Atomic Layer Deposition of L-Alanine Polypeptide

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Ⓢ *Supporting Information*

ABSTRACT: L-Alanine polypeptide thin films were synthesized via atomic layer deposition (ALD). Instead of using an amino acid monomer as the precursor, an L-alanine amino acid derivatized with a protecting group was used to prevent self-polymerization, increase the vapor pressure, and allow linear cycle-by-cycle growth emblematic of ALD. The successful deposition of a conformal polypeptide film has been confirmed by FTIR, TEM, and Mass Spectrometry, and the ALD process has been extended to polyvaline.

Atomic Layer Deposition (ALD) is a burgeoning technology that has enabled the precision 'one-atomic-layer by one-atomic-layer' fabrication of thin films and free-standing materials. To date, a wide range of materials have been prepared by ALD including oxides, nitrides, sulfides, and pure noble metals.[1,2] In addition to inorganic materials, ALD of organic, polymeric materials has been reported recently by George et al. and Yoshimura et al.[3−6] In our previous work, we described ALD of hybrid organic−inorganic polysilsesquioxane thin films[7] and their conversion to high flux, high selectivity microporous membranes by subsequent removal of bridging organic groups from the organosilicate framework. Overall there is a continuing need to extend ALD to new materials classes and structures with tailored properties. In this regard the fabrication of biologically inspired or biomimetic materials has gained increasing attention for applications in areas such as medicine, environmental monitoring and remediation, energy conversion, and high flux membranes mimicking natural ion channels.[8] Thus, demonstrating the feasibility of using ALD to fabricate biomimetic materials is of immediate interest.[9] Here we report the ALD of L-alanine polypeptide thin films.

Polypeptides are polymers composed of amino acid subunits. Various polypeptides have been synthesized by conventional liquid-phase or solid-phase processes, which can be time and labor intensive[10,11] and, in particular for hydrophobic peptides such as L-alanine, not generally suitable for depositing conformal thin films. ALD is a vapor-phase deposition process. Because vapor phase species have higher diffusivities, molecular transport limitations are greatly reduced compared to solid or liquid phase synthesis procedures. Also, since ALD precisely builds up materials layer-by-layer in successive self-limiting reaction cycles, it can be performed automatically with each cycle requiring only seconds. Thus, it should be possible in principle to build a long chain of polypeptide with a designed chain structure. However, ALD of a polypeptide is very challenging in practice due to two related issues: (1) The building blocks/precursors for polypeptides are amino-acids, whose vapor pressures are extremely low due to inherent hydrogen bonding interactions between amine groups and carboxyl groups in their molecular structure, therefore requiring heat to achieve a sufficient vapor pressure for ALD. (2) Since each amino acid molecule contains both an amine group and a carboxyl group, spontaneous self-polymerization will occur when amino acids are heated in the precursor bottle or in the ALD chamber before reaching the sample surface. Self-polymerization precludes both the attainment of a sufficient vapor pressure and the ability to achieve precise layer-by-layer deposition.

To resolve the low vapor pressure and self-polymerization issues, N-(*tert*-Butoxycarbonyl)-L-alanine (Boc-L-alanine) was used as the ALD precursor in our experiments, instead of a regular amino acid monomer. In Boc-L-alanine, the amine groups are "protected" by Boc groups that prevent the amine groups from reacting with carboxyl groups, thereby avoiding the problem of "self-polymerization". Moreover, compared to other common protecting groups, such as carbobenzyloxy (Cbz), benzoyl (Bz), fluorenylmethyloxycarbonyl (FMoc), etc., the Boc group comprises three methyl ligands, which is advantageous in improving the molecular vapor pressure due to the weak bonding between methyl groups and other molecular constituents. The vapor pressure of the L-alanine amino acid is $\sim 1.1 \times 10^{-7}$ Torr at 25 °C, while that of Boc-L-alanine increased by 500 times to 6.3×10^{-5} Torr at 25 °C. The melting point also drops from 314 °C for L-alanine to 84 °C for Boc-protected L-alanine, suggesting that the vapor pressure of Boc-protected L-alanine can be increased further upon heating above 25 °C.

