Structure of Collagen-Glycosaminoglycan Matrix and the Influence to its Integrity and Stability

Yuying Bi, Prabir Patra, Miad Faezipour

Abstract— **Glycosaminoglycan (GAG) is a chain-like disaccharide that is linked to polypeptide core to connect two collagen fibrils/fibers and provide the intermolecular force in Collagen-GAG matrix (C-G matrix). Thus, the distribution of GAG in C-G matrix contributes to the integrity and mechanical properties of the matrix and related tissue. This paper analyzes the transverse isotropic distribution of GAG in C-G matrix. The angle of GAGs related to collagen fibrils is used as parameters to qualify the GAGs isotropic characteristic in both 3D and 2D rendering. Statistical results included that over one third of GAGs were perpendicular directed to collagen fibril with symmetrical distribution for both 3D matrix and 2D plane cross through collagen fibrils. The three factors tested in this paper: collagen radius, collagen distribution, and GAGs density, were not statistically significant for the strength of Collagen-GAG matrix in 3D rendering. However in 2D rendering, a significant factor found was the radius of collagen in matrix for the GAGs directed to orthogonal plane of Collagen-GAG matrix. Between two cross-section selected from Collagen-GAG matrix model, the plane cross through collagen fibrils was symmetrically distributed but the total percentage of perpendicular directed GAG was deducted by decreasing collagen radius. There were some symmetry features of GAGs angle distribution in selected 2D plane that passed through space between collagen fibrils, but most models showed multiple peaks in GAGs angle distribution. With less GAGs directed to perpendicular of collagen fibril, strength in collagen cross-section weakened. Collagen distribution was also a factor that influences GAGs angle distribution in 2D rendering. True hexagonal collagen packaging is reported in this paper to have less strength at collagen cross-section compared to quasi-hexagonal collagen arrangement. In this work focus is on GAGs matrix within the collagen and its relevance to anisotropy.**

I. Introduction

As an important component in Extracellular Matrix (ECM), collagen contributes as scaffold to integrate and support the tissue. The elasticity and strength of the tissue are determined by the structure and function of collagen matrix [1]. Understanding the geometry, mechanical properties and cross-link of collagen, precisely collagenous biomaterials can be produced and used in tissue culturing and repairing.

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Collagen is a highly structured hierarchical protein [2]. The basic unit of collagen, tropocollagen(TC) molecule, is approximately 1.5nm in diameter and 300nm in length [3-5]. The two major classes of collagen, fibrillar and network (non-fibrillar), are divided by the distribution of TC molecules from the twenty-eight founded types of collagen [2]. Non-fibrillar collagens (such as type IV, VI, etc) form a network with individual TC monomers cross-linked by covalent intermolecular bond [2,6,7]. And the fibrillar collagen (such as type I, II, III, etc) is a highly-ordered structure with three levels: TC, microfibril, and fiber [8] (Fig. 1). The diameter of fibrils are various in different tissues;, most of them range from 50nm to 200nm. The distance between two TC monomers plus the overlap distance together is called D-period which is approximately 67nm and may vary with the degree of hydration. Within D-period, the gap usually is 0.54D and overlap is 0.46D (Fig. 2). The actual TC length is 4.46D [2,8]. The staggered structure provides high strength and stability to collagen fiber due to the high energy dissipation needed during deformation [9]. The arrangement of TC monomers in fibril is a quasi-hexagonal shape with five TC monomers [2,10].

Glycosaminoglycan (GAG), a negatively charged polysaccharide, is one of the most prevalent cross-links that not only affects the mechanical properties, but also the fibril formation in collagen. Germany researchers from the University of Munster studied the role of GAG chain of single chain GAG of decorin in collagen fibrillogenesis by using 3D fibroblast culture model. They found that the more Decorin is presented during the early stage of fibrillogenesis, smaller collagen fibrillars were composed [11]. GAG is bound every 67 nm at the D-period band gap in the collagen fibril surface. The single GAG chain that attaches with decorin is 69nm in length 23nm deviation as a Gaussian distribution [12]. GAG as cross-link enhances the strength of collagen, and the symmetry of the material is also an important feature for the durability of collagen, thus isotropy of collagen is studied in this paper to provide a parameter for future matrix building.

