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Review

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Glycosaminoglycans in Tendon Physiology, Pathophysiology, and Therapy

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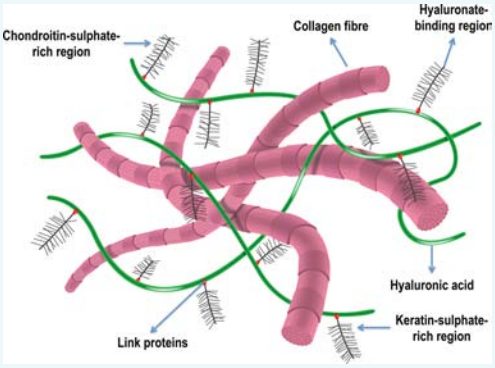
ABSTRACT: Although glycosaminoglycans constitute a minor portion of native tissues, they play a crucial role in various physiological processes, while their abnormal expression is associated with numerous pathophysiologies. Glycosaminoglycans have become increasingly prevalent in biomaterial design for tendon repair, given their low immunogenicity and their inherent capacity to stimulate the regenerative processes, while maintaining resident cell phenotype and function. Further, their incorporation into three-dimensional scaffold conformations significantly improves their mechanical properties, while reducing the formation of peritendinous adhesions. Herein, we discuss the role of glycosaminoglycans in tendon physiology and pathophysiology and the advancements achieved to date using glycosaminoglycan-functionalized scaffolds for tendon repair and regeneration. It is evidenced that glycosaminoglycan functionalization has led to many improvements in tendon tissue engineering and it is anticipated to play a pivotal role in future reparative therapies.



INTRODUCTION

Tendon injuries constitute a poorly addressed clinical need with significant socioeconomic consequences that are expected to rise further in the years to come, given the ever increasing active life style, putting a further financial strain on healthcare systems.[1−4] Optimal extracellular matrix (ECM) structural and mechanical integrity is of paramount importance for normal function; thus, biomaterial-based approaches should display similar properties to native tissues to ensure functional repair and regeneration.[5,6] The incorporation of glycosaminoglycans (GAGs) into scaffolds is associated with improvement of the structural, mechanical, and degradation properties as well as cell phenotype maintenance.[7−11] Collagen-based scaffolds functionalized with chondroitin sulfate or hyaluronic acid are under intense investigation for various clinical targets, with very promising results in vitro,[12−15] in preclinical setting,[15−18] and in clinic.[19−21]

GAGs are long, nonbranched mucopolysaccharides that are composed of repeating disaccharide regions of uronic acid (D-glucoronic acid or L-iduronic acid) and an amino sugar (D-galactosamine or D-glucosamine).[22−24] They are distinguished from each other by the type of hexose, hexosamine, or hexuronic acid unit present and by the geometry of the glycosidic linkage between the repeated units.[22,24,25] The most common classification is based on the degree of sulfation (Figure 1). Sulfated GAGs include chondroitin sulfate (CS), dermatan sulfate (DS), keratan sulfate (KS), and heparin sulfate (HS), while hyaluronic acid (HA) constitutes the only nonsulfated GAG.[24] The biosynthesis of GAGs involves several enzymes that assemble the GAG backbone and subsequently sulfate them at specific positions.[26−28] HS and CS are O-linked to a core protein to form proteoglycans[27,28] in the Golgi, while HA is synthesized by an integral plasma membrane synthase, which secretes the emerging chain.[29] Chains are synthesized by the attachment of a tetra-saccharide linker, where four enzymes transfer the four monosaccharides. Following attachment of the linker, transfer of a glucosamine or a galactosamine to the chain determines whether a HS or CS chain is produced, respectively. Following elongation, sulfation occurs. In the case of CS, chains can be sulfated in three different positions: 4-O and 6-O sulfation of galactosamine and 2-O sulfation of uronic acid.[30] Different concentrations of individual GAGs are found throughout individual tissues in the body (Table 1). For example, KS in cornea is more than 10-fold greater than the amount present in cartilage and 2−4 orders of magnitude greater than the amount of KS present in other tissues,[31] clearly demonstrating the specificity of GAGs to tissue function.

