Visualizing collagen fibril growth

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Collagen fibrils are among the most abundant protein polymers in living organisms. While the longitudinal packing of molecules in the fibrils has been agreed on for many years, there is continuous disagreement over the lateral packing. In this work, we describe a set of computer graphics programs that can be used to visualize fibril packing and simulate fibril growth. An example of a model and simulation are presented.

Keywords: computer graphics, collagen fibrils, visualization, molecular modeling

INTRODUCTION

Type I collagen fibrils are found in skin, tendons and bone, and are among the largest and most abundant protein polymers in living organisms. The fibrils resemble long tubelike structures and are made up of smaller molecules called *monomers*. Each monomer is a flexible rod (cylinderlike), about 300 nm long and 1.0 nm in diameter. The hydrophobic side chains of amino acids located on the surface of the monomer divide it into 4.4 segments or **D**-periods (**D** = 67 nm). Electron micrographs of the collagen fibril have revealed alternating band patterns in the longitudinal direction (Figure 1).[1] This pattern suggests a head-to-tail staggered packing of monomers with a gap of 0.6 **D** between them. There is also an overlap region (3.4 **D**), leading to a bricklike packing pattern, demonstrated in Figure 1. The high degree of order in the longitudinal packing of monomers has been agreed on for many years. However, there is continuous disagreement over the lateral packing of the monomers[1–4] in the tubelike fibril. Because these structures are three-dimensional (3D), visualizing the problem and specifying growth is difficult.

One view of lateral packing is that fibrils consist of "compressed" substructures called *microfibrils*. A microfibril is a helically coiled pentametric group of staggered monomers.[1] Other experiments suggest that the arrangement of molecules in the fibril is crystallinelike.[3,5–7] X-ray diffraction patterns have yielded different possibilities to account for the orientation of the overlap and gap regions[6,7] as did electron microscopy data.[3] Other observations[8] show the alignment of plates of apatite crystals in the gap region. Still other models[9] describe a helically coiled fibril and a coiled set of ropelike structures.[10,11] Recently, experiments have shown that initially the fibrils have one blunted and one pointed end, and growth proceeds unidirectionally from the pointed tip (α-tip).[2] Figure 2 demonstrates this observation. Later, the blunted end also forms a tip, β-tip, and growth continues in both directions. Both tips were near parabolic in shape, with the α-tips having a slope of approximately 17 molecules per **D** period (17 m/**D**) with the N-terminus of the monomers pointed toward the tip. This observation suggests symmetric and orderly lateral growth. Therefore, it is likely that lateral packing is directed by an intermediate structure that controls addition of monomers to the pointed tip but limits the diameter of the fibril.[2]

Our goal

Our goal is to use computer graphics and visualization techniques to simulate and test new theories for collagen fibril growth. Most of the experimental observations (X-ray, [EM]) yield two-dimensional (2D) information and, therefore, many of the resulting proposed models are also inherently 2D. Generally, only schematic sketches of the 3D placement were given or just the 2D cross-section. Extrapolation to three dimensions is very difficult, as is specifying the 3D growth rules. In this realm, computer graphics techniques provide an easy way to experiment and propose new

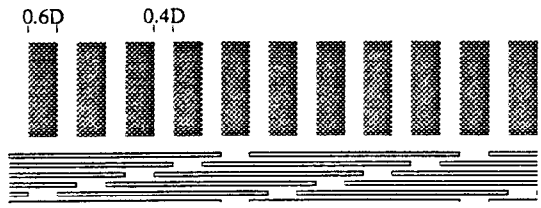

*Figure 1. Band pattern (top) similar to those seen in electron micrographs. The dark areas are 0.6 **D** wide and are reflected by gaps in the monomer staggering (bottom). The distance between the dark regions is 0.4 **D**.*

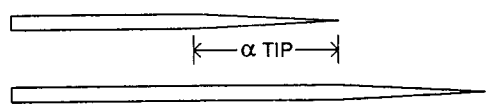

Figure 2. Growing collagen fibril: The fibril has been observed to grow in stages from a pointed α-tip. At a much later time, a tip forms on the blunt end as well.