Having identified an appropriate precursor, the second step was to design an ALD reaction cycle. Although the introduction of the Boc group increases the vapor pressure and avoids self-polymerization, the protective Boc group must be removed after achieving one layer of chemisorbed Boc-L-alanine, to expose activated amine groups for the subsequent deposition cycle(s). In conventional solution-phase polypeptide synthesis, strong acids such as trifluoroacetate (TFA) and HCl are usually used to remove Boc groups. However, ALD takes place within a vacuum chamber. The strong acids may corrode the ALD vacuum system and create hazardous byproducts. In our approach, phosphoric acid (H_3PO_4) was used to remove Boc and deprotect the amine groups.[12] H_3PO_4 is a mild acid and considered safe at low concentration. In the conventional liquid phase synthesis of polypeptides, H_3PO_4 is believed to be ineffective in removing Boc groups due to its weak acidity. But in ALD, where the reaction is carried out at elevated temperatures, such as 100−200 °C or higher, the weak acidity of phosphoric acid can be compensated for by the exponentially increased reaction rate at elevated temperatures. For these reasons, Boc removal by H_3PO_4 has been adopted as the deprotection strategy in our ALD reaction design.

Figure 1 shows a schematic of the L-alanine ALD reaction cycle. ALD was carried out in an *Angstrom-dep* dual-chamber
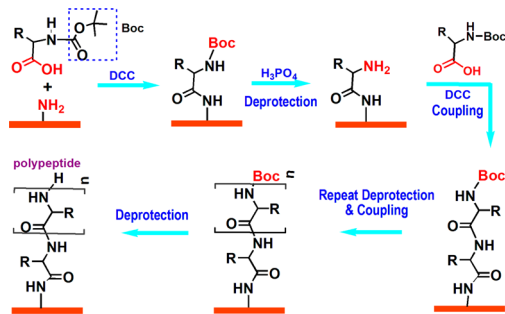

Figure 1. Schematic L-alanine polypeptide ALD process, R = CH_3.

ALD system with an agitated powder ALD chamber. The deposition chamber was a 250 mL Pyrex container. The base vacuum was ~10 mTorr. Ar was used as the carrier gas as well as the purging gas. Both self-assembled mesoporous silica nanoparticles and films prepared by evaporation induced self-assembly[13,14] were used as the substrates for ALD. Silica nanoparticles were used because it is easy to observe the thickness of the ALD coating by transmission electron microscopy (TEM). Mesoporous silica was used because its surface displays reactive silanol groups needed for subsequent ALD reactions, and its high surface area and porosity allow FTIR detection of monolayer or multilayer deposition after initial cycles of ALD and facile dissolution of the silica to remove the film for mass spectrometry or other analysis. Before ALD, the silica particle or thin film substrates were pretreated with 3-aminopropyltrimethoxysilane (APTMS) needed to form a reactive amine monolayer to initiate subsequent steps of peptide polymerization (see Supporting Information for further experimental details).

As mentioned above, Boc-L-alanine was used as the ALD precursor and maintained at 110 °C. Phosphoric acid was used as the deprotection agent and heated to 70 °C. Additionally, *N,N*-dicyclohexylcarbodiimide (DCC) was used as a coupling agent and heated to 100 °C. The ALD temperature was maintained at 130 °C. ALD was performed according to the following steps:

