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Computational Simulation and Analysis of the Transverse Isotropic Property of Glycosaminoglycan in Collagen

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***Abstract* —Glycosaminoglycans (GAGs) are disaccharides chains that are linked to a polypeptide core that serves as a cross-link in collagen to provide strength and persistency for collagen and related tissue. GAGs serve in two roles in Collagen-GAGs matrix. One is to connect collagen fibers in the matrix, and the other one is to contribute in the mechanical properties of the matrix. This paper summarizes the study of the isotropy of GAGs distribution in transverse plane of aligned collagen fibrils based on the roles of GAGs. The angle of GAGs related to collagen fibrils were used as parameter to qualify the GAGs isotropic characteristic in both 3D and 2D rendering. Statistical results included that over one third percentage of GAGs were closed to perpendicular direction 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 peak located at orthogonal plan was deducted by decreasing collagen radius. There were some symmetry features of GAGs angle distribution in selected 2D plane 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 influence GAGs angle distribution in 2D rendering. True hexagonal collagen packaging was reported in this paper to be less strength at collagen cross-section compared with quasi-hexagonal collagen arrangement.**

Keywords— Tropocollagen; Collagen-GAG matrix; 2D plane; 3D plane; isotropy; cross-link

I. INTRODUCTION

Presently, Tissue engineering is being rapidly developed. And the use of extracellular matrix (ECM) contributes to both clinical treatment of soft tissue damage and laboratory cell/tissue growth. As one of the most important protein in ECM formation, collagen has been widely studied during the past century. As the most abundant and widely distributed protein in living organisms, collagen forms about 25% of the human proteome. Collagen is also one of the most functional protein in living organisms with its hyperelasticity, flexibility, and persistency [1]. Understanding the geometry, mechanical properties and cross-link of collagen, precisely collagenous biomaterials can be produced and used in tissue culturing and repairing.

A. Collagen Geometry

Collagen is a highly structured hierarchical protein [2]. The basic unit of collagen, tropocollagen(TC) molecule, is configured as a right-handed triple helical structure. TC is approximately 1.5nm in diameter and 300nm in length [3-5]. TC molecules are composed by three parallel polypeptides with XaaYaaGly sequence. Every third amino acid in the polypeptide chain is Glycine(Gly). Although Xaa and Yaa can be any type of animo acid [2], proline (Pro) and hydroxyproline(Hyp) are the most common type.

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) usually 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 highly-ordered structure with three levels: TC, microfibril, and fiber [8](Fig. 1). TC monomers staggered each other to form fibril, then parallel arrayed fibrils form fiber. 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 called D-period which is approximately 67nm and may varied 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 provide high strength and stability properties to collagen fiber due to high energy dissipation needed during deformation [9].The arrangement of TC monomers in fibril is quasi-hexagonal shape with five TC monomers [2,10].

B. Mechanical Properties

Collagen in tissue such as bone and tendons play an important role for the mechanical properties of those tissues as well as their hierarchical structures[11]. The elasticity of TC monomer has been studied by various methods (Table I). As early as 1977, R. Harley et. Al measured the young's moduli of tropocollagen from rat tail tendon by Brillouin Scattering Spectra as 9.0 Gpa[12]. At 1996, Sasaki and Odajima described the stress-strain curve and young's modulus 2.9 GPa from bovine achilles tendon by using X-ray diffraction[13]. From 2002 to 2004, Sun and Luo et. al determined the persistence length of both collagen I and II thus get young's modulus via calculation. The result they got varies from 0.35 GPa to 12.2 GPa due to the differences of collagen radius[14,15]. From 2004, many works done by using atomistic molecular simulation. Lorenzo and Caffarena reported at 2004 that the young's modulus of TC monomer is 3.8 GPa to 5.8 GPa by using Steered Molecular Dynamics (SMD) simulation[16]. And then at 2009, Gautieri, Buehler, and Redaelli found out the young's modulus changes when the pulling rate during simulation changes. By use the same method (SMD), they get the young's modulus of TC monomer from 4.0GPa at less than 0.5 m/s pulling to 15.0Gpa at more than 100 m/s pulling[11].

