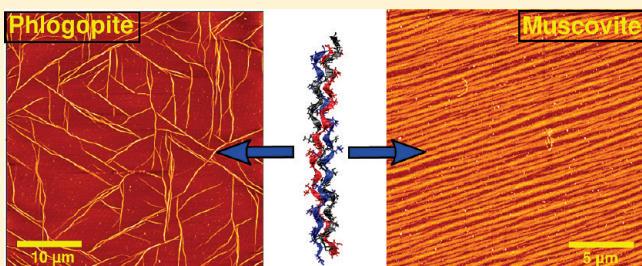


Epitaxially Guided Assembly of Collagen Layers on Mica Surfaces

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ABSTRACT: Ordered assembly of collagen molecules on flat substrates has potential for various applications and serves as a model system for studying the assembly process. While previous studies demonstrated self-assembly of collagen on muscovite mica into highly ordered layers, the mechanism by which different conditions affect the resulting morphology remains to be elucidated. Using atomic force microscopy, we follow the assembly of collagen on muscovite mica at a concentration lower than the critical fibrillogenesis concentration in bulk. Initially, individual collagen molecules adsorb to mica and subsequently nucleate into fibrils possessing the 67 nm D-periodic bands. Emergence of fibrils aligned in parallel despite large interfibril distances agrees with an alignment mechanism guided by the underlying mica. The epitaxial growth was further confirmed by the formation of novel triangular networks of collagen fibrils on phlogopite mica, whose surface lattice is known to have a hexagonal symmetry, whereas the more widely used muscovite does not. Comparing collagen assembly on the two types of mica at different potassium concentrations revealed that potassium binds to the negatively charged mica surface and neutralizes it, thereby reducing the binding affinity of collagen and enhancing surface diffusion. These results suggest that collagen assembly on mica follows the surface adsorption, diffusion, nucleation, and growth pathway, where the growth direction is determined at the nucleation step. Comparison with other molecules that assemble similarly on mica supports generality of the proposed assembly mechanism, the knowledge of which will be useful for controlling the resulting surface morphologies.



■ INTRODUCTION

Fibrillar collagens are extremely versatile tissue scaffolds, and they assemble in a hierarchical manner to form ordered arrays in extracellular matrices such as cornea, tendon, bone, and cartilage.^{1–5} Collagens can also self-assemble in vitro, and the resulting matrices are used as two- and three-dimensional scaffolds for cells,^{6,7} to coat nonbiological surfaces for enhanced biocompatibility,⁸ for functionalized surface patterning,^{9,10} and even in microelectronics applications as a template for generating silicon nanowires.¹¹ Better understanding and control of the self-assembly process of collagen thus has both fundamental and practical importance.

In the past two decades, atomic force microscopy (AFM) provided much information about self-assembly of collagen molecules on flat substrates.^{12–15} On muscovite mica, which is the most widely used substrate for AFM, collagens show a variety of assembly morphologies depending on the pH and electrolyte composition of the buffer.^{16,17} Under certain conditions, a unidirectionally aligned layer of collagen forms with the D-periodic band^{16,18} (cf. Figure 1). Hereafter, we refer to a D-periodic band simply as a D-period. Formation of a D-period indicates collagen molecules in a fibril are ordered in a native-like manner.¹⁹ The unidirectional alignment was initially attributed to hydrodynamic flow induced when the solution of collagen was being deposited on mica,¹⁶ but another study suggested that collagen alignment on mica is quasi-epitaxial, induced by the crystallographic orientation of the mica surface.²⁰ However, the

alignment is not always unidirectional, and potassium (K^+) ion is known to have an influence. A checkerboard-like pattern instead of parallel alignment has been obtained with 150–300 mM KCl and at pH 4.3²¹ or pH 8.5.¹⁵ It has been speculated that K^+ may interact with collagens in a specific manner that affects the alignment.²¹ Potassium is also thought to be important for the D-period formation,¹⁵ although this does not appear to be the case for the assembly in bulk solution.²²

To understand the conditions leading to various morphologies of collagen layers on mica, a unifying picture for the self-assembly process is necessary. In the present study, we use AFM to capture stages where individual collagen molecules adsorb on the mica surface and subsequently nucleate into fibrils aligned in parallel. As the initial fibrils are sufficiently separated, the alignment is more likely to be guided by the underlying mica lattice rather than by interaction between the nucleating fibrils, which supports the quasi-epitaxial growth mechanism.²⁰ We then compare the assembly of collagen on muscovite mica with that on a less widely used phlogopite mica. Phlogopite is known to preserve the surface hexagonal symmetry, whereas muscovite does not.^{23,24} On phlogopite, collagens assembled into a novel triangular network where individual fibrils possess D-periods. By comparing changes in the assembly morphology on both types of