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Received 7 May 1993; accepted 12 October 1993

theories in two and three dimensions. We have built a set of fibril modeling programs to develop a set of growth rules and simulate 3D fibril growth. Using a graphics model, one can compare the different observations in more detail and also formulate new theories for fibril growth. Furthermore,

- the addition of a large amount of monomers can be simulated (thousands)
- a full 3D model can be viewed with rotations, scaling, etc.
- 3D quantifications can be computed, such as volume, slope, and mass, for correlation to experimental observations
- surgical techniques can be performed, including slicing or peeling (for additional correlations)

In order to create a visualization tool to model fibril growth, some basic building blocks and simple rules were defined. These are described in the next section, followed by the data structure and software. In our example, a model is presented that follows the observations of Kadler et al.[2] and the use of the tools to visualize the model is demonstrated. Additional applications of the software are also discussed.

TERMINOLOGY

In order to implement a fibril-growing/visualization tool, a set of simple 2D and 3D structures must be defined. These structures form the building blocks from which complex rules can be established. The tools and growth programs are geometrical (i.e., monomers are treated as cylinders) and growth rules are based on positions (i.e., chemical/biological interactions are modeled as geometry).

Monomers (rods) are modeled as directional cylinders divided into 4.4 **D** segments as shown in Figure 3 (the right is the N-terminus and the left is the C-terminus). They bind to each other by overlapping. The most common is a 3.4 **D** overlap (or a 1-**D** stagger), which can be stacked to form strands. In two dimensions, a strand resembles the staircase pattern shown in Figure 4 and in Figure 1. The strands can also be 2 **D** staggered, etc. The overlap or stagger can be defined to the right (positive) or left (negative) and up or down. For example, the monomers in a strand can also be viewed from top to bottom as having a 3.4 **D** overlap. A collection of staggered strands each at 0.6 **D** gaps forms a bricklike pattern as shown in Figure 5. This is sometimes referred to as a *sheet* of monomers.[9,12] The gaps cause the bands in the electron microscopy images (Figure 1).

Growth rules

A set of growth rules can be specified using the overlap and stagger definitions described earlier. However, this will result in a 2D "fibril." Additional qualifications must be given—notably, how the monomers should be placed in three dimensions. For example, if each monomer is offset 72° from its 1 **D**-staggered partner, a pentamer is formed. The sixth monomer of the pentamer comes in along the same line as the first, with a 0.6 **D** head-to-tail gap as seen in Figure 6b. The cross-section is displayed in Figure 6a. This can be distorted and flattened to resemble microfibrils.[1] (Note that the figures show a counterclockwise direction. However, the placement rules and growth are equally valid for the clockwise direction. The program can handle either specification.)

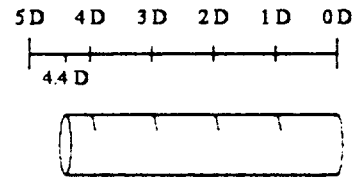

*Figure 3. A cylindrical monomer broken up into **D** segments (4.4 **D**).*

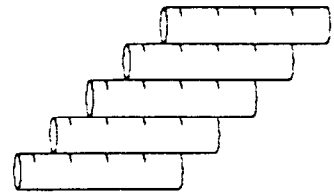

*Figure 4. A simple strand consisting of five monomers. Each successive monomer is offset 1 **D** from the previous monomer (or a 3.4 **D** overlap).*

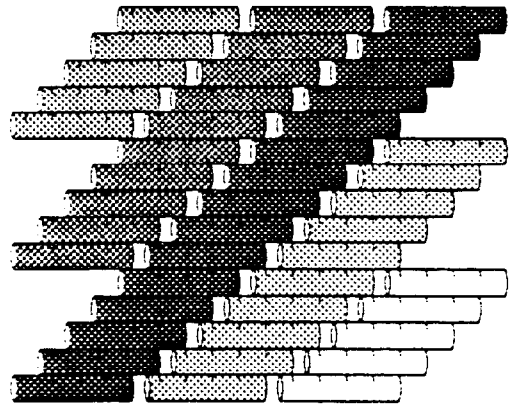

*Figure 5. A sheet of strands forming a bricklike pattern. The gaps between strands is 0.6 **D**.*