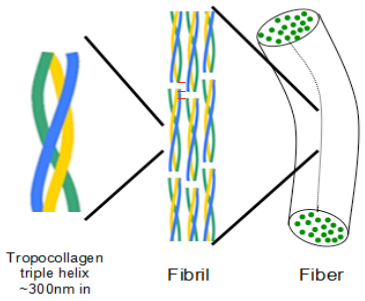


Fig. 1. Schematic view of hierarchical arrangement of collagen from TC triple helix to fiber.

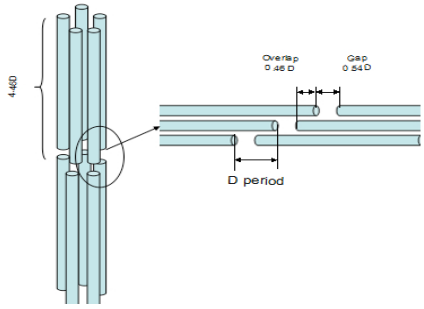


Fig. 2. Schematic view of D-period, which include Gap and overlap between two continuum TC monomer

TABLE I. YOUNG'S MODULUS OF TROPOCOLLAGEN MONOMER FROM DIFFERENT EXPERIMENTAL AND COMPUTATIONAL SIMULATION TECHNIQUES

Year	Scientist	Young's Modulus(GPa)	Techniques
1977	*R. Harley et. al*	*9.0*	*Brillouin Scattering Spectra*
1996	*Sasaki and Odajima*	*2.9*	*X-ray diffraction*
2002-2004	*Sun and Luo et. al*	*0.35-12.2*	*Calculation from persistence length*
2004	*Lorenzo and Caffarena*	*3.8-5.8*	*Steered Molecular Dynamics (SMD) simulation*
2009	*Gautieri, Buehler, and Redaelli*	*4.0-15.0*	*SMD with different pulling rate*

TABLE II. PERSISTENCE LENGTH(LP) FROM DIFFERENT EXPERIMENT METHOD

Year	Experimental method	Persistence Length(Lp)
1973-1983	*Hydrodynamic properties*	*130-180nm*
1984	*Electro microscopy*	*57±5nm*
2002	*Optical tweezer*	*14.5 ± 7.3nm*
2007	*Molecular Dynamics bending*	*23.4nm*
2010	*MARTINI coarse-grain model*	*51.6± 6.7nm*

Aside to elasticity, persistence length is also an important mechanical property for quantifying the stiffness of collagen. The smaller persistence length stands for lower stiffness and higher flexibility. Persistence length can be measured either by measuring the tangent angle and distance or calculating from known Young's Modulus. Since the value of TC molecule Young's modulus is various from different types of experiments, persistence length of TC molecule is not fixed value (Table II). Persistence length value from optical tweezers experiment was 14.5 ± 7.3nm [14]; from Molecular Dynamics(MD) bending simulations, value 23.4nm was calculated [5]; after MARTINI coarse-grain model simulation, the persistence length of TC molecule was determined as 51.5 ±6.7nm [17]; value from electro microscopy experiment was 57±5nm [18]; and from hydrodynamic properties analysis, persistence length was 130-180nm [19,20].

Persistence length has a direct relationship with bending stiffness (Equation 1) which can be expressed by Young's

Modulus (Equation 2). Thus persistence length can be expressed by Young's Modulus (Equation 3).

$$L_P = \frac{Bs}{K_B T} \quad (1)$$

$$B_s = EI = E\frac{\pi r^4}{4} \quad (2)$$

$$L_P = \frac{E\pi r^4}{4k_B T} \quad (3)$$

Where Lp is the persistence length, Bs is bending stiffness, kB is Boltzmann's constant (1.38*10^23 J/K), r is TC molecular radius, T is absolute temperature. In most situation, T is considered as 300K, and r is 0.7nm, when E is around 1.0-1.5 GPa, Lp is 45-68 nm. So in the simulation model, I used 50nm as slice thickness to study the isotropy of GAGs within TC molecular persistence length.

C. Cross-link and GAGs

The deformation mechanical properties of collagen is regulated by its covalent intermolecular cross-link[21]. Buehler proved when the cross-link density less than 10 GAGs/unit volume, the yield strain is lower then 10% and lead to high elasticity but low strength of collagen fibril. While cross-link density increasing, fibrils become less elastic and more strong until reach 25 GAGs/unit volume, at which point the elasticity and strength do not change with increasing density. [22].