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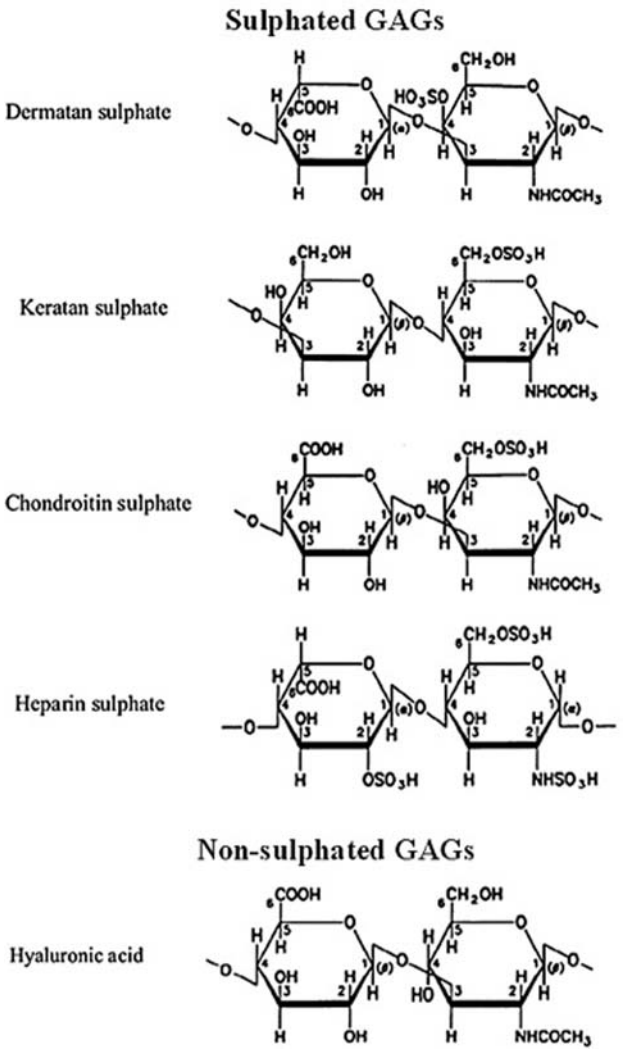

Figure 1. Classification of GAGs based on the degree of sulfation.

GAGs are negatively charged molecules and it is this negative charge that is responsible for the structural integrity of the ECM.[40,41] This negative charge increases osmotic pressure, which results in increased tissue hydration and subsequently influences the tissue viscoelastic properties.[40,42] Negatively charged GAGs covalently bind to the protein core of proteoglycans (e.g., aggrecan), and subsequently sodium and water are attracted, providing the tissue with stability and resistance.[43,44] Matrix that is negatively charged is shielded by the positive ions at equilibrium, producing osmotic swelling pressure. An electric potential is created as positive ions enter and leave the matrix during dynamic loading, also known as streaming potentials, which are used to detect the depletion of proteoglycans.[44] Under normal physiological hydrated conditions, sulfate groups and carboxylic acid become deprotonated, affording GAGs with very negative charge densities, which are responsible for the creation of a water molecule shell around them. Upon mechanical loading, these water molecules are released and subsequent removal of the applied load allows the outer shell to reform.[36,45−48] DS is the main GAG present in mature tendon and skin, controlling collagen fibril assembly and diameter, which in turn is crucial for the elasticity and viscoelasticity of the tissues.[32,49−52] CS is present in relatively high proportions in tendon and plays a key role in ECM organization and aggregation of protein ligands to proteoglycans.[7,35,36] It also contributes to the resistance of compressional forces on the ECM in tissues such as cartilage.[53] KS is particularly important for the maintenance of tissue hydration in the cornea, which is required for transparency.[34,54] It has also been shown to regulate cellular recognition of protein ligands and axonal guidance.[55−57] HS functions as anticoagulant and is also necessary for the attachment of active proteins to collagen structures, including growth factors, cytokine enzymes, and cell adhesion molecules.[24,58−60] HA is significantly involved in joint lubrication, modulation of inflammatory responses, and wound healing.[61−65] This multifaceted functionality of GAGs has made them key ingredients in biomaterials design for tissue engineering and regenerative medicine applications.[66−72] This review will discuss the role of GAGs in tendon physiology and pathophysiology (Table 2) and the advancements achieved to-date using GAG-functionalized scaffolds for tendon repair and regeneration.

