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Taking Advantage of Tailored Electrostatics and Complementary Hydrogen Bonding in the Design of Nanostructures for Biomedical Applications

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Summary: Four-armed, star-shaped poly(D,L-lactide) (PDLLA) was synthesized and terminally-functionalized with either adenine or thymine complementary hydrogen bonding groups (PDLLA-A and PDLLA-T, respectively). The strong hydrogen bonding led to increased viscosity below the dissociation temperature of the hydrogen bonds. Rheology confirmed that these bonds were thermally reversible, with a sharp reduction in viscosity near 100 °C. PDLLA, PDLLA-A, and PDLLA-T were melt electrospun with no significant change in fiber diameter (all between 3.6 and 4.0 μm). However, a blend of PDLLA-A and PDLLA-T formed fibers with an average diameter of 9.8 ± 2.0 μm, resulting from the hydrogen bond associations. Also, the phospholipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE) was melt electrospun at 200 °C and formed uniform fibers with average fiber diameter of 6.5 ± 2.0 μm.

Keywords: hydrogen bonding; melt electrospinning; phospholipids; poly(D,L-lactide); thermoreversible

Introduction

Biology provides great inspiration for polymer scientists in terms of the exquisite structure and function of biological molecules. The hydrogen bond is ubiquitous in biological systems, particularly in the construction of proteins,[1] DNA[2] and RNA. In all cases, hydrogen bonding performs the role of establishing a reversible structure, which allows such processes as replication and transcription in DNA and a myriad of enzymatic reactions. Hydrogen bonding is central to many molecular recognition events in living organisms, notably the biotin-streptavidin interaction.[3] In DNA, hydrogen bonding performs in concert with numerous other non-covalent interactions such as electrostatic interactions and π-stacking to yield the double helix structure and to impart dynamic properties into DNA.[4] One goal of modern polymer science is to incorporate such functionality and dynamics into synthetic macromolecules.

In our laboratories, we have investigated the effects of regiospecific hydrogen bonding sites,[5] topology, and the use of hydrogen bonding to influence rheological and mechanical performance.[6] The strength of these interactions is a strong function of temperature, solvent, humidity and pH, thus allowing control of properties through a number of environmental parameters. The strength of hydrogen bonding associations is further tunable via structural parameters and molecular design of the hydrogen bonding sites. Nucleobases possess association strengths near 100 M^{-1},[7] which lead to highly dynamic interactions.

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Electrospinning provides a means to create tailored nanostructures from polymer solutions and melts.[8–11] The application of a large electric potential to polymeric fluid results in the deformation of the solution towards a grounded or oppositely charged electrode. For dilute solutions or melts of low molecular weight, the fluid will contract into droplets and deposit on the opposite electrode target (electrospraying). In the presence of sufficient entanglements, viscosity, or molecular weight, the fluid will elongate into a continuous filament and deposit on the target as a continuous, electrospun fiber.[12,13] In its simplest form, electrospinning generates a nonwoven mat of randomly oriented fibers with diameters on the order of 10 nm to 1 μm. Previous reports have demonstrated that electrospun mats can be successfully used in biomedical applications as drug-delivery scaffolds, cell growth scaffolds, and tissue and organ engineering scaffolds.[9,10,14] Our group has recently developed semi-empirical relationships relating solution rheology to electrospun fiber diameter for neutral, non-associating polymers.[12] Random copolymers with self-complementary multiple hydrogen bonding groups shwoed positive deviations from this relationship due to aggregation in solution.[15] Polyelectrolytes electrospun from water showed negative deviations from this relationship.[16] The addition of salt to the polyelectrolyte solutions was demonstrated to reduce this deviation.

We have previously reported the synthesis of a series of star-shaped poly(D,L-lactide) (PDLLA) polymers end-functionalized with adenine and thymine complementary base pairs.[17] These polymers demonstrated significant aggregation in solution based on complementary base-pair hydrogen bonding between the adenine and thymine groups. We report herein an investigation of the aggregation of these PDLLA stars in the melt state, as well as melt electrospinning to generate biocompatible and biodegradable fibers. We have also reported recently that the intermolecular attractions and aggregation of phospholipids in solution can be harnessed to electrospin amphiphilic fibers from asolectin.[18] The electrostatic (hydrophilic) and hydrophobic interactions of these low molar mass amphiphiles promote aggregation into cylindrical or wormlike micelles that entangle much like polymer solutions and can be electrospun. We demonstrate in this report that a well-defined phospholipid, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine, can also be electrospun from the melt state.