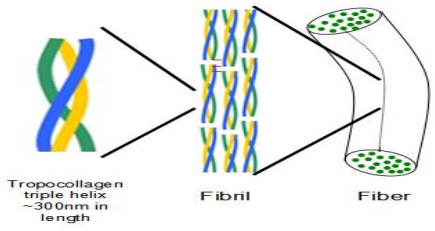

Fig. 1. Schematic view of hierarchical arrangement of collagen from TC triples helix to fiber.

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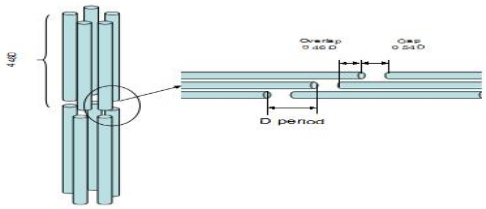

Fig. 2. Schematic view of D-period, including gap and overlap between two continuum TC monomer.

II. Material and Methods

A. GAGsim3D

In this study, a previously developed simulation program; GAGsim3D; was used to build a 3D model of GAG distributed C-G matrix with variables such as collagen diameter, collage distribution, and GAGs density. The distribution of collagen is hexagonal to quasi-hexagonal with various jitter numbers, where 0 stands for true hexagonal and 0.2 deviates from realistic collagen packaging. GAGs connect the nearby fibrils from the point located in the each separation line which has constant distance as D-period on collagen fibril (Fig. 3a). A thin slice from co-axial plane with collagen fibril can be intersected from the matrix and projected into 2D rendering for the study of GAGs alignment (Fig. 3b).

B. C-G Model Parameter

In order to create a 3D collagen/GAGs model, certain data is needed. In this study 1000×1000×1000nm cube was used as field of view and slice thickness was 50nm. Collagen fibrils radius was changed with the range from 50nm to 200nm, which occupy the total transverse cross-section area range from 40% to 70% (2,8). Jitter was used to determine the degree of deviation from true hexagonal distribution.

The study of cross-linking GAGs showed that the length of single GAG chain was complied with Gaussian curve with means around 69nm and 23nm deviation. Seed is a random number for generating a unique GAG distribution, and "0" was used in all models. Separation on collagen fibrils as D-period bands was labeled every 67nm along the fibrils. GAGs were oriented from a D-period band and projected to another D-period band on neighbor fibril and generate an angle (Fig. 3a).

C. C-G Model Generation

Three variables were used in this simulation: collagen radius, jitter, and GAG density. Since other collagen fibrils were neither too fragile nor too rigid. The collagen radius were divided into five sections: 50-80nm; 80-110nm; 110-140nm; 140-170nm; and 170-200nm. In this group, two cross-section of each model was used to analyze the influence of collagen fibril radius for its isotropy. The second group had constant value of collagen radius at 110-140nm and collagen density at 15 per unit volume, and jitter was divided to 0, 0.1 and 0.2. In this group, we still had two cross-sections of each model to analyze the influence of collagen fibril distribution to its isotropy. The last group had collagen radius and jitter constant at 110-140nm and 0.1, and GAGs density divided to 5, 15, and 25 to analyze the influence of GAGs density to collagen isotropy, and two cross-sections from each model were analyzed in this group.

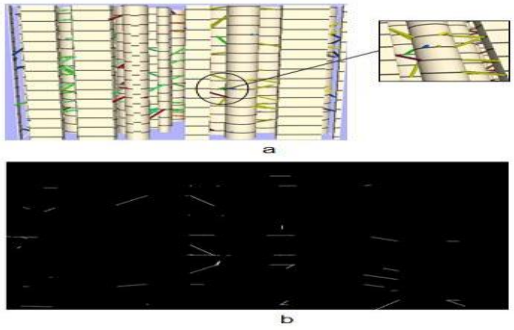

Fig. 3. Co-axial plane view of collagen/GAGs matrix model. (a) Represents the GAGs connection between nearby collagen fibrils from separation line. (b) Represents the 2D projection from 3D layer.