GAGS IN TENDON PHYSIOLOGY AND AGING

Proteoglycans are glycoproteins with a core protein structure and are covalently attached to GAGs.[83] Decorin is commonly associated with DS,[84] aggrecan has a combination of DS and KS chains,[85] and versican is coupled with CS.[84] These complex structures form the regular gel-like structure that surrounds collagen fibrils,[86] as has been demonstrated by small-angle X-ray diffraction.[87] Glycoproteins play a pivotal role in the structural stability of collagen fibrils and consequently in tendon tissue integrity,[88,89] through mechanical restraint, electrostatic interactions, and/or by means of water-binding capacity.[90]

Although they form a minor proportion of the tendon ECM, they influence several physiological processes, including collagen fibrillogenesis, fiber diameter, and mechanical integrity.[91−93] As collagen fibrillogenesis has been shown to

Table 1. Tissue Distribution and Function of GAGs in the Body

GAG	structure	molecular weight	major function	location	ref
DS	$C_{14}H_{21}NO_{15}S$	15−40 kDA	controls collagen fibril diameter and formation	tendon, ligament, skin, blood vessels, heart valves	32
KS	$C_{28}H_{48}N_2O_{32}S_4$	4−19 kDA	corneal transparency, cellular recognition of protein ligands, axonal guidance, cell motility, embryo implantation	cornea, cartilage, tendon, brain tissue	33,34
CS	$C_{13}H_{21}NO_{15}S$	5−50 kDA	formation of proteoglycan aggregates with protein molecules, matrix organization	cartilage, bone, tendon, ligament, aorta, spinal cord, brain	7,35,36
HS	$C_{14}H_{23}NO_{21}S_3$	10−70 kDA	prevents blood clotting, needed in FGF binding	most mammalian cells, Blood vessels, muscle	37,38
HA	$C_{16}H_{27}NO_{12}$	4000−8000 kDA	lubrication and shock absorption, wound repair, cell signaling	ECM of loose connective tissue, synovial fluid, vitreous humor, tissue repair post injury	39

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Table 2. GAG Distribution in Normal and in Pathological Human Tendons

GAG	normal tendon	ruptured tendon	tendinopathy	overused tendon
DS	situated between adjacent collagen fibrils throughout tendon[73] regulates collagen fibril structure[32]	increased concentration[74,75]	increased concentration[76,77]	increased concentration[78]
KS	situated in tendon areas subject to compressional force[79]	increased concentration[74]	no change[77]	increased concentration[78]
CS	situated in tendon areas subject to compressional force, mainly terminal areas[80]	increased concentration[74]	increased concentration[76,77]	increased concentration[78]
HS	situated on the cell surface of every cell,[40] highest amount in myotendinous junction[81]	increased concentration[74]	increased concentration[76,77]	increased concentration[78]
HA	situated in extracellular space[80]	increased concentration[82]	increased concentration[76,77]	increased concentration[78]