Experimental Part

Synthesis of PDLLA

Adenine- and thymine-terminated, four-arm star-shaped PDLLAs were synthesized as reported previously.[17] In short, acrylate terminated star-shaped polymers were derivatized with adenine and thymine using a Michael addition reaction. ^{1}H NMR spectroscopy confirmed the quantitative addition of terminal adenine and thymine end groups, and size exclusion chromatography (SEC) confirmed the absence of side reactions during functionalization.

Melt Electrospinning

The melt electrospinning apparatus consisted of a glass pipette wrapped in an electrical heating band and grounded. The positive lead of a high voltage power supply (Spellman CZE1000R, Spellman High Voltage Electronics Corp.) was connected to a piece of aluminum foil wrapped around the target and placed approximately 10 cm below the pipette. Polymer or phospholipid powder was placed in the pipette, and the pipette was purged with nitrogen gas to inhibit oxidative degradation and promote fluid flow. The pipette was heated above the melting temperature of the sample and flow began, the target was charged with 30 kV and electrospun fibers were collected.

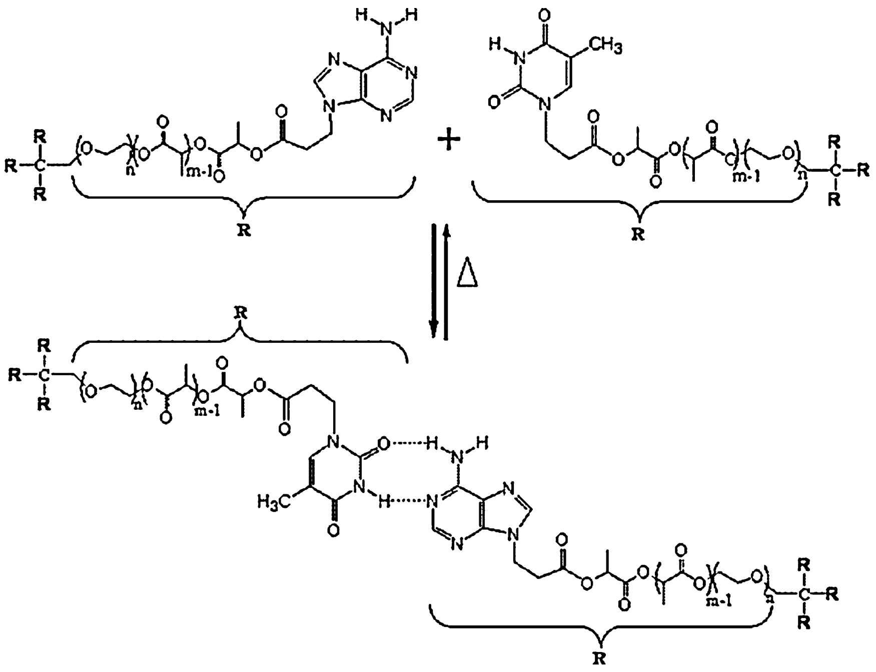
Characterization

Molar mass and molar mass distribution for PDLLA were determined by SEC using a Wyatt 717 Autosampler at 40 °C in THF. The SEC was equipped with a Waters 2410 refractive index detector, a Wyatt Technology MiniDAWN MALLS detector, and a Viscotek 270 viscosity detector. Melt rheological data were collected using a TA Instruments AR 1000 stress-controlled rheometer with a 25 mm parallel plate geometry. Strain amplitudes were limited to the linear viscoelastic regime over a frequency range of 0.1 to 100 Hz. The temperature was varied from 60 to 180 °C. The fiber diameter and morphology of melt electrospun fibers were analyzed using a LEO 1550 field emission scanning electron microscope (FESEM). Fibers for FESEM analysis were collected on a ¼" by ¼" stainless steel mesh, mounted on an SEM disc, and sputter-coated with a 10 nm Pt/Au layer to reduce electron charging. Thermogravimetric analysis (TGA) was performed under N_2 atmosphere at a heating rate of 10 °C/min using a TA Instruments Hi-Res TGA 2950.