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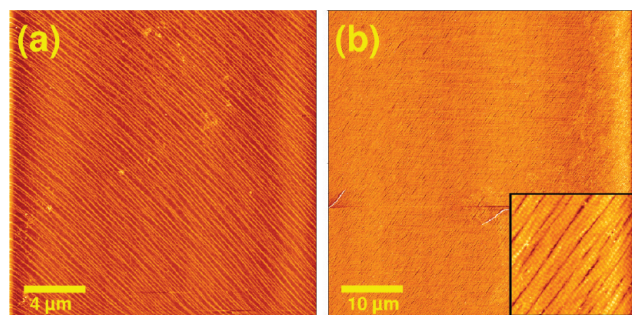


Figure 1. Highly aligned collagen fibrils on muscovite mica. [KCl] = 200 mM. The concentration of collagen and incubation time on mica are (a) 10 $\mu\text{g/mL}$ collagen and 1 min of incubation and (b) 50 $\mu\text{g/mL}$ and 108 min of incubation. Inset: 2 μm scan showing ribbon-like fibrils with D-periodic bands of size 65.2 ± 5.1 nm.

mica at different KCl concentrations, we find that K^+ affects the assembly by binding to the mica, which reduces the binding affinity of collagen and enhances surface diffusion of the weakly adsorbed collagen molecules. These results suggest that the assembly of collagen on mica occurs via the initial adsorption to mica, surface diffusion, nucleation, and growth into a 2-dimensional network of fibrils. The mica lattice determines the growth direction of fibrils during the nucleation step, while potassium affects surface adsorption and diffusion of collagen molecules by neutralizing the mica surface. The proposed mechanism of collagen assembly is analogous to that for the assembly of A β 25-35 derived from Alzheimer's β peptide on mica^{25,26} and provides insight into understanding surface-guided assembly of a wide variety of filamentous structures such as silk-elastin-like protein polymer (SELP),^{27,28} and organic photonic materials including oligothiophenes and oligophenyls.^{29–35}

MATERIALS AND METHODS

Sample Preparation. Collagen stock was prepared using solubilized type I collagen derived from rat tail tendon (BD Biosciences 354236) (3.74 mg/mL) with >90% purity by SDS–PAGE. The aliquot was prepared by diluting the stock with 0.1% acetic acid to 1.65 mg/mL (pH 2.5) and stored at 4 $^{\circ}\text{C}$ and was used for experiments for up to 3 months. A collagen sample was prepared from collagen stock diluted with a buffer to a given concentration containing 30 mM Na_2HPO_4 and 10 mM KH_2PO_4 at pH 7. The concentration of KCl differs among experiments and is mentioned separately in each case.

Muscovite mica disks (grade V1) were purchased from Ted Pella, Inc. (Redding, CA). Phlogopite mica sheets were custom-ordered from Axim Mica (New Hyde Park, NY) and also provided as a generous gift from Frank Balzer at the University of Southern Denmark. A mica sheet was affixed on a metal disk and freshly cleaved by sticky tape before each AFM experiment. A 30 μL aliquot of the solution was deposited onto mica and incubated at room temperature for a given amount of time in a moisture chamber to minimize evaporation. The sample was then flushed twice with 70 μL of deionized water in multiple flow directions. Kimwipes were used to carefully remove excess water from the edges of the mica. The mica disk was then left to air-dry for more than 10 min in ambient conditions (room temperature), half-covered with a plastic dish. The dried sample was used for AFM imaging.

AFM Imaging. The AFM instrument (Veeco CP-II, Camarillo, CA) was operated in air at room temperature with a $100 \times 100 \mu\text{m}^2$ piezoelectric large-area scanner. An antimony-doped silicon probe (Veeco Probes, FESP7) with a nominal force constant of 2.8 N/m