(1) introduce Boc-L-alanine vapor to the chamber; (2) inject DCC vapor to trigger the coupling reaction between carboxyl groups in Boc-L-alanine and -NH_2 groups on sample surface, forming chemisorbed Boc-L-alanine; (3) purge the ALD chamber with Ar flow to remove residual Boc-L-alanine, DCC, and byproducts, leaving only a monolayer of chemisorbed Boc-L-alanine on the sample surface; (4) introduce phosphoric acid vapor to strip off Boc protective groups from the chemisorbed Boc-L-alanine, exposing −NH_2 groups; (5) purge the chamber with Ar to remove residual phosphoric acid and byproducts, generating a new −NH_2 terminated surface that is ready for another layer of chemisorption; (6) repeat the coupling and deprotection processes (steps 1−5) to obtain the desired polypeptide film thickness. The ALD parameters used for each of these steps are specified in section III of the Supporting Information (SI) where we also present 'simplified saturation curves' used to establish the reaction and purge times (30 s) needed to achieve self-limiting ALD conditions.

The formation of a polypeptide was verified by ATR-FTIR. Figure 2 shows the FTIR spectra of (a) the original mesoporous
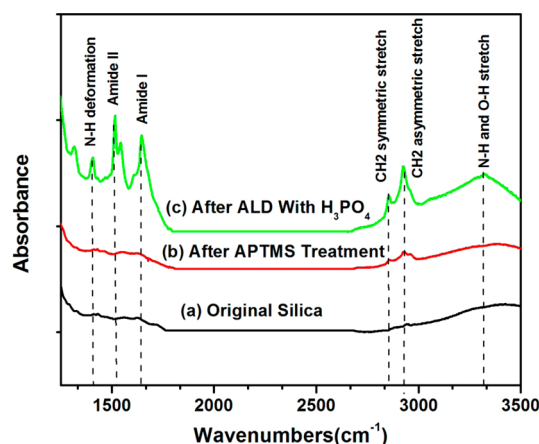

Figure 2. ATR-FTIR of (a) original nanoporous silica; (b) nanoporous silica after APTMS treatment; and (c) nanoporous silica after subsequent 60 cycles of ALD deposition using Boc-L-alanine precursor.

silica sample; (b) mesoporous silica after APTMS treatment to form an amine monolayer or multilayer; and (c) mesoporous silica after APTMS treatment and 60 cycles of ALD. In all spectra, the background between 1800 and 2600 cm^{-1} has been subtracted. For the sample after APTMS treatment (spectrum b), there are two small absorptions at 2854 and 2925 cm^{-1}, attributed to symmetric and asymmetric C−H stretching vibrations of the −CH_2−CH_2−NH_2 ligand of the propyl amine, suggesting that the sample surface has been successfully modified with −NH_2 groups (absorption by −NH_2 groups is not distinctive in this spectrum, consistent with reports by other researchers).[6] For the sample after polypeptide ALD (spectrum c), three more absorption bands appear at ~1400, 1647, and 1516 cm^{-1}. The absorption at 1400 cm^{-1} can be assigned to the N−H deformation mode.[5] The absorptions at 1647 and 1516 cm^{-1} are assigned to amide I and II bands, respectively,[15] typical of peptide bonds. The amide I absorption is governed by the stretching vibrations of the C=O (70−85%) and C−N groups (10−20%).[16] The amide II absorption arises primarily from in-plane bending vibrations of the N−H bond in the amide structure. We note that the original Boc-L-alanine precursor exhibits similar amide I and II absorptions in IR (see Figure S2 in

the SI), but the position and shapes of these bands, in particular the amide I band, are very different. This reflects that the amide I vibration is very sensitive to the conformation and hydrogen bonding of the polypeptide backbone, which exists for the ALD sample but not the monomer. For the ALD sample we also observe increased absorptions of the C−H stretching vibrations at 2854 and 2925 cm^{-1} (spectrum c), consistent with the proposed polypeptide structure, e.g., Figure 1, where R = CH$_3$.