Glycosaminoglycan (GAG), a negatively charged polysaccharide, is one of the most prevalent cross-link affect not only the mechanical properties but also the fibril formation in collagen. GAGs is one of the component of Proteoglycans (PGs), in PGs, GAGs serve as branches attached with a core protein (Decorin, Perlecan, Biglycan, etc) (Fig. 3). Those core proteins in PGs play multiple roles in connective tissues, such as regulation of collagen fibril formation, and affecting the mechanical properties of the tissue. Decorin is one of the most prevalent core protein in PGs located to the surface of collagen fibrils. Decorin is a curve patterned molecule with only one GAG chain and stand astride to a single collagen triple helix. It is bound every 67 nm at the D-period band gap in the collagen fibril surface. The single GAG chain attaches with decorin is 69nm in length 23nm deviation as Gaussian distribution[23]. GAG as cross-link enhance the strength of collagen, and the symmetry of the material is also an important feature for the durability of collagen and the related connective tissue, thus isotropy of collagen is studied in this paper to provide a parameter for future matrix building. If a plane has the same construction, constituent, and material properties during test from different directions, it considered as an isotropy plane. The isotropy in the transverse plane of collagen in which the fibril as axis is concerned to be related to its mechanical properties[23].

D. Computational modeling

Computational simulation now play an indispensable role as a mathematical tool in biological tissue and molecular study for its high efficiency and low expense [5,7]. Several computational techniques can be used to study the structure and function of collagen: Molecular Dynamics (MD) is able to bring out the perfect biological molecular model based on known protein arrangement and environmental parameters and run a series of force-movement simulations under various force field. Through collagen MD simulation, stress-strain curve, Young's Modulus, and persistence length can be calculated.[7,16]

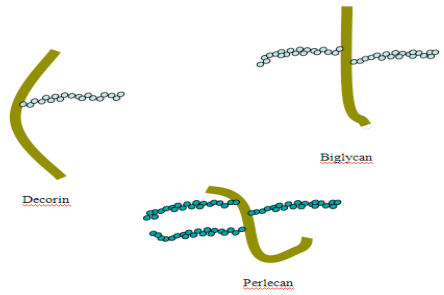


Fig. 3. Three different types and structure of PGs. The green rod in the middle is core protein: Decorin, Biglycan, and Perlecan. The blue branches are GAGs.

Finite Element (FE) modeling is used to study the mechanical properties of collagen under certain elasticity data from MD study. Different from MD modeling which is used to study the micro-scale level of collagen such as TC molecule, FE modeling work with larger scale object such as collagen fiber and study the structure movement as a whole.

Pre-configured programs such as GAGsim3D, WinFiber3D, etc are built to study the details of a certain tissue. Those programs collects data from imaging techniques such as Transmission Electron Microscopy (TEM) and builds basic model perviously. In this paper, a three dimensional C++ collagen matrix model program - GAGsim3D developed by University of Utah, was used to analyze and interpret the constructional symmetry in transverse plane of collagen. The isotropy of GAGs distribution can be determined by analyze the angle distribution in the configured model. The symmetry of angle distribution in 3D reflex the condition that quantity in the same directions of force from GAGs connections compare with other directions thus, isotropy in 3D model can be determined [23].

The hypothesis of this paper is to statistically verify the isotropy for distribution of GAG in transverse collagen plane.

II. MATERIAL AND METHOD

A. GAGsim3D

In this study, a previously developed simulation program - GAGsim3D was used to build a 3D model of changeable parameters such as collagen diameter, GAGs length, density, and more. With certain parameter, a complete 3D collagen/GAGs matrix can be observed. The distribution of collagen is hexagonal to quasi-hexagonal with various jitter number that 0 stands for true hexagonal and 0.2 deviate from realistic collagen packaging. GAGs connect the nearby fibrils from the point located in the each separation line which in constant distance as D-period on collagen fibril (Fig. 4a). 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. 4b).

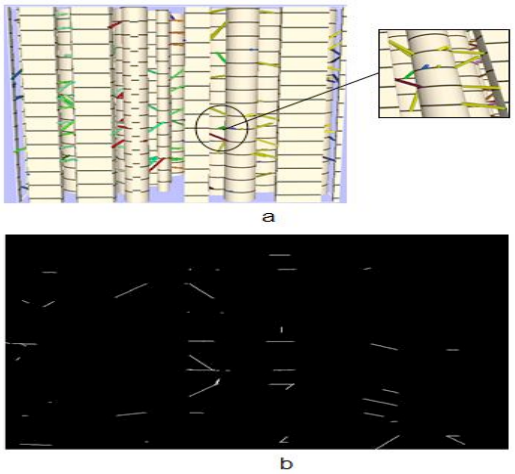


Fig. 4. Co-axial plane view of collagen/GAGs matrix model. a represent the GAGs connection between nearby collagen fibrils from separation line. b represent the 2D projection from 3D layer.