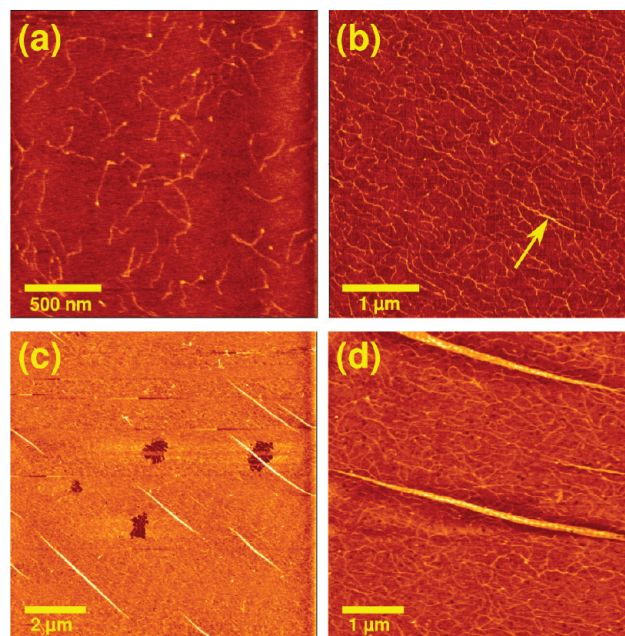


Figure 2. Early stages of collagen assembly on muscovite, 0.5 $\mu\text{g/mL}$ collagen with 200 mM KCl: (a) 1 min of incubation, showing randomly adsorbed collagen monomers, (b) 18 min of incubation (a nascent fibril is marked by an arrow), (c, d) 30 min of incubation, showing emergence of fibrils aligned in parallel. D-periods are visible in (d).

was used. Imaging was performed in the non-contact tapping mode (NCM) with a driving frequency near the nominal resonance frequency of the cantilever (~ 75 kHz). The NCM amplitude was about 70 nm, and the signal-to-noise ratio was maintained higher than 10. The scanning speed was 1 Hz for small-area scans ($< 2 \mu\text{m}$) and was decreased when larger areas were being scanned to ensure image quality.

Image analysis and processing were performed with the di-SPMLab software. Only basic image processing including leveling and adjustment to the contrast histogram was done. Line analysis was performed to measure the heights and lengths of the collagens. The fibril height was averaged over 10 measurements, and the length of a D-period was obtained from an average over 10 measurements where each measurement was on a straight region of a fibril with about 10 pitches. Data are presented as the average $\pm$ standard deviation. The lengths of the initially adsorbed molecules in Figure 2a were measured using ImageJ by following their contour.

RESULTS

Assembly of Unidirectionally Aligned Collagen Layers on Muscovite Mica. With 10 $\mu\text{g/mL}$ collagen and 200 mM KCl, highly aligned fibrils appeared within 1 min of incubation on muscovite mica (Figure 1a). At 50 $\mu\text{g/mL}$, in less than 2 h the aligned collagen fibrils covered almost the entire mica domain spanning more than hundreds of micrometers (Figure 1b). A closer inspection reveals formation of a D-period (Figure 1b, inset), but it is not as evident at 1 min (Figure 1a). Thus, after aligned fibrils form, individual collagen molecules may diffuse axially to find the D-period stagger that is believed to be an energetically favored state.^{36,37} These layers have a thickness of 1.3 ± 0.93 nm at 1 min (Figure 1a) and 2.17 ± 0.33 nm at 30 min (Figure 1b). By comparison, the diameter of a single hydrated collagen molecule is 1.3–1.5 nm,³⁸ and it is ~ 3 nm for a native collagen microfibril.³⁹ This suggests that collagen molecules

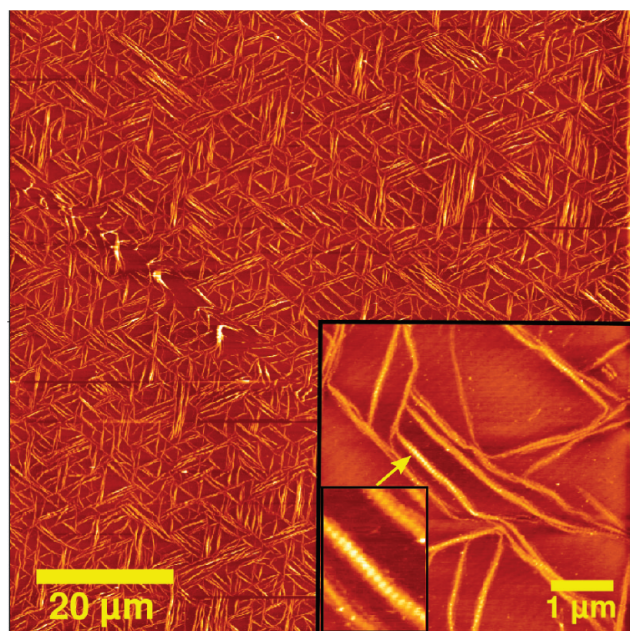


Figure 3. Triangular patterns of collagen fibrils on phlogopite mica, 10 $\mu\text{g/mL}$ collagen with 150 mM KCl, incubated for 2 h, 90 μm scan. Inset: 5 μm scan after 5 h of incubation, showing D-periods (67.73 ± 11.31 nm) and bending of collagen fibrils. The fibril height is 7.08 ± 1.87 nm.