Results and Discussion

PDLLA Synthesis and Characterization

The synthesis and characterization of the adenine- and thymine-functionalized, four-arm PDLLA (PDLLA-A and PDLLA-T, respectively) was described in our recent report.[17] In the previous report, a significant increase in the solution viscosity of a hydrogen-bonded complex consisting of a 1:1 wt:wt mixture of the PDLLA-A and PDLLA-T was observed. In addition, the thermoreversibility of the PDLLA adenine and thymine hydrogen-bonded complexes (PDLLA-(A-T)) in toluene was demonstrated. In this report, we focus on the melt properties of PDLLA-A and PDLLA-T polymers and their melt electrospinning behavior. The structures of PDLLA-A and PDLLA-T are illustrated in Scheme 1 below, along with an illustration of the PDLLA-(A-T) complex.



Scheme 1.
Schematic representation of PDLLA-A, PDLLA-T and PDLLA-(A-T) (1/1 w/w PDLLA-A/PDLLA-T).

TGA was performed on the A- and T-modified, star-shaped PDLLAs and their hydrogen-bonded complex ($M_n = 45600$ g/mol, $M_w/M_n = 1.37$) to determine weight loss as a function of temperature. The onset of weight loss for PDLLA is known to occur at temperatures above 200 °C.[19,20] Dynamic TGA experiments revealed that all PDLLA precursors and complexes did not exhibit appreciably weight loss below 200 °C. In fact, the onset of degradation occurred at 200 °C with a minimal weight loss of 0.7%. Isothermal degradation experiments were also performed at 180 °C for 1 h under nitrogen. The A- and T-terminated, star-shaped PDLLAs, and the hydrogen-bonded complex remained relatively stable under these conditions with approximately 5.5% weight loss observed for the blended samples.

Melt rheological characterization was performed on the four-arm, star-shaped PDLLA-(A-T) complex and a non-functionalized PDLLA of identical molar mass distribution. Figure 1 shows the complex viscosity (η^*) as a function of frequency (ω) at 70 and 120 °C (above and below the dissociation temperature of the adenine-thymine base pair). At 70 °C, different viscosity profiles were observed for the non-functionalized PDLLA and the PDLLA-(A-T) complex, even though both samples were of the same molar mass. The complex viscosity of the complex was consistently higher than that of the unfunctionalized PDLLA precursor, reflecting the formation of

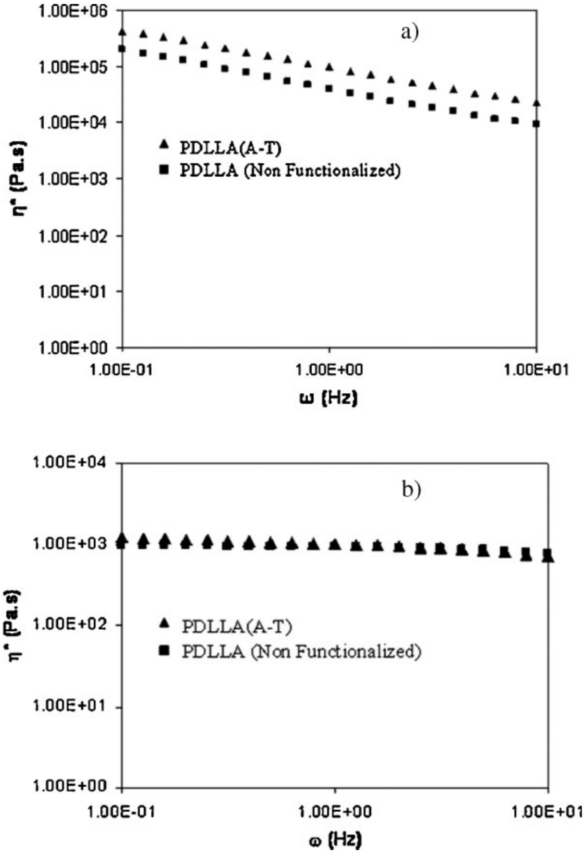


Figure 1.