be influenced by GAGs, the diameter of collagen fibers may vary due to the difference in the concentration of the individual GAGs present in tendons.[94] Fibrils with a diameter of >150 nm are found in tissues mainly containing DS, 60 to 150 nm fibrils are found in tissues containing CS, and fibrils <60 nm are found in tissues dominated by HA.[77] In tendon, decorin is noncovalently bound to GAG chains and connects adjacent fibrils every 68 nm; this provides the mechanical integrity enforced by the hierarchical structure of tendons.[95] GAGs have also been suggested to act as molecular cross-links between collagen fibrils, playing a significant role in tendon elastic and viscoelastic behavior.[96−99] However, recent studies have demonstrated that depletion of CS and DS, the two main GAGs found in tendon, has no significant effect on the elasticity and viscoelasticity of tendons,[100−102] and further finite element analysis indicates that the remaining HS and KS is not associated with the elasticity and viscoelasticity of tendons.[50] Computational models have been developed to assess the role of GAGs in the load transfer mechanics, with studies demonstrating that elastic modulus depends on the fibril length, fibril diameter, and interfibrillar distance.[103] Experimental analysis has shown that removal of 79% of the GAGs did not significantly change the tendon modulus, energy dissipation, stress at break, and strain at break, suggesting that GAGs cannot be considered mediators of tensile force transmission in human patellar tendon.[104] Finite element analysis has shown that relatively small quantities of GAGs, acting as collagen cross-linking elements, could provide mechanical integrity to tendons, while their partial enzymatic depletion should result in mechanical changes that are not reflected in analogous experimental testing, suggesting that GAG side chains of small leucine-rich proteoglycans are not a primary determinant of tensile mechanical behavior in mature rat tail tendons.[105] Using a modified shear-lag model, it has been demonstrated that the nonlinear mechanical response of GAGs leads to a distinct toe region in the stress−strain response of tendon, and when the fibril lengths are significantly larger than this length scale, the mechanical properties of the tendon are relatively insensitive to deletion of GAGs.[106] Yet again, as tendon tissue ages, a reduction in cellular concentration and/or reduction in cellular activity occurs, leading to a higher overall collagen concentration, lower GAG content, and a reduction in tissue elasticity,[107] continuing the debate whether directly or indirectly GAGs contribute to tissue elasticity/viscoelasticity. GAG content has also been shown to be developmental stage, aging, and activity dependent. For example, CS and HA dominate fetal tissues, while DS is the main GAG present in mature tendons.[108] Further, DS and CS content of the supraspinatus and bicep tendons gradually decrease with age, while HA content remains relatively constant.[77] Aging has been shown to be associated with a decline in physical activity and GAG content, whereas exercise is capable of reversing the age related decline of GAGs.[67,109] GAG composition may also be linked with the individuals' physical activity, as immobilization has been shown to be associated with reduction of GAG content.[110] The turnover of mature collagen and cross-links increases with heavy exercise;[111] collagen fibrils thicken and GAGs with galactosamine content increase,[112] resulting in an associated increase in tendon stiffness.[113] However, collagen turnover in response to exercise is much higher in immature tendon and is associated with a reduction in collagen maturation[111] and altered concentrations of HA.[114] Collectively, these studies demonstrate that GAG content is tissue, developmental stage, aging, and physical activity dependent, indicating that recapitulation of native tissue function through scaffold functionalization strategies is far more complex than currently considered.