initially assemble laterally on mica to form a single-molecule-thick layer and later form quasi-hexagonal microfibrils with a D-period. Previous AFM studies in liquid report formation of a layer very similar to that in Figure 1b yet with a 3 nm thickness.^{16,18} Thus, the reduced thickness of the layer in our case may have been caused by drying. However, it is unlikely that the overall morphology of a mature layer is affected by drying, since collagen fibrils are strongly bound to mica and even withstand rinsing with 1 M HCl. As shown below, the morphology of the layer depends on the incubation time of the collagen solution on mica, which further indicates that it is determined while the collagens assemble.

Although there are reports on the early stages of the assembly process, they are either in regimes where fibrils have already grown extensively^{18,20} or at the protofibrillar level without an extensive ordering.^{13,40} Noting that the ordered fibrils appear almost instantaneously upon deposition of the collagen solution on mica (Figure 1a), we used a concentration of 0.5 $\mu\text{g/mL}$, well below the critical concentration for collagen fibrillogenesis in bulk solution (4.73 $\mu\text{g/mL}$ at 29 $^{\circ}\text{C}$).⁴¹ With 200 mM KCl, we incubated the solution on mica for various durations and imaged with AFM (Figure 2). At 1 min, randomly adsorbed molecules are visible (Figure 2a). They are approximately 230–320 nm in length, corresponding to the length of a single collagen molecule.^{19,39} At 18 min, straight collagen fibrils appear, corresponding to nucleation events (Figure 2b, arrow), which become more abundant and grow in size by 30 min of incubation (Figure 2c). Their height is 1.1 ± 0.15 nm, consistent with a single-molecule-thick layer as in Figure 1a. Although D-periodic bands are visible (Figure 2d), the periodicity is 88.3 ± 11.97 nm (measured over four filaments in two images), again indicating that the molecules in a fibril do not have the native-like packing at this stage.

Since we used a subcritical concentration of collagen, it is unlikely that the nascent fibrils as in Figure 2b are formed in

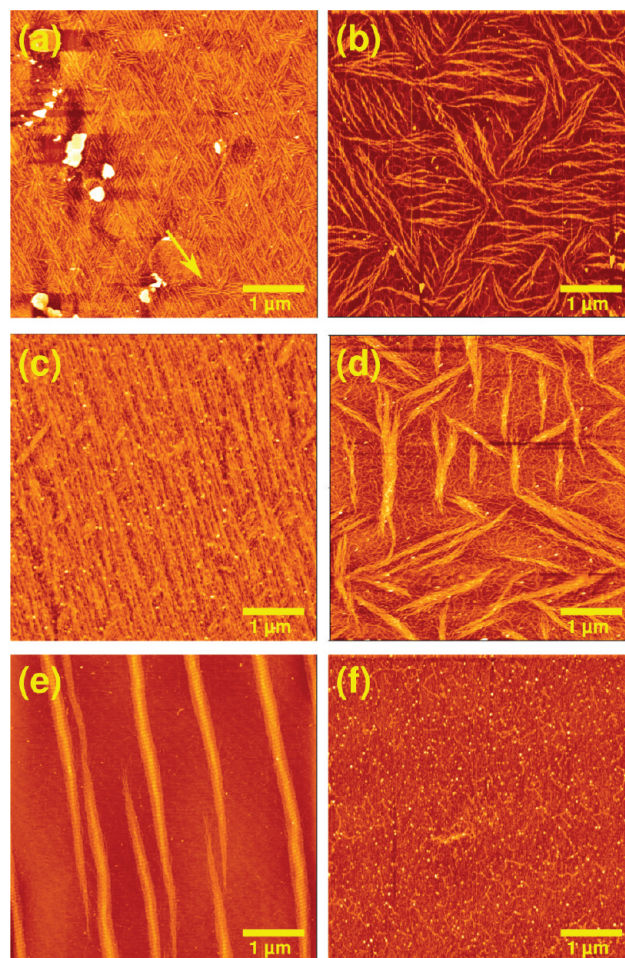


Figure 4. Effect of K^+ on collagen assembly: (a, c, e) muscovite, (b, d, f) phlogopite. All images are with 5 $\mu\text{g/mL}$ collagen and a 30 min incubation time. Other conditions and characteristics: (a, b) 50 mM KCl, (a) checkerboard-like, (b) triangular patterns [the arrow in (a) marks collagen fibrils aligned in a third direction]; (c, d) 100 mM KCl, (c) mostly unidirectional alignment, (d) bundling of collagen fibrils in the triangular pattern; (e, f) 400 mM KCl, (e) growth of parallel, thicker fibrils with D-periods, (f) no extensive assembly on phlogopite mica, suggesting weak adsorption.