Comparison of complex viscosity (η^*) master curves of PDLLA(A-T) hydrogen-bonded complex and PDLLA (non functionalized) at a)70 °C and b) 120 °C.

physical crosslinks through complementary hydrogen bonding. An increase in viscosity and an increase in apparent molar mass was observed at 70 °C. However, the two PDLLA samples exhibited similar η^* profiles at 120 °C, suggesting complete dissociation of the hydrogen-bonding interactions in the PDLLA-(A-T) complex. Furthermore, the PDLLA-(A-T) hydrogen-bonded complex displayed relatively higher η^* and a greater dependence of viscosity on temperature than the non-functionalized PDLLA precursor (Figure 2). The significant decrease in viscosity with temperature was consistent with dissociation of the hydrogen bonding interactions at higher temperatures and suggested the continuous disengagement of the associated hydrogen-bonded chains as the temperature was increased from 70 to 130 °C.

Melt electrospinning was used to prepare micron-sized fibers from the thermoreversible star-shaped PDLLA. The four-arm PDLLA, PDLLA-A, PDLLA-T, and PDLLA-(A-T) complex were melt electrospun at 180 °C and 30 kV with a working distance of 6 cm. The electrospinning temperature was chosen to allow complete dissociation of both the complementary hydrogen bonding interactions between the adenine and thymine units as well as any self-association of the nucleobases. As the polymer sample was heated above the dissociation temperature and the intensity of the electric field was increased, the polymer melt flowed and elongated from the tip of the pipette to form a conical shape known as the Taylor cone prior to jet initiation. As the discharged polymer jet traveled through the ambient air, the polymer cooled and reassociation of the adenine and thymine units was expected before fiber solidification.

Electrospun fibers with an average fiber diameter of 3.6 ± 1.0 μm were obtained from the non-functionalized PDLLA precursor (Figure 3a). Upon functionalization with adenine and thymine end-groups, the average fiber diameter remained statistically unchanged at 4.0 ± 0.6 μm and 4.4 ± 1.0 μm, respectively (Figures 3b and 3c). The slight increase in the electrospun fiber diameter after functionalization with the nucleobases suggested minimal self-association of the adenine and thymine groups. In addition, the slightly higher fiber diameter of the PDLLA-T relative to PDLLA-A was consistent with the stronger self-association of the thymine groups ($K_{a(A\text{-}A)} = 2.4\ M^{-1}$, $K_{a(T\text{-}T)} = 3.5\ M^{-1}$ in $CDCl_3$).[21,22] A significant increase of the average fiber diameter to 9.8 ± 2.0 μm was observed for the PDLLA-(A-T) hydrogen-bonded complex (Figure 3d). The higher

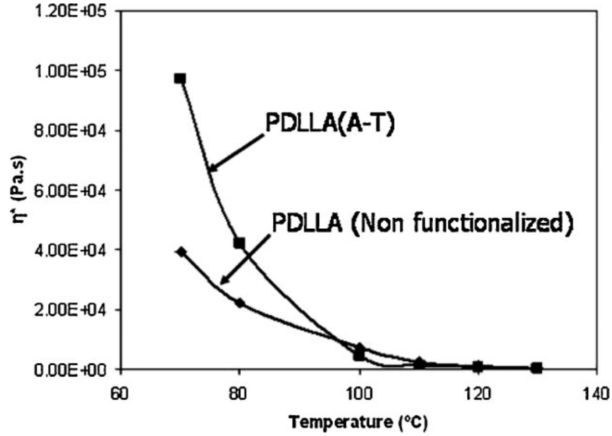


Figure 2.
Comparison of complex viscosity (η^*) of PDLLA(A-T) hydrogen-bonded complex and PDLLA (non functionalized) as a function of angular frequency at 70 °C.