B. Collagen/GAGs 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% (2,8,24). Jitter was used to determine the degree of deviation from true hexagonal distribution.

The study of cross-linking GAGs showed the length of single GAG chain was complied with Gaussian curve with means around 69nm and 23nm deviation. The parameters were constant. Seed is a random number for generation to create a unique GAG distribution, and "0" was used in all models. GAG length followed Guassian distribution with 69 $\pm$ 23 (mean $\pm$ standard deviation). Separation on collagen fibrils as D-period bands 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. 4a).

The variables in this study assembled all simulation models into three groups. In first group, jitter was set constant as 0.1 which is the median between true hexagonal and deviation from realistic collagen packaging, and GAGs density was set at 15 so the various by location, structure and age of tissue. Studies showed when GAGs density smaller than 10 per unit volume, collagen fibril was fragile and hyperelastic. And before the density reach 25 per unit volume, distance between D-period bands on collagen fiber was 67nm as separation (23). The density of GAGs was collagen fibril became stronger and less elastic with increasing density. As soon as the density reach 25 per unit volume, the deformation mechanism of collagen fibril was based on molecular fracture thus the strength and elasticity not change by density of GAGs (22).

C. Collagen/GAGs model generation

Three variables were used in this simulation: collagen radius, jitter, and GAG density, and other collagen fibrils was neither too fragile nor too rigid, and I divided collagen radius 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, I still had two cross-section 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.

D. Statistic 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 et. al.), and line grams were created based on the discrete bins. In a certain model, 3D data include all the GAGs in whole model. After sorting out the 3D angle data into discrete bins, percentage was calculated instead of the number of GAGs for better inspection in line gram. In the selected plane for each model, 3D GAGs graphic were projected into 2D image, 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 across through the collagen fibril (Fig. 5a,b) and another plane located between fibrils (Fig. 5c,d). 2D plane angles also sorted into discrete bins and analyzed the isotropy within the selected plane.

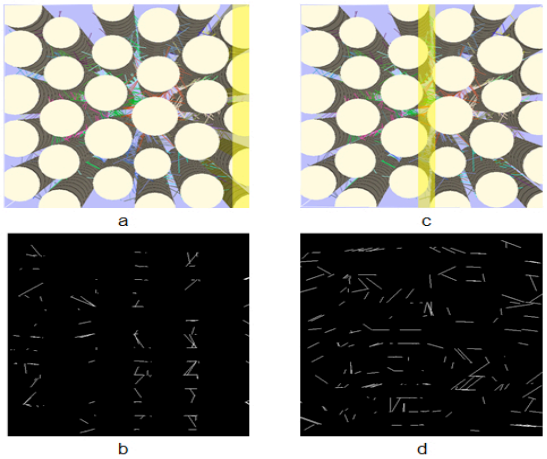


Fig. 5. Selected plane from simulation model. a represent the plane across through collagen fibrils. b represent the 2D projection from a plane. c represent the plane located between collagen fibrils. d represent the 2D projection from c plane.

III. RESULT AND DISCUSSION

A. Collagen status analysis

In three groups, collagen radius and volume ratio in simulation models changed with variables. With increasing collagen radius range while other two variables remain unchanged, the average of collagen radius and the ratio of collagen cross sectional area to total model cross sectional area were both increasing (Table III), thus space for GAGs was decreased by increasing collagen radius. When collagen radius was 50-80nm, the collagen volume ratio was 55.23%; while the collagen radius increased to 140-170nm, the collagen volume ratio increased by 9.05% which was the highest ratio in this group. While the collagen radius increased to 170-200nm, the collagen volume ratio decreased a little as in 63.98% (Table III). When jitter as the only changing variable, the average collagen radius and collagen volume ratio decreased with increased jitter number (Table IV). True hexagonal distribution of collagen (jitter=0) led to the highest collagen volume ratio which was 78.21%, and the boundary of deviation from realistic collagen packaging (jitter=0.2) led to the lowest collagen volume ratio which was 48.78%. Jitter changing from 0 to 0.2 result in nearly 30% change in collagen volume ratio thus led to big change of the space for GAGs. When the collagen radius and jitter remained unchanged, both average collagen radius and collagen volume ration were not changing with the variation of GAGs density. With the changing of collagen volume ratio and GAGs density, the GAGs angle distribution can be analyzed by both variables.