solution and then deposited on mica. The fibrils appear in parallel with distances comparable to or larger than their own lengths, which precludes an alignment mechanism due to the liquid crystalline behavior of collagen as observed in tissues or at very high concentrations (>20 mg/mL).^{42–44} Initial random adsorption of collagen molecules and dependence of the assembly process on the incubation time on mica also exclude the possibility of alignment due to hydrodynamic flow induced when the solution is deposited on mica. Considering these factors, the most likely mechanism for the alignment is guidance by the underlying mica lattice.

Formation of Triangular Collagen Networks on Phlogopite Mica. If the alignment direction of the growing collagen fibrils is determined by the underlying mica lattice, it should be possible to obtain different alignment patterns when a substrate with a different lattice symmetry is used. Muscovite is a dioctahedral mica possessing a tetrahedral tilt, which breaks the hexagonal symmetry of the surface lattice. On the other hand, phlogopite is a trioctahedral mica with less lattice distortion (cf. Figure 5a–d).^{23,24,30,34} With 150 mM KCl, collagens indeed

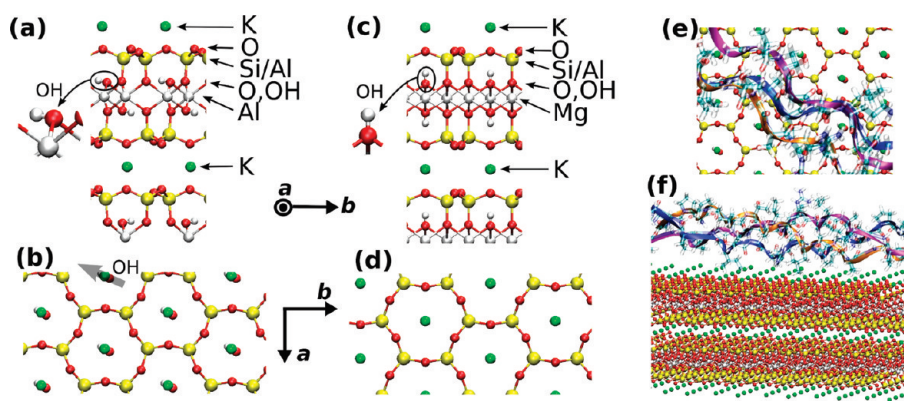


Figure 5. Mica and collagen structures: (a, b) muscovite, (c, d) phlogopite, (e, f) model of a collagen triple helix along the [110] direction of the muscovite mica. (a, c) View from the *a* axis of the unit cell. The middle Al layer of muscovite is a dioctahedral sheet, whereas the Mg layer in phlogopite is a trioctahedral sheet. In the Si/Al layer beneath the cleavage plane (K layer), replacement of the Si atom with Al causes the surface to be negatively charged.⁴⁷ Atoms are vertically aligned in phlogopite compared to muscovite. The two K layers are separated by (a) 9.98 Å and (c) 10.24 Å. The insets show different orientations of the OH groups in the two lattices. The proximity and direction of the OH group to the K⁺ atom in phlogopite reduce its binding affinity.⁴⁹ (b, d) View from above the cleavage plane (*ab* plane). Only the top layers including K, O, Si/Al are shown for clarity. In (b), the OH group is also shown to indicate its direction of tilt (transparent gray arrow). (e) Model of a collagen triple helix oriented along the [110] direction on the muscovite surface.²⁰ Backbones of the triple helix are represented as tapes, and amino acid side chains are rendered transparent. (f) Same system shown with two mica sheets. While the top K layer is shown fully occupied, in reality, the exposed layer has vacancies where collagen interacts with the K⁺ binding pockets. X-ray data for muscovite⁴⁷ and phlogopite⁴⁸ were used to construct the lattices shown. For collagen, the Protein Data Bank, 1BKV,⁵⁰ was used. Structures are rendered by using VMD.⁵¹

assembled on phlogopite into an extensive triangular network (Figure 3). Collagen fibrils had the native-like 67 nm D-period, and longer fibrils bent along the triangular pattern (Figure 3, inset). The height of the fibrils was 7.08 ± 1.87 nm, which is much greater than that on muscovite mica under the same conditions (1.79 ± 0.37 nm). This is likely because collagens bundle more extensively since they have a lower affinity for phlogopite than for muscovite.