D. Statistical Analysis

The data from GAGsim3D contains both 3D and 2D projected GAGs length and angle towards collagen fibril. The angle data was equally divided into nine discrete bins with 20 degrees in each discrete bin (0-20^o; 20-40^o, and so on), and line grams were created based on the discrete bins. In a certain model, 3D data included all the GAGs in the whole model. After sorting out the 3D angle data into discrete bins, the percentage was calculated instead of the number of GAGs for better inspection in the line gram. In the selected plane for each model, 3D GAGs graphic were orthographic projected into 2D image (1), and the plane angle was used to analyze the symmetric and isotropy within the selected plane. Two planes were selected from each simulation model in which one plane was across through the collagen fibril and the other plane located between fibrils. These two planes represent the GAGs distribution related to different collagen distribution. 2D plane angles were also sorted into discrete bins and the isotropy was analyzed within the selected plane.

$$\begin{bmatrix} B_x \\ B_y \end{bmatrix} = \begin{bmatrix} S_x & 0 & 0 \\ 0 & 0 & S_z \end{bmatrix} \begin{bmatrix} A_x \\ A_y \\ A_z \end{bmatrix} + \begin{bmatrix} C_x \\ C_y \end{bmatrix} \quad (1)$$

Where A_x, A_y, A_z indicate the coordinate of 3D point, B_x and B_y indicate the projected 2D coordinate. Vector S is an arbitrary scale factor, and vector C is an arbitrary offset.

III. Results and Discussion

A. Baseline Model

Among the three variables, the median number of each variable was combined as baseline data to be used as control group to analyze the variations. Parameters for the baseline model are: collagen radius 110-140nm; jitter 0.1; and GAGs density 15 per unit volume. The average collagen radius of baseline model was 116.29nm and the ratio of collagen cross sectional area to total model cross sectional area was 61.90%. The result of 3D angles from baseline model showed that GAGs near the coaxial plane of collagen fibrils were 0% of total number of GAGs in the model. The percentage of GAG angles were constantly increasing before reaching the orthogonal plane (80-100^o). Additionally, the peak

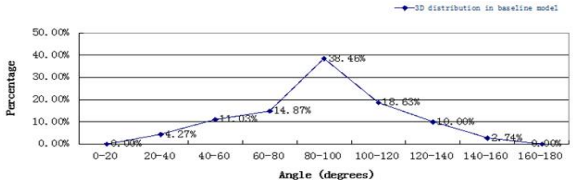

Fig. 4. Histogram and percentage of GAGs 3D angle distribution from baseline model.

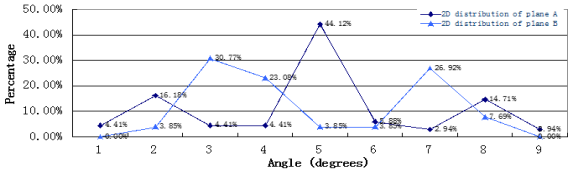

Fig. 5. Histogram and percentage data of GAGs 2D angle distribution from baseline model.

percentage which was located in the orthogonal area occupied more than one third of the total number of GAGs (Fig. 4). Data showed that the GAG angle was symmetrically distributed with single peak at the axis 80-100$^{o.}$ The angle distribution of 2D projection of selected planes (plane A and B) were analyzed for symmetry. Although both planes appear to have a certain degree of symmetry, they resulted in different shapes (Fig. 5). Plane A showed a large proportion in orthogonal area (80-100^{o}) and had similar curve between 0-80^{o} and 100-180^{o}. Plane B, on the contrast, had only 3.85% located in the orthogonal area. The large proportion of plane B was located in 40-60^{o}, 60-80^{o}, and 120-140^{o}. When 80-100^{o} was the axis in this histogram, there was asymmetry between 60-80^{o} and 100-120^{o}.

From the baseline model, the GAGs angle distribution of 3D was close to perfect symmetrical distribution with 80-100^{o} degrees as axis, and over one third of GAGs were directed to the orthogonal plane from collagen fibrils. 2D distribution of GAGs angle appeared as wave with three axes located in 80-110^{o} and two symmetry intervals aside 80-110^{o}. The 2D plane crossing through collagen fibrils presented more symmetry than the 2D plane passing through the space between fibrils.