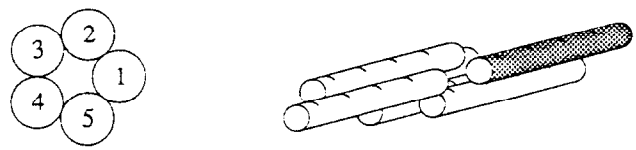

Figure 6. (a) A pentamer cross-section, head-on view (left image) and (b) a pentamer/microfibril (right image).

The rules in three dimensions can be either angular specification relative to the monomer on which the addition is taking place or can be more general "growing" rules (i.e., the smallest angle that does not intersect any other monomer). This rule is used for coiling strands about each other. Rules must also be specified to start new strands, stop strands from growing and account for collision of monomers, possible strand-strand contact rules (if ropelike penta-

mers are formed), tilting of monomers, growth rates for strands, etc. Some of these are described in later sections. Because complete specification of 3D rules is difficult, intermediate structures are assumed to guide growth (e.g., strands, pentamers, etc.). Currently, rules are specified by writing a "program" for each fibril model to be simulated. Rules are treated as separate function calls. These programs generate a full 3D fibril by filling in the data structure (described in the next section). The programs can be quite complex, as they have to account for varying growth rates and collisions. We are currently working on a more interactive set of rule specification. Tools to aid in rule design are described later.

DATA STRUCTURES

The data structure used represents a cylindrical **D** tiling of 3D space. It is defined based on a circular or radial tiling, with modifications for pentagonal or hexagonal tiling. Monomers are stored as cylinders in a set of arrays defining a volume of space. Each array is a specific core in the fibril (Figure 7). Each cell is 1 **D** × 1 **D** (for efficient coloring by **D** segment and slicing). The columns represent the length of the fibril. The number of rows varies among cores and is equal to the number of full monomers that can fit in the core. Polar coordinates are used to specify core and radial position. If the first core is assumed to be a pentamer, the radius for the *nth* core, r_n, can be computed by using the equation $r_n = \rho + 2Rn$, where R is the radius of a monomer (cylinder) and ρ is the pentamer radius (a constant) equal to $1.7R$ (Figure 8).

A monomer's position consists of three values: the core, the angle in that core, and the starting **D** segment. Each monomer fills five cells (4.4 **D**). Both intersection and conflict testing can be easily determined. Furthermore, this data structure easily accommodates weights (for binding interaction between monomers or on **D** segments) and twisting of individual monomers (to maintain a tilt off the fibril axis)[1,9,12] as shown in Figure 9. In addition to the **D** cells, the actual 3D coordinates (x, y, z) are stored together with the 2D location, time of placement, strand identification, and rule used. This information is necessary for rendering.

For a pentagonal or hexagonal lattice, subcores are used to designate radial positions. For a pentagonal lattice, subcores are five-sided and increase by five monomers. The monomers are always 72° apart with increasing radial distance. For an hexagonal lattice, there are six monomers per subcore, 60° apart. In both of these cases, the resulting fibrils are not circular but more crystalline.

RENDERING

Using the data structure just described and a rule program, a stepwise fibril model is created that can be rendered as a full 3D model (*GrowthView*) or as 2D slices (*SliceView*). In *GrowthView,* the individual monomers are represented by cylinders, and can be colored by **D** segment or strand (or microfibril, where applicable). The monomers grow one at a time and the simulation can be halted, restarted or reversed. The 3D fibril can be rotated, translated and scaled so that different perspectives are seen. Multiple views of the fibril growth process can be created (e.g., a longitudinal view and a head-on view). The 2D (flat) representation of rules can also be viewed. (Note that this representation corresponds to a type of "unraveling" of the fibril—a mapping from three to two dimensions—and is helpful for computing the slope.) A screen-dump of the program and user interface is shown in Figure 10 (a growing fibril with the 2D rule placement). In