Role of Potassium in Controlling Surface Adsorption and Diffusion. While epitaxy explains the orientational order of collagen layers on both types of mica, as mentioned above, in the case of muscovite, the K⁺ concentration is also an important factor affecting the unidirectional versus checkerboard-like orientations. Within the mica lattice, there is a layer of potassium atoms between silicate sheets that becomes the cleavage plane (cf. Figure 5a,c).⁴⁵ Since about half of the potassium atoms will be removed after cleavage, there will be empty potassium binding pockets, resulting in a partially negatively charged surface. On the other hand, collagen molecules (pI 9.3¹⁶) are positively charged at neutral pH. Therefore, if the buffer contains an insufficient amount of K⁺ ions, collagen molecules will adsorb more strongly to the mica and diffuse less. This tendency has been demonstrated for the assembly of the 10-residue-long A β 25-35 peptide.^{25,26} It assembled into fibers in three directions on muscovite mica. At higher concentration of cations, adsorption was reduced and filaments did not form. For the inhibitory effect, about 10-fold less KCl was required than NaCl, suggesting that K⁺ binds more tightly to the mica surface.²⁶ This also shows that Cl⁻ does not significantly affect the binding affinity to the mica surface.²¹

We varied the concentration of KCl and compared the morphologies of the collagen network on muscovite and phlogopite after 30 min of incubation (Figure 4). At 50 mM KCl, a checkerboard-like pattern emerged on muscovite whereas on phlogopite individual fibrils arranged into a triangular pattern (Figure 4a,b). Compared to muscovite, less collagens adsorbed

to phlogopite and the fibrils grew longer, indicative of a lower affinity and higher surface diffusion of collagen molecules on phlogopite. On muscovite, due to the lower level of diffusion, two directions of growth may be possible as collagen oligomers at the prenucleation stage (“protofibrils”) may not be able to rotate to energetically the most favorable direction. Consistent with this, a small number of collagen fibrils grow in a third direction (arrow in Figure 4a).

At 100 mM KCl, extensive alignment occurred on muscovite, suggesting that protofibrils of collagen were able to rotate and find the most favorable direction on mica (Figure 4c). The enhanced surface diffusion manifests as extensive bundling of fibrils on phlogopite (Figure 4d). Finally, at 400 mM KCl, thick collagen fibrils grew on muscovite,¹⁸ likely due to the even higher level of diffusion that leads to the coalescence of collagen molecules and oligomers into fewer fibrils (Figure 4e). The presence of a D-period also suggests that collagen molecules diffuse axially after forming the fibril. On the other hand, no extensive growth was observed on phlogopite (Figure 4f). The already weak interaction between collagen and phlogopite may have been reduced further by neutralization of mica by the surface-bound K⁺. Only small aggregates are present, which may be either collapsed monomers or oligomers. They could be in adsorption–desorption equilibrium with the incubating solution or may eventually nucleate fibrils with a prolonged incubation time. These results indicate that binding of K⁺ on mica reduces adsorption and enhances surface diffusion, which in turn affects the morphology of the collagen layer. Our experiments also suggest that the optimal KCl concentration for extensive ordering of collagen fibrils with a D-period is 200–400 mM on muscovite and $\sim$ 150 mM on phlogopite.

CONCLUDING DISCUSSION

Assembly Pathway of Collagen on Mica. In bulk solution, collagen is believed to assemble in three steps: nucleation, initial

axial growth, lateral growth.^{22,46} The present results suggest that collagens assemble on mica through a similar pathway, where collagen molecules adsorb, perform surface diffusion, nucleate a fibril, and grow. While this is shown most clearly at a low concentration of collagen (Figure 2), assembly in higher collagen concentrations is likely to be similar. This is because collagen fibrils nucleate more easily on mica than in bulk, as we have shown using a solution of collagen with a concentration lower than the critical concentration in bulk. Therefore, surface nucleation and growth of collagen fibrils will dominate over the adsorption of fibrils preformed in solution. In the latter case, the preformed fibrils should be small enough to rotate and align on mica, since otherwise the ordering as shown in Figures 1 and 3 may not be possible.