B. 3D Angle Distribution with Changing Variables

In all the 3D geometric models, the GAGs angles distributed symmetrically with a single peak at axis 80-100^{o}. Models with constant jitter, GAGs density had nearly identical trends for GAGs angle distribution with increasing collagen radius range (Fig. 6). The same trends were shown in models with changing GAGs density or collagen distribution while the other two variables remained constant (Fig. 7, 8). All of the models in these three groups had symmetrical distribution with largest GAGs amount located in orthogonal plane at 30% to 40%, and near 0% in coaxial plane. The results indicate that the 3D GAGS angle distribution was isotropic and not influenced by collagen radius, collagen distribution, and GAGs density.

C. 2D Angle Distribution with Changing Variable

Unlike 3D, 2D GAGs angle distribution was variant with changing parameters. The data from baseline model indicated

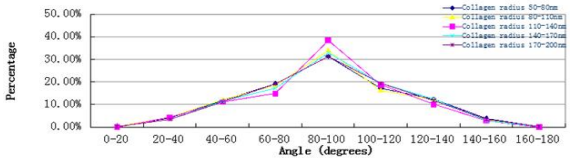

Fig. 6. 3D GAGs angle distribution with various collagen radius values.

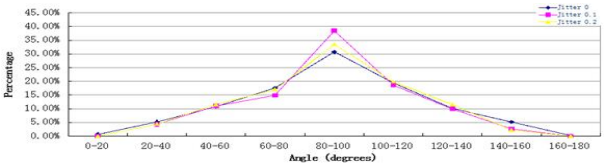

Fig. 7. 3D GAGs angle distribution with changing collagen distribution.

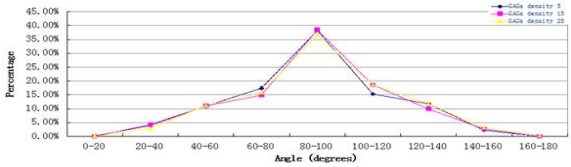

Fig 8. 3D GAGs angle distribution with changing GAGs density.

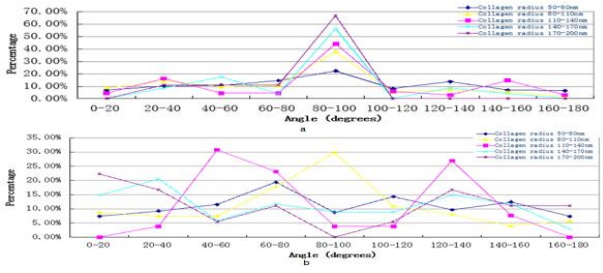

Fig. 9. 2D GAGs angle distribution with increasing collagen radius. (a) is the distribution in the plane crossing the collagen fibrils. (b) is the distribution in the plane passing through the space between fibrils.

that the GAGs angle distribution was slightly asymmetry from the 2D plane A and more symmetrical from the 2D plane B. Fig. 9 represents the trend of GAGs angle distribution with increasing collagen radius of both planes A and B. The distributions from plane A was close to ideal distribution with slight waves on both sides. By increasing collagen radius, the GAGs on orthogonal plane (80-100^{o}) was increased from 22.32% to 66.67%, while GAGs angle from other intervals maintained relatively stable from 0% to 15%. The distributions of plane B was irregular and had multiple peaks. When the collagen radius was 80-110nm, the GAGs angle distribution was close to a ideal distribution with a peak at orthogonal plane 29.89%. For the 110-140nm collagen radius which was the baseline model, there were two peaks at 40-60^{o} and 120-140^{o} intervals.

For true hexagonal distributed collagen (jitter=0), GAGs angle in plane A was symmetrically distributed with a small peak as 21.95% located at orthogonal plane (Fig. 10). When collagen distribution deviated from true hexagonal to semi-hexagonal, more GAGs perpendicular was directed to fibrils. All the three models in plane A had symmetric GAGs angle distribution. The GAGs angle distribution in plane B was less symmetrical and irregular than plane A. All three graphs in plane B had two peaks located in different intervals.