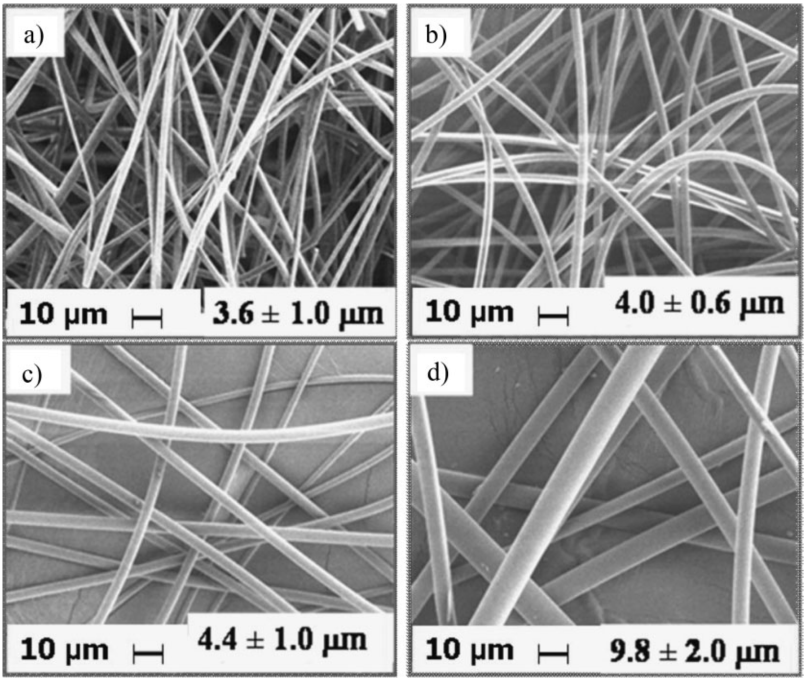


Figure 3.
FESEM images of electrospun fibers produced from four-arm, star-shaped, PDLLA (MW = 45600 g/mol, Mw/Mn = 1.37 a) non-functionalized PDLLA, b) PDLLA-A, c) PDLLA-T, d) PDLLA-(A-T) (1/1 w/w PDLLA-A/PDLLA-T).

average fiber diameter was consistent with the increased η^* of the complex relative to the non-functionalized PDLLA at an identical temperature. Furthermore, the formation of intermolecular hydrogen-bonding interactions between complementary adenine and thymine units in the electrospinning jet is proposed to occur based on melt rheological data. These results suggest that thermoreversibility, as well as the strength of the hydrogen-bonding interactions between the end groups of the tailored star PDLLA-based supramolecular polymers, control the fiber diameter in the melt electrospinning process.

Melt Electrospun Phospholipids

Amphiphilic molecules are known to aggregate into specific structures in solution and melt states based on hydrophobic and hydrophilic interactions. The shape of the structure is dominated primarily by the chemical structure of the amphiphile, and the most common aggregate is the spherical micelle. Certain molecules will form cylindrical aggregates or micelles under some conditions. These aggregates in solution have been studied in depth, and their dynamic and rheological properties are analogous to those of polymers. Recently, we demonstrated that reverse cylindrical micelles of phospholipid in solution can be electrospun.[18,23] These solutions demonstrated rheology similar to polymer solutions, and a critical concentration for entanglements was identified. Above this concentration, uniform fibers could be electrospun.

We have found that a well-defined phospholipid, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), forms long range aggregates in the melt state. When heated to 200 °C in the electrospinning apparatus, the POPE began to flow. The electrospinning jet solidified during flight and solid, amphiphilic fibers were deposited. FESEM images of the electrospun POPE, as well as the chemical structure, are shown in Figure 4. Using this electrospinning

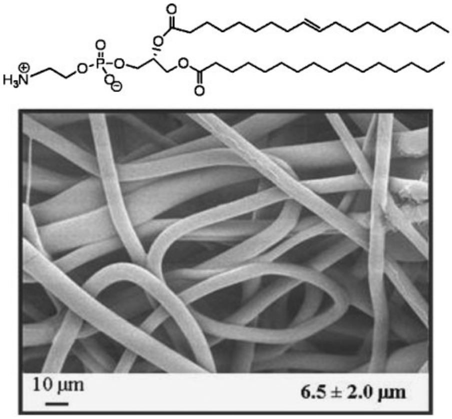


Figure 4.
Chemical structure and melt electrospun fibers of POPE phospholipid.

technique, fibrous scaffolds of biologically-derived phospholipid can be formed.