Comparison between Muscovite and Phlogopite Structures. Atomic structures of muscovite and phlogopite are shown in Figure 5a–d.^{47,48} The distortion of the lattice on muscovite^{23,24,30,34} is apparent as the atoms lack vertical alignment (Figure 5a), which contrasts with the more symmetric structure of phlogopite (Figure 5c). In Figure 5a,c, the Si/Al layer contains about 75% Si⁴⁺ and 25% Al³⁺. The replacement of Si⁴⁺ by Al³⁺ renders the surface to be negatively charged, which is compensated for by K⁺.^{45,47} Another important difference between the two types of mica is the orientation of the OH group in the octahedral sheet (insets in Figure 5a,c). In muscovite, its tilting leads to misalignment of its dipole field with the K⁺ above, whereas in phlogopite it is perpendicular to the cleavage plane and points toward K⁺. The O–H...K⁺ distance is also shorter in phlogopite, 3.3 Å, while it is 3.9 Å in muscovite. With the H atom in the hydroxyl group having a partial positive charge, K⁺ binds less strongly to phlogopite,⁴⁹ which is consistent with the weaker binding and higher thickness of collagen fibrils on it (Figures 3 and 4).

Factors Affecting Surface Adsorption and the Growth Direction. Different assembly morphologies on muscovite and phlogopite unambiguously show that the growth direction of collagen fibrils is guided by the underlying mica lattice. On phlogopite, since the network has three directions, it is also clear that the order is not generated by hydrodynamic flow or liquid crystalline behaviors. As Figure 4 shows, K⁺ can affect the assembly process by neutralizing the mica surface, on which collagens adsorb less and have higher diffusivity. Earlier experiments showed that the surface K⁺ occupancy on muscovite varies from more than 50% to near 100% as [KCl] increases from 10 to 500 mM.⁴⁷ Thus, in our experiments where [KCl] is at most 400 mM, the muscovite surface will contain unoccupied K⁺ binding pockets. As mentioned above, due to the vertical orientation of the OH group in phlogopite (Figure 5c), K⁺ will be more mobile and bind less strongly,⁴⁹ which will also be the case for collagen. Furthermore, on muscovite, K⁺ preferentially binds to lattice sites with two underlying Al atoms rather than those with one Al atom, which results in stronger binding of K⁺ on alternating rows of the mica lattice.⁵² This may provide additional guidance in the parallel alignment of collagen where individual molecules locate between the rows of strongly bound K⁺ and the positively charged amino acid side chains⁵³ interact with the empty K⁺ binding pockets of mica. Another possible effect of increased [KCl] would be enhanced electrostatic screening, which may assist with bundling of collagen molecules as in Figure 4e. However, in the 50–400 mM range of KCl, the Debye screening length (including other electrolytes in the buffer) varies from 7.9 to 4.3 Å, which is fairly short even at

low [KCl]. Changes in the level of electrostatic screening are thus unlikely to be significant. For both collagens¹⁵ and amyloid A β 25–35,^{25,26} since K⁺ promotes the surface assembly more strongly than Na⁺ does, the high K⁺ selectivity of mica⁵⁴ may play a greater role.

Binding of collagen on the mica surface will be mediated by van der Waals forces across the entire molecule and more locally by electrostatic attraction between positively charged amino acid side chains and empty K⁺ pockets on mica. Importantly, since hydration shells are formed around these surfaces,^{38,50,55} unless binding is strong (as occurs in low-K⁺ cases), the lubricating effect of hydration shells⁵⁵ will promote surface diffusion, as in the case of high K⁺ in Figure 4.