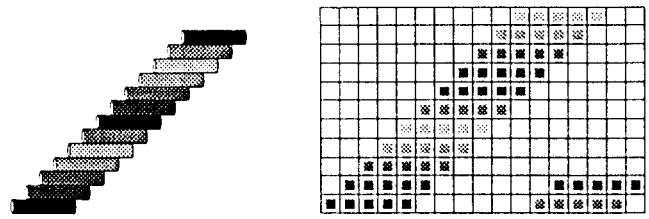

Figure 7. Strand in 2D formation along with its representation in the data structure. The array is for the second core.

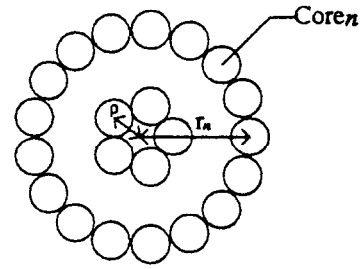

Figure 8. Helical core model.

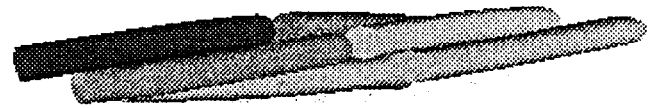

Figure 9. A twisted microfibril—to maintain a tilt of the monomers relative to the fibril's main axis.

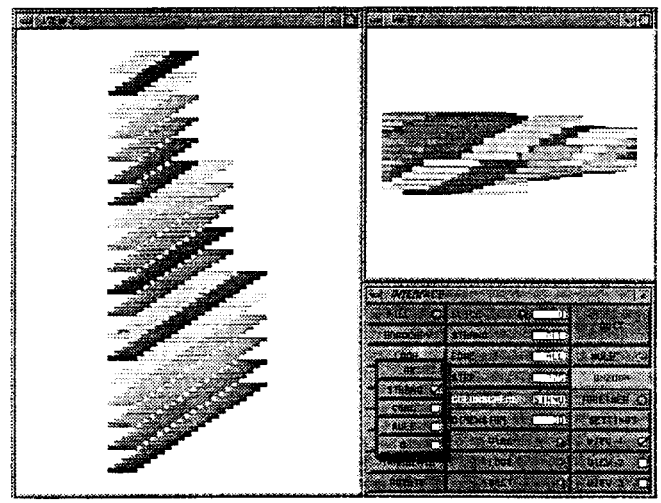

Figure 10. Screen dump of the GrowthView *program containing windows for the 2D representation and 3D model.*

addition, outer cores can be rendered transparent to allow viewing of inner cores or they can be totally removed to reveal the inner core structure. This is specified using the menu box.[13]

SliceView

SliceView works with the *GrowthView* program to allow surgical processes to the 3D model. Individual or ranges of cores and strands can also be analyzed separately. The fibril can be sliced (widthwise) to generate images similar to those in electron microscopy[3] or X-ray diffraction and color coded or labeled by **D** segment (Figure 11). This is important in attempting to correlate different models to experimental observations, since most of the literature describes lateral packing based on partial slices. The dialog box of Figure 11 can be used to choose slices along the fibril. The slices can also be made 3D as shown in Figure 12.

Fibril computer-aided design (CAD)

Programs were also developed to aid in the formulation of new rules. These programs create fibrils by allowing the user to add monomers one at a time. Monomers can either be added to a slice (by **D** segment specification), to the 2D rule representation or to an actual 3D fibril (CADlike). In the 2D rule representation, the program attempts to create a 3D model where possible.

In the 3D CADlike program, the user can place a monomer anywhere on a growing fibril. Placement is limited to the tiling of space selected (radial, hexagonal, etc.). An equivalent 2D placement map is also constructed.