Conclusions

A new family of electrospun biodegradable membranes were prepared from thermoreversible, four-arm, star-shaped PDLLA with terminal complementary hydrogen bonding units or phospholipids. The influence of intermolecular hydrogen bonding on the melt electrospinning process was investigated. Adenine- and thymine-functionalized, star-shaped PDLLA were blended to obtain PDLLA-based supramolecular polymers through hydrogen bonding interactions. Linear viscoelastic measurements confirmed the formation of a hydrogen-bonded complex with significantly higher melt viscosity compared to the non-functionalized PDLLA precursor and a strong dependence of melt viscosity on temperature. Thermoreversibility of the adenine and thymine complexation controlled the fiber size in the melt electrospinning process. As a consequence, the star-shaped, PDLLA-based supramolecular polymers produced fibers with significantly larger diameters compared to the single component fibers due to the intermolecular hydrogen bonding of the PDLLA-A and PDLLA-T in the molten electrospinning jet. Melt electrospinning of low molar mass amphiphiles was also demonstrated for the first time. This technique can be applied to prepare a wide range of structured, biologically-compatible membranes for biomedical applications.

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[1] A. Aggeli, M. Bell, N. Boden, J. N. Keen, P. F. Knowles, T. C. B. McLeish, M. Pitkeathly, S. E. Radford, *Nature* **1997**, *386*, 259.

[2] D. Voet, J. G. Voet, "*Biochemistry*", John Wiley and Sons. New York: **1995**, p. 1361.

[3] P. C. Weber, D. H. Ohlendorf, J. J. Wendoloski, F. R. Salemme, *Science* **1989**, *243*, 85.

[4] J. Barciszewski, *Biotechnologia* **1993**, *3*, 138.

[5] B. D. Mather, J. R. Lizotte, T. E. Long, *Macromolecules* **2004**, *37*, 9331.

[6] K. Yamauchi, J. R. Lizotte, D. M. Hercules, M. J. Vergne, T. E. Long, *J. Am. Chem. Soc.* **2002**, *124*, 8599.

[7] Y. Kyogoku, R. C. Lord, A. Rich, *Proc. Natl. Acad. Sci. USA* **1967**, *57*, 250.

[8] D. H. Reneker, I. Chun, *Nanotechnology* **1996**, *7*, 216.

[9] R. Vasita, D. S. Katti, *Intl. J. Nanomedicine* **2006**, *1*, 15.

[10] W.-E. Teo, W. He, S. Ramakrishna, *Biotechnol. J.* **2006**, *1*, 918.

[11] D. Li, Y. Xia, *Adv. Mater.* **2004**, *16*, 1151.

[12] M. G. McKee, G. L. Wilkes, R. H. Colby, T. E. Long, *Macromolecules* **2004**, *37*, 1760.

[13] S. L. Shenoy, W. D. Bates, H. L. Frisch, G. E. Wnek, *Polymer* **2005**, *46*, 3372.

[14] J. Venugopal, S. Ramakrishna, *Appl. Biochem. Biotechnol.* **2005**, *125*, 147.

[15] M. G. McKee, C. L. Elkins, T. E. Long, *Polymer* **2004**, *45*, 8705.

[16] M. G. McKee, M. T. Hunley, J. M. Layman, T. E. Long, *Macromolecules* **2006**, *39*, 575.

[17] A. S. Karikari, T. E. Long, *Biomacromolecules* **2007**, *8*, 302.

[18] M. G. McKee, J. M. Layman, M. P. Cashion, T. E. Long, *Science* **2006**, *311*, 353.

[19] C. C. Chien, Y. C. Ju, H. Tseng, M. H. Haw, Y. L. Sheng, *Biomaterials* **2003**, *24*, 1167.

[20] H. Abe, N. Takahashi, J. K. Kang, M. Mochizuki, Y. Doi, *Biomacromolecules* **2004**, *5*, 1606.

[21] J. Sartorius, H.-J. Schneider, *Chem. Eur. J.* **1996**, *2*, 1446.

[22] S. Sivakova, D. A. Bohnsack, M. E. Mackay, P. Suwanmala, S. J. Rowan, *J. Am. Chem. Soc.* **2005**, *127*, 18202.

[23] M. T. Hunley, M. G. McKee, T. E. Long, *J. Mater. Chem.* **2007**, *17*, 605.