GAGS IN TENDON PATHOPHYSIOLOGY

Injured equine superficial digital flexor tendons have increased cellularity and angiogenesis; loosely and irregularly packed collagen fibers; and increased DS and decreased HA and CS content.[115] However, human patients affected by chronic tendinitis exhibited considerably increased HA concentrations, increased DS content, and a relatively smaller increase in CS,[116] clearly indicating that GAG content is species and pathophysiology dependent. Degeneration of the equine superficial digital flexor tendons[117,118] and human Achilles tendons[119] is associated with increased levels of sulfated GAGs, collagen III, ECM turnover, and cellularity. Tendon tissues of athletes suffering from insertion tendinopathy were characterized by markedly increased amounts of acid mucopolysaccharides, degeneration of the collagen fibers, focal mineralization, and necrosis; while at the reparative zones, proliferating capillaries with collapsed lumen and prominent endothelial cells and basement membranes were evidenced.[120] In chronic Achilles paratenonitis of athletes, a slight inflammatory cell reaction was evidenced; the fatty areolar tissue was thickened, oedematous, with necrotic areas and profound adhesions; the blood vessels were degenerated with necrotic zones; increased anaerobic glycolysis was evident; and an increased amount of both neutral and acid mucopolysaccharides was found in the inflamed paratenon.[121] In a rat rotator cuff overuse model, sulfated GAG content was increased after 4 weeks and remained elevated for up to 16 weeks. Further, upregulation of decorin; versican; aggrecan; biglycan; collagen type I, type II, type III, and type VI; and SOX9 mRNAs and protein levels of heparin affine regulatory peptide, known to regulate developmental chondrocyte formation; were increased,[84] suggesting a shift toward chondrogenic phenotype. In a surgically induced supraspinatus

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tear in a rat model, collagen type I and type XII were greatly increased after injury and then decreased with time; collagen type III was detected in the scar tissue formed and remained for months; decorin and biglycan were increased initially and then decreased; aggrecan and collagen type II were detected and were associated with the expression of sulfated GAGs.[122] In human subjects, sulfated GAG content of normal supraspinatus tendon (major GAG was CS; small amount of DS and KS) was between three and ten times greater than that in the biceps tendon (major GAG was DS; small amount of CS; no KS). Although there was no difference in the concentration of HA between the tendons and as a function of age, in the supraspinatus tendon, there was a significant decrease in CS and DS as a function of age. In supraspinatus tendons with chronic tendinitis, HA, CS, and DS were significantly increased, while no difference was observed in KS.[77] Collectively, these data indicate that GAG content is tissue, age, and pathophysiology dependent, providing potential targets to prevent the progression of the pathology.

GAG-LOADED SCAFFOLDS

Given the importance of GAGs in normal and pathophysiological conditions, numerous qualitative and quantitative assays have been developed over the years, with a variable degree of accuracy (Table 3). Further, GAG-based functionalization strategies (Table 4) are at the forefront of tissue engineering and regenerative medicine, as GAGs have the ability to bind and modulate cytokines, growth factors, and ECM proteins; improve mechanical properties; and enhance cellular activities, such as attachment, proliferation, migration, and phenotype maintenance or direction toward a specific lineage.[123−126] In the following sections, advancements in scaffold functionalization with GAGs for tendon repair and regeneration will be discussed.

Table 3. Methods for GAG Qualitative and Quantitative Assessment in Tissues, Scaffolds, and Monolayers

	qualitative assessment	quantitative assessment
assessment of individual GAG content	lectin immunohistochemistry[127,128]	enzyme-linked immunosorbent assay[129,130]
assessment of total GAG content	alcian blue stain[131,132]	liquid chromatography (HPLC)[131]
	safranin-o stain[133,134]	mass spectrometry (MS)[135]
		dimethyl-methylene blue assay (DMMB) (sulfated GAGs only)[136,137]
		lectin microarrays[135,138]
		glyco-arrays[130]

Sulfated GAGs in Tendon Repair and Regeneration. Polysulfated GAGs have been studied as a beneficial therapy in the treatment of tendon injury and tendinitis, particularly in horses.[150−152] They have been shown to inhibit collagenase-induced degradation of proteoglycans, to increase fibroblast-mediated synthesis of hyaluronate, and to reduce inflammation and associated pain.[153−155] Preclinical studies, however, have presented conflicting results, which brings a level of doubt to the effectiveness of such therapy for tendon repair. For example, in one study injection of polysulfated GAGs into the superficial digital flexor tendons of rabbits following collagenase-induced injury reduced inflammation and restored ultimate strength, yield strength, and maximum absorbed energy to failure to uninjured levels.[152] However, no benefit was seen after treatment with polysulfated GAGs in Arabian horses with collagenase-induced tendinitis.[151] This highlights the necessary caution when translating small animal studies to large animal/human studies.