Slice growing

For the correlation of the fibril to experimental observations, a slice-growing program is available (it is part of the *SliceView* tool set). An empty cross-section can be filled in with **D** segments by the user (with labels 1–5 representing positions along the monomer) and rendered in three dimensions assuming a 0.6 **D** gap region. The program will also attempt to complete the slice if so desired. The rules for completion are chosen from the regular suite of growing options, such as a 1-**D** stagger or alternating and nonalternating cores. (Total completion is not necessary for the 3D rendering.) In rendering the slice as a 3D part of a fibril, the program grows each cross-section of a monomer according to its **D** segment. Additional monomers are added after these monomers to give the slice width. This can be seen in Figure 13. This partial slice has a section with a crystallike substructure.[3,13]

Comparisons to experimental observations

After the model is formulated and created, it can be measured. These measurements can be used to help correlate the computed model with experimental observations. For example, mass, slope, distances between monomers, cross-sectional distances and configurations, radius, volume, etc., can be computed. We are currently enhancing the system to accommodate other measurements and renderings to mimic the experimental platforms.

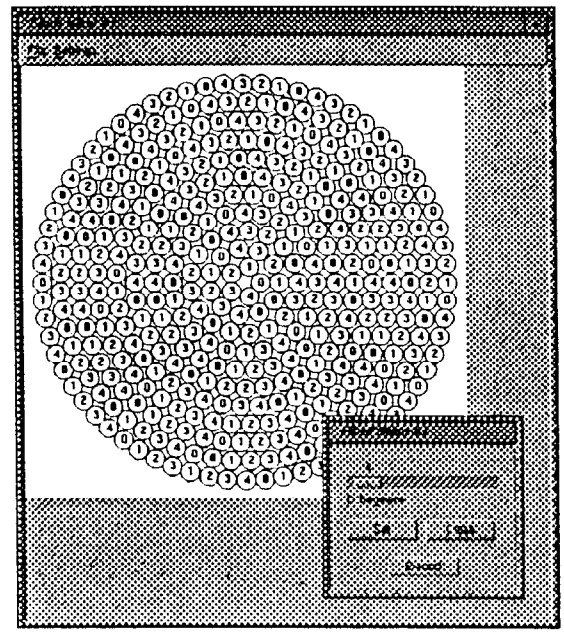

Figure 11. An axial slice from the SliceView *program. **D** segments are labeled (0–4).*

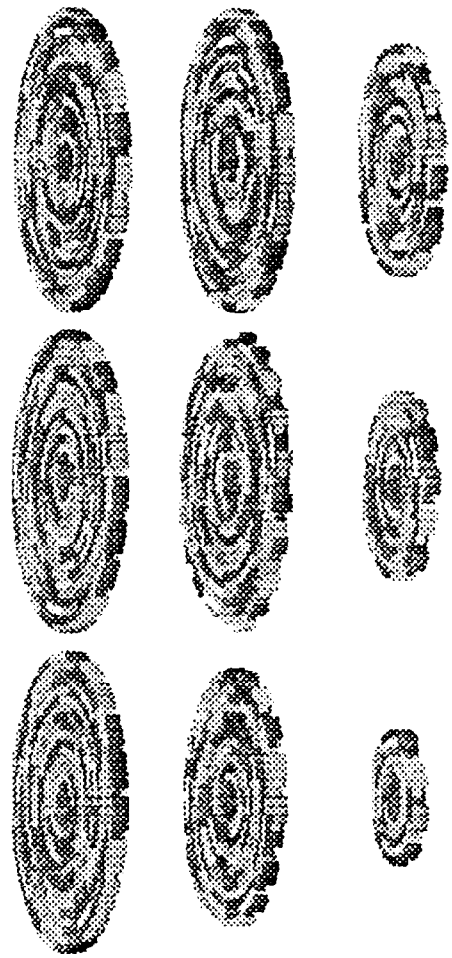

*Figure 12. Slices at different cross-positions in the fibril. The slices are colored by **D** segment and are 1 **D** wide.*