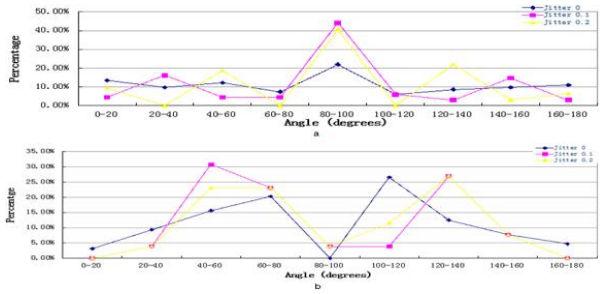

Fig. 10. 2D GAGs angle distribution with different collagen distribution. (a) is the distribution in the plane crossing the collagen fibrils. (b) is the distribution in the plane passing through the space between fibrils.

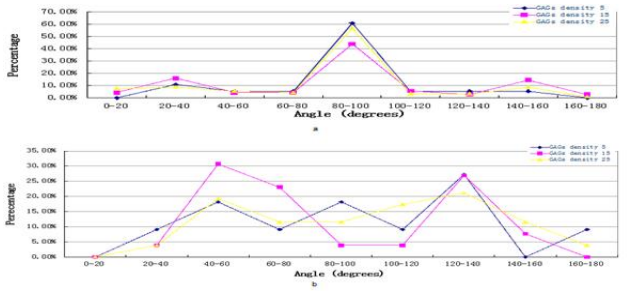

Fig. 11. 2D GAGs angle distribution with increasing GAGs density. (a)The distribution in plane crossing the collagen fibrils. (b) The distribution in plane passing through the space between fibrils.

For jitter 0, the two peak intervals were 60-80^{o} and 100-120^{o}; and for the other two models, two peaks were located at 40-60^{o} and 120-140^{o}. In the model with semi-hexagonal collagen distribution, more GAG had perpendicular connection then the model with true hexagonal collagen distribution. Changing of jitter did not affect the GAGs angle at orthogonal plane in which a lower peak appeared in all three models.

For comparing the C-G matrix model with different GAGs densities, the GAGs angle distribution in plane A was assembled in the orthogonal interval (Fig. 11a). The graph of the baseline model had a slightly small peak with 44.12% while the other two graphs had peaks around 60%. All of the three graphics in plane A were symmetrically distributed.

The GAGs angle distribution graphic with increasing GAGs density for plane B had multiple peaks with no specific axis. Other than the baseline model, the two graphs had waves between 20-160^{o} and near to 0 at two ends (Fig. 11b).

IV. Conclusion and Future Recommendation

This study reported the isotropy characteristics of GAGs in Collagen-GAG matrix in both 2D and 3D rendering by computational simulation. In 3D rendering, one third of GAGs was symmetrically distributed and directed perpendicularly with collagen fibrils. As in Collagen-GAG matrix of 1000nm cubic, 3D rendering of GAGs distribution was stable and isotropic, without being influenced by collagen radius, GAGs density, and collagen distribution. In 2D rendering, GAGs distribution was complex. With 50nm thick transverse specimen, GAGs distribution was isotropic in the selected plane crossing through collagen fibrils with more orthogonal direction in larger collagen fibrils matrix and was not significant influenced by collagen distribution and GAGs density. In the selected plane passing the spaces between collagen fibrils, GAGs had an irregular distribution with multiple peaks that indicated less strength on the transverse plane in C-G matrix. Collagen radius was the major influence of the distribution of GAGs. There were some symmetry presented with radius 80-110nm and 110-140nm, and with other collagen radius, GAGs were distributed asymmetrically. The deviation from true hexagonal collagen distribution result the shift of the peak in graph from perpendicular to coaxial in 20 to 40 degrees. Moreover, GAGs density did not have a significant influence on GAGs distribution in the 2D plane that passed through space between collagen fibrils.

The computational simulation and modeling analyzed the GAGs distribution and its influence for the stability of overall Collagen-GAGs matrix. This study could be further expanded by extending the subjects type of collagen from different tissue samples. Also, with specific tissue observation and image processing techniques, the isotropy as a major characteristic of GAGs cross-link from collagen could be better explained. Furthermore, mechanical simulation with force field analysis could provide data of the strength distribution during tissue movement. We are in the process of using Finite Element modeling to study Collagen-GAG matrix strength with respect to force field distribution.

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