, G.; Ingber, D. E.; Whitesides, G. M. *Langmuir* **2001**, *17*, 6336–6343; (h) Chapman, R. G.; Ostuni, E.; Takayama, S.; Holmlon, R. E.; Yan, L.; Whitesides, G. M. *J. Am. Chem. Soc.* **2000**, *122*, 8303–8304.
4. Sato, Y.; Yoshioka, K.; Tanaka, M.; Murakami, T.; Ishida, M. N.; Niwa, O. *Chem. Commun.* **2008**, 4909–4911.
5. (a) Ishihara, K.; Ueda, T.; Nakabayashi, N. *Polymer J.* **1990**, *22*, 355–360; (b) Ishihara, K.; Nomura, H.; Mihara, T.; Kurita, K.; Iwasaki, Y.; Nakabayashi, N. *J. Biomed. Mater. Res.* **1998**, *39*, 323–330.
6. Iwasaki, Y.; Ishihara, K. *Anal. Bioanal. Chem.* **2005**, *381*, 534–546.
7. Ahmed, S. A.; Tanaka, M. *J. Org. Chem.* **2006**, *71*, 9884–9886.
8. (a) Guivisdalsky, P. N.; Bittman, R. *J. Org. Chem.* **1989**, *54*, 4643–4648; (b) Rosenthal, A. F. *J. Lipid Res.* **1966**, *7*, 779–785.
9. Halter, M.; Nogata, Y.; Dannenberger, O.; Sasaki, T.; Vogel, V. *Langmuir* **2004**, *20*, 2416–2423.
10. Walczak, M. M.; Popenoe, D. D.; Deinhammer, R. S.; Lamp, B. D.; Chung, C.; Porter, M. D. *Langmuir* **1991**, *7*, 2687–2693.
11. (a) Chen, S.; Zheng, J.; Li, L.; Jiang, S. *J. Am. Chem. Soc.* **2005**, *127*, 14473–14478; (b) Chen, S.; Liu, L.; Jiang, S. *Langmuir* **2006**, *22*, 2418–2421; (c) Zhang, Z.; Zhang, M.; Chen, S.; Horbett, T. A.; Ratner, B. D.; Jiang, S. *Biomaterials* **2008**, *29*, 4285–4291.
12. (a) Tegoulia, V. A.; Rao, W.; Kalambur, A. T.; Rabolt, J. F.; Cooper, S. L. *Langmuir* **2001**, *17*, 4396–4404; (b) Chung, Y. C.; Chiu, Y. H.; Wu, Y. W.; Tao, Y. T. *Biomaterials* **2005**, *26*, 2313–2324.
13. Ishihara, K.; Aragaki, R.; Ueda, T.; Watenabe, A.; Nakabayashi, N. *J. Biomed. Mater. Res.* **1990**, *24*, 1069–1077.
14. Herrwerth, S.; Eck, W.; Reinhardt, S.; Grunze, M. *J. Am. Chem. Soc.* **2003**, *125*, 9359–9366.
15. Iwasaki, Y.; Yamasaki, A.; Ishihara, K. *Biomaterials* **2003**, *24*, 3599–3604.
16. Ishihara, K.; Fujiike, A.; Iwasaki, Y.; Kurita, K.; Nakabayashi, N. *J. Polym. Sci., Part A: Polym. Chem.* **1996**, *34*, 199–205.
17. Itaya, K. *Prog. Surf. Sci.* **1998**, *58*, 121–248.
18. Schönenberger, C.; Sondag-Huethorst, J. A. M.; Jorritsma, J.; Fokkink, L. G. J. *Langmuir* **1994**, *10*, 611–614.
19. Tegoulia, V. A.; Cooper, S. L. *J. Biomed. Mater. Res.* **2000**, *50*, 291–301.