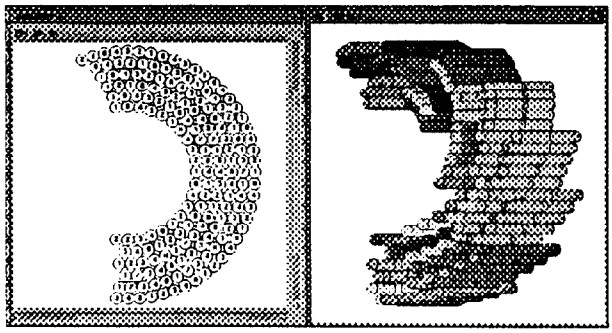

Figure 13. Growing a slice based on user input specifications.

Software

A 3D fibril model is created by executing the growth program with a set of "growth rules." The model is stored as a text file with the position of each monomer. A model can also be grown interactively using the *GrowthView* program (with the same set of rules). Both *GrowthView* and *SliceView* will analyze a completed model. The growing and analysis routines were written in the C programming language. The 3D *GrowthView* visualization program was written using Graphics Library (GL) on a Silicon Graphics workstation. *SliceView,* which is a 2D visualization program, is written using OSF/Motif™ graphical user interface libraries and runs under the X Window System.™

EXAMPLE: SIMULATING A POINTED TIP GROWTH MODEL

In this section, we present an example of creating a model using the growth programs. The model was to account for the pointed tip observation of Kadler et al.[2] and is fully described in Silver et al.[4] To simplify the growth possibilities, the following set of assumptions were made:

(1) The fibril is probably assembled using a limited number of binding steps.
(2) A core nucleus is formed that then propagates as growth progresses (i.e., growth is not random).
(3) The nuclei are most likely coiled or helical.

Furthermore, the model must maintain an α-tip of a slope of approximately 17 molecular **D** segments per **D** period, all monomers should be oriented in the same direction and the contour should remain constant as the fibrils grow. The growth model proposed was based on a central microfibril core and strands of monomers coiling about it as shown in Figure 14. Basically, two rules are used that dictate growth. One rule causes the fibril to lengthen (3.4 **D** overlap rule) and the second causes widening (-0.4 **D** overlap).

The first strand forms an inner pentametric core that consists of five monomers with a 1-**D** stagger (Figure 15). New strands wind about this center core forming concentric helical layers (Figure 14). Strands in each layer are interlocked by a -0.4-**D** overlap (on the opposite side). This is represented by a monomer filling in the blunt end of the fibril and is shown in Figure 16. This new strand will

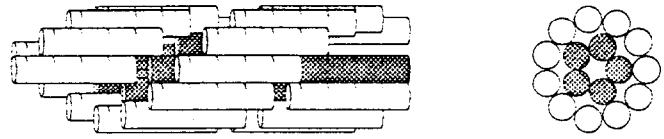

Figure 14. Axial (left) and lateral (right) views of growing fibril. The darker monomers are part of the inner core.

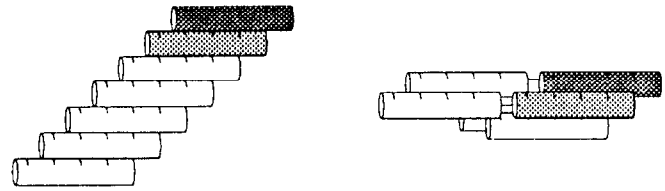

*Figure 15. Two-dimensional representation (left) and the 3D equivalent (right). Note the addition of the monomers with a 3.4 **D** overlap (a 1-**D** stagger).*

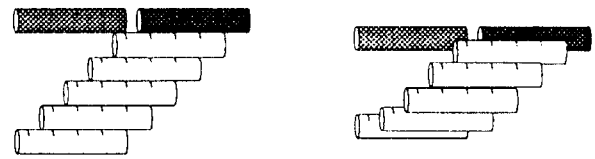

*Figure 16. Growth of a new strand—the 0.4 **D** overlap rule in two dimensions (left) and the 3D representation (right).*

continue to form and grow about the existing fibril. The first two layers (first three strands) are shown in Figure 14. A head-on projection is also displayed (a rotation of the fibril so that the tip is pointed toward the viewer). The next new strand to be created will form a third concentric, helical layer winding about the existing fibril structure. When new monomers are added, if there is a conflict, the new monomer is placed in the next core still maintaining a 1-**D** stagger.