B. Baseline model

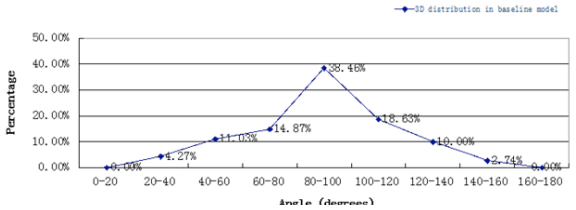
Among the three variables, the median number of each variable was combined as baseline data that used as control group to analyze the variations. Parameters for the baseline model are: collagen radius 110-140nm; jitter 0.1; and GAGs density 15 per unit volume. The average collagen radius of baseline 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 GAGs near the coaxial plane of collagen fibrils were 0% to total number of GAGs in the model. The percentage of GAG angles were constantly increasing before reach the orthogonal plane (80-100^o). And the peak percentage which located in orthogonal area occupied more than one third for the total number of GAGs (Fig. 6). Data showed the angle distribution was symmetrical with the axis 80-100^o and the shape of graphic close to parabola. The angle distribution of 2D projection of selected planes (plane A and B) were analyzed for symmetry. Although both planes appears a certain degree of symmetry, they showed in different shapes (Fig. 7). 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, in the contrast, had only 3.85% located in the orthogonal area. The large proportion of plane B located in 40-60^o, 60-80^o, and 120-140^o. When 80-100^o was axis in this histogram, there was asymmetry between 60-80^o and 100-120^o.

Collagen radius (nm)	Jitter	GAGs density (per unit volume)	Average collagen radius (nm)	Collagen volume ratio (%)
50-80	0.1	15	62.53	55.23
80-110	0.1	15	89.09	59.40
110-140	0.1	15	116.29	61.90
140-170	0.1	15	143.33	64.28
170-200	0.1	15	170.55	63.98

Collagen radius (nm)	Jitter	GAGs density (per unit volume)	Average collagen radius (nm)	Collagen volume ratio (%)
110-140	0	15	131.42	78.21
110-140	0.1	15	116.29	61.90
110-140	0.2	15	101.78	48.78

TABLE III. AVERAGE COLLAGEN RADIUS AND COLLAGEN VOLUME RATIO FROM MODELS WITH DIFFERENT COLLAGEN RADIUS PARAMETER

TABLE IV. AVERAGE COLLAGEN RADIUS AND COLLAGEN RATIO



From 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 directed to the orthogonal plane from collagen fibrils. 2D distribution of

Fig. 6. Histogram and percentage of GAGs 3D angle distribution from baseline model.

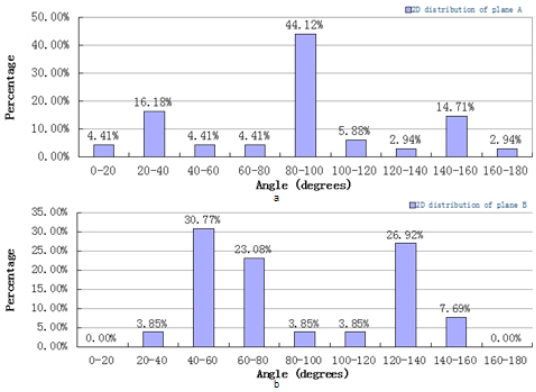


Fig. 7. Histogram and percentage data of GAGs 2D angle distribution from baseline model. a represent the distribution for plane a which cross through the collagen fibrils. b represent the distribution for plane b which pass through the space between fibrils.

GAGs angle appeared as wave with an three axises located in 80-110^{o} and two symmetry intervals aside 80-110^{o}. The 2D plane cross through collagen fibrils presented more symmetrical than the 2D plane pass through the space between fibrils.