Compared to other molecules that grow epitaxially on mica, a single collagen molecule is significantly larger, which is a triple helix of α -chains, ~ 1.4 nm in diameter and 300 nm in length³⁹ (Figure 5e,f). By comparison, unit cells of the surface lattice in muscovite and phlogopite mica are about 0.5×0.9 nm² ($a \times b$).^{24,47,48} The mechanism by which mica guides the growth direction of the large and flexible collagen molecules is unclear. In a related vein, latex beads a few hundred nanometers in diameter also align on muscovite, which is known to be due to electric dipole interactions between the bead and the mica.^{56,57} Earlier studies show the presence of a surface electric dipole field on muscovite that is at a 15° angle relative to the high-symmetry direction of the lattice.^{30,45} In the case of *p*-hexaphenyl (a linear chain of six phenyl rings), each molecule aligns with the dipole field and assembles laterally in the [110] and $[1\bar{1}0]$ directions of the mica lattice.^{29,30} This means that individual molecules form a $\sim 75^\circ$ angle with the filament axis. Likewise, A β 25–35, which also exhibits an epitaxial growth on mica,^{25,26} forms the so-called amyloid “cross- β ” structure where each peptide is perpendicular to the fibril axis.⁵⁸ By contrast, collagen molecules lie along the length of a fibril and assemble in a staggered manner. If collagen fibrils align with one or more of the symmetry axes of the mica lattice, primarily in the [110] direction as previously suggested (Figure 5e),²⁰ they may be nearly perpendicular to the surface dipole field, but the role of the surface dipole in guiding the alignment of collagen is unclear. The intrinsic dipole moment or polarizability of collagen molecules⁵⁹ could be important. Another feature of muscovite mica that may help with alignment is the surface groove or topographic features due to its broken hexagonal symmetry.^{23,24,30,34,35} However, the groove is only 0.7–0.8 Å in depth, nearly 1/20 of the diameter of a collagen molecule. Thus, compared to small molecules that are comparable in size to the mica lattice,^{34,35} the role of surface geometry for the alignment of collagen fibrils would be minor. In reality, it is likely that the growth direction is determined by the interplay between multiple factors, including surface geometry and electrostatics.

Dependence of the Assembly Morphology on pH. The proposed mechanism for the epitaxial assembly of collagen on mica provides insight into the morphology of layers obtained under different experimental conditions. For example, at pH 8.5, collagens assembled into a checkerboard-like pattern on muscovite mica even though 200 mM KCl was used.¹⁵ Since collagen is less charged at pH 8.5, electrostatically driven selection for the major growing direction such as the surface dipole³⁰ or rows of K⁺⁵² may not be effective, and fibrils may grow in two directions guided more by the surface topography. In this case, collagen fibrils possess a D-period,¹⁵ suggesting that the checkerboard-like pattern is not a result of reduced surface diffusion, unlike in

our case (Figure 4a). In another study of collagen assembly on muscovite at pH 4.3, checkerboard-like patterns formed only when the KCl concentration was above 100–150 mM, below which thin disordered fibrils formed.²¹ At pH 4.3, collagen molecules have higher positive charges and tend to possess globular morphology instead of forming fibrils,¹⁶ which, without KCl, would adsorb to mica in a disordered manner (the mica surface is negatively charged at all pH values^{49,60}). A certain amount of K^+ would allow collagen molecules to interact and form ordered fibrils via surface diffusion. However, as the K^+ concentration increased further, the pattern became more unidirectional, and at 400 mM KCl, fibrils adsorbed to the surface only sparsely and mostly parallel to each other,²¹ which is consistent with our observation that KCl reduces adsorption and increases alignment (Figure 4).

Implications for the Surface Assembly of Other Systems. A β 25–35, which has a single positively charged lysine residue (Lys28) in the middle, appears to have a rotational degree of freedom when Lys28 binds to the K^+ pocket of mica, resulting in growth in all three directions on muscovite.^{25,26} Regarding SELP, it assembles into nanofibers on muscovite mica but not in bulk solution.²⁷ The molecule has a high proportion of hydrophobic residues and flexible glycine residues on every other position in its amino acid sequence. It thus may not have any particular preference in the growth direction and forms a network of randomly oriented nanofibers, as in Figure 4a of ref 27. The rotational freedom allows additional control of the growth direction by mechanical stimulus.²⁸ Surface adsorption of SELP is weak, and no ordered network was formed at a NaCl concentration as low as 10 mM. It would be interesting to see if adding a small amount of KCl results in a more aligned network.

In contrast to biomolecules that require a solvent for self-assembly, organic photonic molecules assemble via vapor deposition, yet they exhibit very similar network morphologies, i.e., parallel alignment or bidirectional growth on muscovite and a triangular network on phlogopite.^{29–31,34,35} On muscovite, in the absence of solvent or added K^+ ions, the deposition temperature may control surface diffusion of molecules adsorbed to mica, where parallel alignment is observed at higher temperature, and conversely, the growth is bidirectional at lower deposition temperature. In the absence of additional K^+ ions that form parallel rows, the alignment should be driven more by surface dipoles and grooves.^{30,34} More generally, elucidating factors that affect each step of the assembly pathway—surface adsorption, diffusion, nucleation, and growth—would be helpful for controlling the resulting morphology for a wide variety of systems.

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