The ALD of a polypeptide film was further confirmed by TEM analysis, using the samples prepared on silica nanoparticles in the same ALD batches as for films. Figure 3a is the TEM image of the
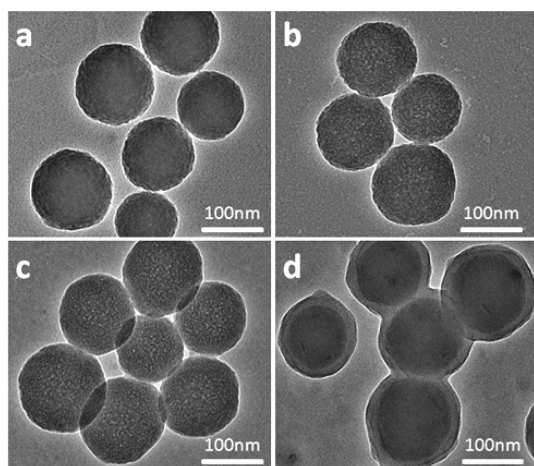

Figure 3. TEM image of (a) original silica nanoparticles; (b) particles after modification with APTMS; (c) particles in (b) after subsequent 60-cycle polypeptide ALD without H$_3$PO$_4$; and (d) particles in (b) after subsequent 60-cycle polypeptide ALD with H$_3$PO$_4$.

original silica nanoparticle, where the particle surface is free of any coating except for some features related to surface roughness. Figure 3b is the TEM image of the silica nanoparticles after APTMS treatment. Here no substantial changes can be observed for the nanoparticles, in agreement with our expectation that five cycles of APTMS/H$_2$O modification (see SI) should result in a monolayer or ultrathin multilayer unresolvable by TEM. Figure 3d is the TEM image of silica particles after 60 ALD cycles. In this image, a 12 nm thick coating can be observed on the surface of the silica nanoparticles. The coating is uniform and conformal to the silica surface, consistent with the typical morphology of ALD-prepared coatings.

After confirming the formation of a polypeptide film, it was important to confirm that the deposition of polypeptide was achieved in a layer-by-layer manner as proposed in Figure 1, where the deposition of each new layer relies on the removal of the Boc protecting group by H$_3$PO$_4$. Cava et al. reported that the Boc group attached to some amino acids may not be thermally stable and that "nonspecific" deprotection may take place at elevated temperatures.[17] If this occurs to the Boc-L-alanine precursor, the deposition of a coating would occur even in the absence of the deprotectant H$_3$PO$_4$ via uncontrolled spontaneous self-polymerization as opposed to controlled layer-by-layer ALD. To help confirm our proposed synthetic scheme (Figure 1) we conducted an additional ALD experiment where we performed the exact same sequence of exposure and purge cycles but with the deprotectant H$_3$PO$_4$ valve closed. Figure 3c shows a TEM image of the silica nanoparticles after being treated with APTMS and subsequent ALD without H$_3$PO$_4$. Here, the silica nanoparticles appear identical to those in Figure 3b,

suggesting that, although the Boc-L-alanine and DCC reactants were introduced into the deposition chamber at the same temperature, ALD was completely suppressed. This observation establishes that the Boc groups are thermally stable in the Boc-L-alanine precursor and that Boc deprotection by H$_3$PO$_4$ is necessary for activation of amine groups after each ALD cycle, thereby enabling controlled layer-by-layer ALD.

To further support the idea that polyalanine deposition is occurring by ALD and to quantify the growth rate, we monitored the film thickness with TEM. Figure 4a shows representative
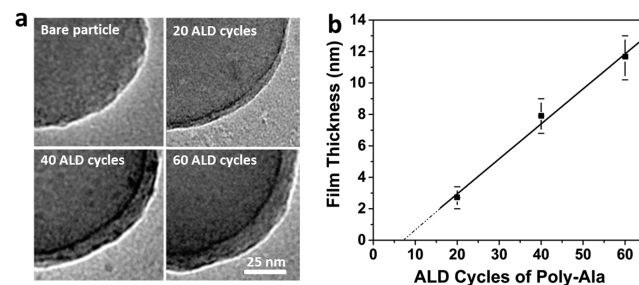

Figure 4. (a) TEM images of polyalanine films after different numbers of ALD cycles; (b) plot of the film thickness vs the ALD cycle number.