Because successive layers must differ by approximately 2π molecules and the **D** offset requires multiples of five, larger (than 0.6 **D**) head-to-tail gaps are introduced between some of the strands in a layer. These gaps are not propagated between layers. An arbitrary choice was made to coil strands about a central core. A different model was also created with strands being true spirals and coiling from layer to layer. This model does not dramatically change the essential discussion of the slope and programs.[13]

Slope and growth rates

Several assumptions were made to accommodate growing fibrils: growth is orderly (i.e., each strand has a growth rate) and strands cannot overtake previous levels (no overhanging). Time is counted in steps with one or more molecules being added at each step. Rates are specified on a per-step basis. For example, if all strands were given the same rate (each strand grows at each time step), the result is the 2D diagram shown in Figure 17. The slope of this fibril is 1 m/**D** (rounding up).

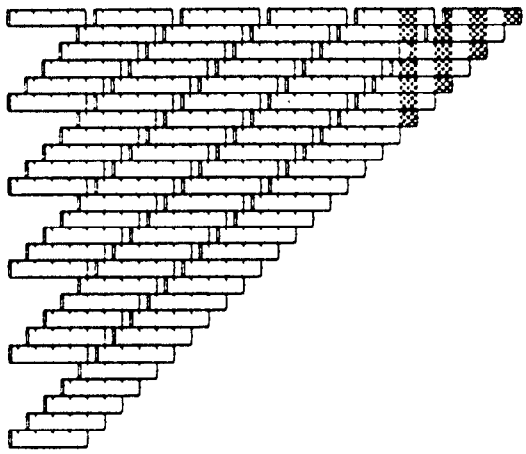

Figure 17. Two-dimensional representation with slope = 1 m/D. This results when the growth rate is equal for all strands.

A slope of s molecules per **D** (for the α-tip, $s = 17$) can be attained if the strands grow in a certain formation. The formation for a slope of $s = 3$ can be seen in Figure 18 and is analogous to any other slope. Basically, for a slope of s, s strands will grow together. Note that when strands grow together, the first strand must "wait" until the later strands (to the left) catch up lengthwise (in the x direction), implying that the later strands have a faster growth rate than the first one.

To explain this phenomenon, several mechanisms were considered. One was a stutter mechanism in which there was a finite probability of nonproductive additions of monomers during the bind steps required to initiate new strands or to propagate a strand. In this case, a slower rate is assigned to the stuttered strand. Because strands cannot overtake or propagate beyond a strand on a previous level, subsequent strands on cores above the stuttered ones will be delayed. If the probability of nonproductive additions is $1/s$, an average slope of s molecules/**D** will result. (Note that because the slope depends on strands at a lower level, strands that are formed after the slower one, but at the same core level, will still grow, similar to strands on higher core levels that do not overlap the slow strand.) Additional assumptions and mechanisms were postulated to account for the different types of tips.[4]

For a slope of 17 (α-tip), one out of every 17 new strands is a nonproductive addition and therefore slower. The others all grow at an equal rate. The faster rate must be at least 80 times (16×5) faster than the slow rate in order for all 16 strands to catch up with the first strand. After a basic nucleus is complete (with one out of every 17 stuttered), axial growth continues and the slope is maintained.