C. 3D angle distribution with changing variables

In all the 3D geometric models, The GAGs angles distribution showed as parabola shape. Models with constant jitter, GAGs density had nearly identical trends for GAGs angle distribution with increasing collagen radius range (Fig. 8). The same trends showed in models with changing GAGs density or collagen distribution while the other two variables remained constant (Fig. 9,10). All of the models in these three groups had

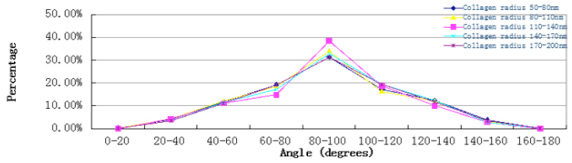


Fig. 8. 3D GAGs angle distribution with changing of collagen radius range.

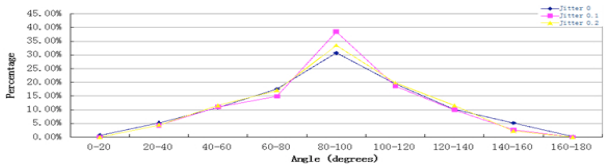


Fig. 9. 3D GAGs angle distribution with changing collagen distribution.

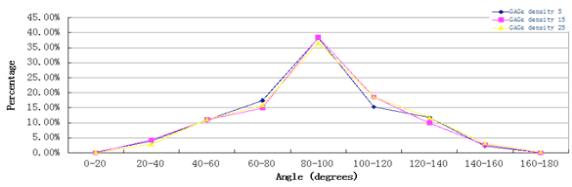


Fig. 10. 3D GAGs angle distribution with changing GAGs density.

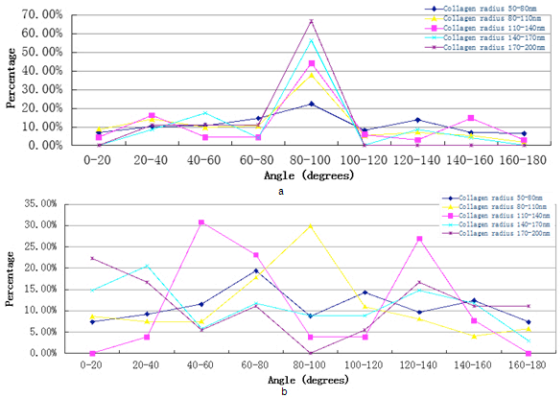


Fig. 11. 2D 2D GAGs angle distribution with increasing collagen radius. a represent the distribution in plane A that cross the collagen fibrils. b represent the distribution in plane B that pass through the space between fibrils.

symmetrical distribution with largest GAGs amount located in orthogonal plane at 30% to 40%, and near 0% in coaxial plane. The results indicate that 3D GAGS angle distribution was isotropic and not influenced by collagen radius, collagen distribution, and GAGs density.

D. 2D angle distribution with changing variables

Unlike 3D, 2D GAGs angle distribution was variant with changing parameter. The data from baseline model indicated that the GAGs angle distribution was slightly asymmetry from the 2D plane that pass through the space between fibrils and basically symmetrical from the 2D plane cross the fibrils. Fig. 11 represent the trend of GAGs angle distribution with increasing collagen radius of both plane A and B. The distributions from plane A was close to parabola shape with slightly wave on both side which were symmetrical. By increasing collagen radius, the GAGs on orthogonal plane (80-100^{o}) was increasing from 22.32% to 66.67% while GAGs angle from other intervals maintained relatively stable from 0% to 15%. The distributions of plane B had some symmetry with fluctuations. There were no specific peak on the graphic of collage radius 50-80nm, 140-170nm, and 170-200nm thus no axis for symmetry. When the collagen radius was 80-110nm, the GAGs angle distribution was close to parabola shape 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.

When collagen fibrils distributed as true hexagonal packaging (jitter=0), GAGs angle in plane A had a little peak as 21.95% located at orthogonal plane (80-100^{o}) and evenly distributed at other intervals (Fig. 12). With collagen distribution deviated from true hexagonal to realistic packaging, GAGs increase the number located at orthogonal plane in the model. All the three models in plane A had symmetric GAGs angle distribution with 80-100^{o} as axis. The GAGs angle distribution in plane B was less symmetrical than plane and only few GAGs was orthogonal direction. All three graphics in plane B had two peaks located in different intervals. For jitter 0, the two peak interval were 60-80^{o} and

100-120°; and for the other two models, two peaks were located at 40-60° and 120-140°. Compare with the graphics for distribution with increasing collagen radius, graphics for distribution with different collagen distribution had more symmetry with axis in 80-100° and two peaks aside. By increasing jitter, GAGs distributed more prone to co-axial direction with collagen fibrils. Changing of jitter did not affect the GAGs angle at orthogonal plane in which a lower peak appeared in all three models.