TEM images of poly-L-alanine films deposited on mesoporous silica particles, and Figure 4b shows the relationship between the thickness of the deposited layer and the number of ALD cycles based on measurements of 100 particles for each data point. For 20−60 cycles, the increase of thickness of the poly-Ala layer is nearly linear with cycle number (the slope of the plot is 0.23 nm/layer, and R^2 is 0.9). As the dimension of the poly-Ala repeat unit is ∼0.38 nm, according to bond length, the growth rate corresponds approximately to 0.5 monolayer/cycle, consistent with the MS results (see following discussion). We attribute the apparent zero growth rate at cycle numbers <8 to ALD deposition within the surface accessible pores of the silica nanoparticles.

To further confirm polypeptide ALD, we performed mass spectrometry (MS) of the films after successive cycles of L-alanine ALD. Films were deposited on mesoporous silica thin films using identical conditions as for particles. After 10, 20, and 40 cycles of ALD, we dissolved the mesoporous silica support using HF and isolated and purified the disjoined polypeptide layers (see SI for experimental details). The time-of-flight MS results presented in supplementary Figure S-3 show discrete M/Z+ envelopes whose mass increases linearly with deposition cycle number, a hallmark of ALD, along with an envelope of low MW fragments typical of polypeptide mass spectra (see Figure S-3C). Analysis of the mass spectra indicates that under the ALD conditions used the average deposition rate is ∼0.5 L-alanine monolayers per cycle consistent with TEM (see Figure 4). Specifically, molecular masses corresponding to polypeptides with 4 and 5 amino acid repeats terminated with an aminopropyl group were observed for the 10-ALD-cycle sample; molecular masses corresponding to polypeptides with 9 and 10 amino acid repeats terminated with an aminopropyl group were observed for the 20-ALD-cycle sample, and molecular masses corresponding to polypeptides with 19−20 amino acid repeats terminated with an aminopropyl group were observed for the 40-ALD-cycle sample. Less than one atomic or molecular layer per cycle is in fact typical of many ALD processes.[4,5,18,19] As to the specific mechanism that governs the ∼0.5 monolayer per cycle deposition, it could result from the efficiency of either the

coupling or deprotection steps, as insufficient adsorption or deprotection could both reduce the deposition rate. Alternatively or additionally, the bulky BOC ligand, beyond serving as a chemical protecting group, could occlude/sterically inhibit accessibility to some of the reactive amine groups of the underlying layer in one cycle and, after deprotection, these sites become accessible in the subsequent cycle.

Despite the somewhat slow rate of polypeptide ALD, we find the surface grafting efficiency to be quite high. To determine the grafting density, we analyzed the ATR-FTIR reflectivity data (see Figure 2 and expanded Figure S-4 in SI) of a 12 nm thick polypeptide film formed by 60 cycles of ALD, applying the Beer–Lambert law and assuming the reported molar absorptivity value of 320 mol^{-1} cm^{-1} for the amide I band. For the reflection geometry depicted in Figure S-4A and a path length of 2×12 nm $\times \sqrt{2}$, we calculate an amide I concentration of 10.1 M, which, assuming 30/amide bonds per polypeptide molecule for 60 cycles of ALD (see MS discussion above), corresponds to a surface concentration of polypeptide chains of 2.3 peptide chains/nm^2 and ∼80% grafting efficiency of the propylamine surface groups.