Simulation and discussion

A set of four frames from a full simulation/animation is shown in Figure 19.[4] The first frame contains 3000 monomers and each subsequent frame contains an additional 3000 monomers. Monomers of each strand have the same color, with shades varying (lighter) as outer layers are formed. A

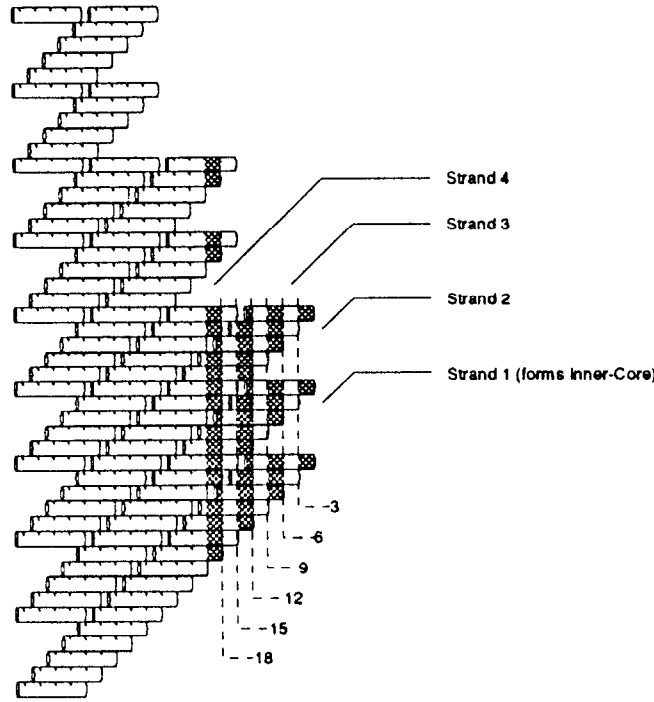

Figure 18. Two-dimensional representation with a slope of 3 m/D.

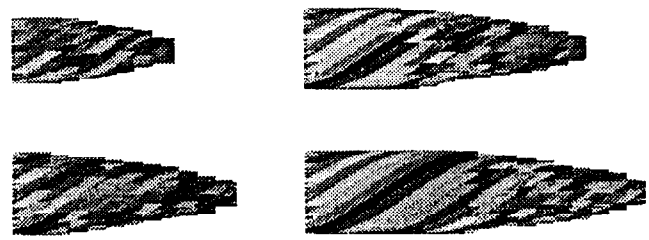

Figure 19. Four stages from a growth simulation. The first view has 3000 monomers and each successive view has an additional 3000 monomers. The final stage contains 12,000 monomers. The monomers are colored by strands.

slope of 17 is maintained throughout. A view of the fibril growing with its 2D representation is shown in Figure 10. (Note that the molecules are not tilted off the fibril axis in this simulation.) Cross-sections of this model are shown in Figure 11.

This model is the result of geometric constraints and simple assumptions based on growth of the fibril. The model is currently being correlated to experimental observations. It does conform to the slope and symmetry results[2] that it was designed to model. Other initial observations show that crystal-like patterns[3,7] and microfibrils[1] are visible in certain areas of the cross-section (Figure 11).

CONCLUSION

Although the problem of fibril formation has been discussed and studied for many years, the exact nature of lateral growth is unknown. Because much of the existing knowl-

edge is seemingly conflicting and, most importantly, is inherently 2D, it is difficult to formulate a complete 3D model. Computer graphics provide the necessary tools and capability to help understand and simulate fibril formation. Full 3D models can be constructed and measured, rules for growth can be specified and correlation to existing observations can be computed. In this work, we have created a set of basic tools to simulate and visualize fibril formation. These tools have aided in the formation of a new model of fibril growth.[4] They are now being used to verify and correlate other models, in addition to being enhanced to incorporate different theories. Geometric simulation and visualization provides a new way to experiment with and understand the nature of collagen fibril growth.

ACKNOWLEDGMENTS

This work was done in collaboration with Dr. D. Prockop, Department of Molecular Biology and Biochemistry at Thomas Jefferson University Medical College, which also supported this project. The equipment used is part of the Laboratory for Visiometrics and Modeling at the CAIP Center, Rutgers University. We are also grateful for discussions with Dr. K. Piez and Dr. D. Hulmes. The 2D and 3D software is available from the authors (either the SGI or SUN versions). Please send e-mail to silver@ caip.rutgers.edu for more information.

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