When the collagen fibril model maintained in the same condition while changing the GAGs density in model, GAGs angle distribution in plane A were assembled in the orthogonal interval (Fig. 13a). The graphic of baseline model had slightly small peak with 44.12% while other two graphics had peaks around 60%. All of the three graphics in plane A were in symmetrical shape with axis at orthogonal plane (80-100°). As the distribution with increasing collagen fibril radius, the GAGs angle distribution graphic with increasing GAGs density for plane B showed more waves with no specific axis or peaks. Other than baseline model, the two graphics were waved

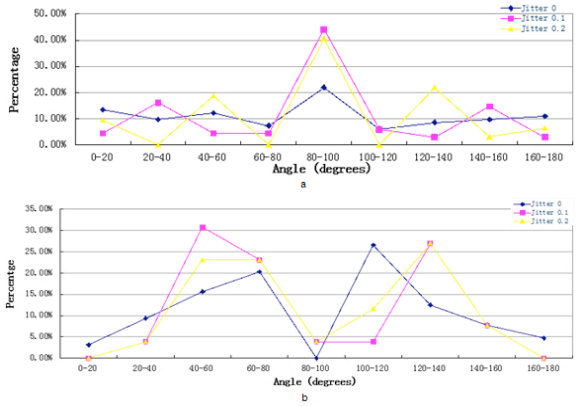


Fig. 12. 2D GAGs angle distribution with different collagen distribution. a represent the distribution in plane A that cross the collagen fibrils. b represent the distribution in plane B that pass through the space between fibrils.

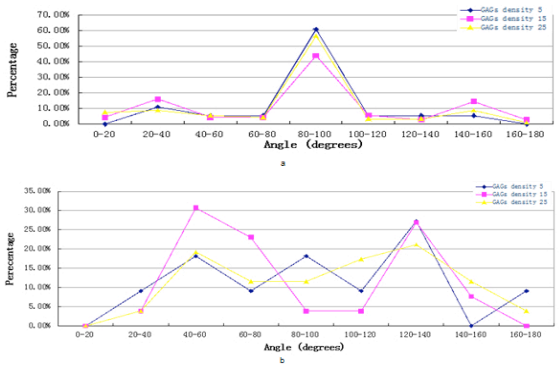


Fig. 13. 2D GAGs angle distribution with increasing GAGs density. a represent the distribution in plane A that cross the collagen fibrils. b represent the distribution in plane B that pass through the space between fibrils.

between 20-160° and near to 0 at two ends (Fig. 13b). Changing of GAGs density from baseline model increased the angle distributed at orthogonal plane.

IV. CONCLUSION AND FURTHER RECOMMENDATION

A. Conclusion

Cross-link provide the structure integrity that allows the system to function. The strength and direction of this connection is an important factor to keep the system functioning in appropriate manner. This study reported the isotropy characteristic of GAGs in Collagen-GAG matrix in both 2D and 3D rendering by computational simulation. In 3D rendering, one third of GAGs was directed perpendicularly with collagen fibrils and total GAGs were distributed symmetrically. As in Collagen-GAG matrix of 1000nm cubic, 3D rendering of GAGs distribution was stable and isotropic, without influenced by collagen radius, GAGs density, and collagen distribution. In 2D rendering, GAGs distribution was complexed. With 50nm thick transverse specimen, GAGs distribution was isotropic in the selected plane cross through collagen fibrils with more orthogonal direction in larger collagen fibrils matrix and no significant influence by collagen distribution and GAGs density. In the selected plane passed the spaces between collagen fibrils, GAGs distributed with multiple peaks instead of parabola which corresponded to both 3D rendering and 2D plane cross through collagen fibrils and indicated high strength on the transverse plane in Collagen-GAG matrix. Collagen radius was the biggest influence to 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. Jitter as collagen fibrils distribution the deviation from true hexagonal distribution caused shift of GAGs from close to perpendicular to coaxial in 20 to 40 degrees. And GAGs density did not have a significant influence to GAGs distribution on 2D plane passed through space between collagen fibrils.

B. Limitation and further recommendation

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

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