In summary, the strategy of using protected amino acid precursors for polypeptide ALD has been demonstrated to be feasible. The use of a protecting ligand can prevent an amino acid derived precursor from self-association through noncovalent interactions and self-polymerization. This allows achievement of a sufficient vapor pressure and enables layer-by-layer ALD-type deposition. In addition, the proper selection of protecting ligand may improve the precursor's vapor pressure, making it more favorable for performing ALD. The protecting ligand may be thermally unstable, and its thermal stability determines the maximum ALD temperature that can be used. In additional experiments (see SI Figure S-5) we have found that performing ALD at or above 150 °C is not feasible due to the decomposition of the Boc ligand and subsequent self-polymerization of the amino acid. Weaker acid deprotectants such as H$_3$PO$_4$ that are not effective in liquid-phase synthesis can be effective for ALD synthesis because of higher temperatures employed in this vapor phase process. This strategy makes it possible to precisely construct polypeptides via the layer-by-layer ALD process. Compared to conventional liquid-phase synthesis, ALD is an alternative approach that could be in general faster and less labor intensive. More importantly as shown here for L-analine it provides a means for depositing a uniform conformal film from insoluble peptides. Potentially, depending on the development of appropriate precursor chemistries and protection/deprotection strategies, ALD could form arbitrary polypeptide materials with structures and properties different from those obtainable from conventional solution phase approaches. Figure S-7 and the surrounding discussion demonstrate extension of this ALD approach to polyvaline.

ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details, characterization details. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ REFERENCES

(1) King, D. M.; Liang, X.; Weimer, A. W. *Powder Technol.* **2012**, *221*, 13−25.
(2) Leskela, M.; Ritala, M. *Thin Solid Films* **2002**, *409*, 138−146.
(3) Yoshimura, T.; Tatsuura, S.; Sotoyama, W. *Appl. Phys. Lett.* **1991**, *59*, 482−484.
(4) Du, Y.; George, S. M. *J. Phys. Chem. C* **2007**, *111*, 8509−8517.
(5) Adamczyk, N. M.; Dameron, A. A.; George, S. M. *Langmuir* **2008**, *24*, 2081−2089.
(6) Ek, S.; Iiskola, E. I.; Niinisto, L. *J. Phys. Chem. B* **2004**, *108*, 9650−9655.
(7) Jiang, Y. B.; Xomeritakis, G.; Chen, Z.; Dunphy, D. J.; Kissel, D.; Cecchi, J. L.; Brinker, C. J. *J. Am. Chem. Soc.* **2007**, *129*, 15446−15447.
(8) Lee, S.; Xie, J.; Chen, X. *Chem. Rev.* **2010**, *110*, 3087−3111.
(9) Hadjichristidis, N.; Latrou, H.; Pitsikalis, M.; Sakellariou, G. *Chem. Rev.* **2009**, *109*, 5528−5578.
(10) Han, S. Y.; Kim, Y. A. *Tetrahedron* **2004**, *60*, 2447−2467.
(11) Venkatesan, N.; Kim, B. H. *Chem. Rev.* **2006**, *106*, 3712−3761.
(12) Li, B.; Berliner, M.; Buzon, R.; Chiu, C. K. F.; Colgan, S. T.; et al. *J. Org. Chem.* **2006**, *71*, 9045−9050.
(13) Jiang, X. M.; Bao, L.; Cheng, Y. S.; Dunphy, D. R.; Li, X.; Brinker, C. J. *Chem. Commun.* **2012**, *48*, 1293−1295.
(14) Brinker, C. J.; Lu, Y. F.; Sellinger, A.; Fan, H. Y. *Adv. Mater.* **1999**, *11*, 579−585.
(15) Miyazawa, T.; Shimanouchi, T.; Mizushima, S. I. *J. Chem. Phys.* **1956**, *24*, 408−418.
(16) Surewicz, W. K.; Mantsch, H. H.; Chapman, D. *Biochemistry* **1993**, *32*, 389−394.
(17) Rawal, V. H.; Jones, R. J.; Cava, M. P. *J. Org. Chem.* **1987**, *52*, 19−28.
(18) Won, S. J.; Kim, J. Y.; Choi, G. J.; Heo, J.; Hwang, C. S.; Kim, H. J. *Chem. Mater.* **2009**, *21*, 4374−4379.
(19) George, S. M. *Chem. Rev.* **2010**, *110*, 111−131.