The majority of work performed using sulfated GAGs in the area of tendon repair and regeneration has been in the development of biomaterial scaffolds. The most extensively investigated is the collagen-GAG scaffold comprising bovine type I collagen and CS derived from shark cartilage.[124,141,156−160] For the treatment of tendon and ligament tissue damage in vitro, CS has been shown to upregulate collagen synthesis, hence suggesting CS accelerates healing following injury.[161] The coprecipitation of CS with collagen improves construct properties and has numerous advantages. Pure collagen gels are susceptible to deformation following manipulation;[143,159,162] alteration of properties with GAGs allows for easy modification of matrix stiffness without affecting other matrix properties.[143,159,162] Highly cross-linked collagen can often result in a weak material; copolymerization with GAGs increases the durability of collagen scaffolds and slows down the rate at which it is degraded in vivo.[163] The advantage of GAGs in biomedical implants is not limited to the alteration of properties and biomechanics of construct structure; it also improves the response of cells and healing to the

Table 4. GAG Functionalization Approaches and Benefits in Tissue Engineering and Regenerative Medicine

GAG	scaffold conformation	clinical target	functionalization method	benefits	ref
DS	membrane	cartilage	conjugation to polymer	can alter pore size and does not alter cross-linking ability	139
	scaffold	cartilage	mixing with polymer	does not alter porosity and scaffold thickness	140
CS	membrane	tendon	mixing with polymer	improves mechanical properties	124,141
	membrane	tendon	adsorption to membrane	does not change morphology	142
	sponge	tendon	mixing with polymer in acid solution	even CS dispersion and increases matrix stiffness	143
HS/ Heparin	membrane	bone	mixing with polymer	equal matrix distribution and increases matrix stiffness does not change membrane morphology and mineral content	86,144
	electrospun nanofibers	general	mixing with polymer	increased heparin incorporation in fibers and increased growth factor binding	145,146
HA	nanotubes	general	conjugation to phospholipids	equal dispersion in solution	147
	electrospun nanofibers	tendon	surface modification of polymer	increase water adsorption and mechanical strength	148
	hydrogel	general	addition of cross-linked HA to polymer	fast gelling and swelling, easier purification and drug loading	149

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construct.[159,164] The incorporation of CS to collagen sponges increases linear stiffness without affecting the structure and also results in upregulation of collagen type I, decorin, and fibronectin gene expression.[143] The addition of an outer collagen-GAG shell to an anisotropic collagen-GAG scaffold further improves mechanical strength without adversely affecting scaffold permeability, tenocyte attachment, proliferation, and viability.[141] More recently, another approach taken by one group was to incorporate extruded collagen fibers into the traditional collagen-GAG scaffold;[165] the scaffolds reinforced with fibers had higher mechanical strength; however, they did not reach the range of native tendon. Previously, collagen-GAG scaffolds were considered unsuitable for tendon repair as they generally have a relative density, which is incapable of withstanding tenocyte-mediated contraction.[141,160] However, recently this has been overcome, resulting in a greater scaffold diameter that correlates with a decrease in cell-mediated contraction.[157] Scaffolds with the greatest relative density and lowest cell-mediated contraction also showed increased gene expression for both scleraxis and tenascin-C, healthy tenocyte markers, thus showing that maintenance of scaffold integrity allows for increased tenocyte proliferation and collagen synthesis. In a chicken flexor model, surrounding the tendon repair site with the collagen-GAG membrane resulted in reduced formation of peritendinous adhesions,[158] while histological analysis showed a reduction in inflammatory cells and ECM fibroblasts from surrounding tissues.

HS and heparin are necessary for the attachment of growth factors to collagen structures[60,166] (Figure 2), and the incorporation of these specific GAGs has an effect on the assembly of collagen fibril structure in vitro.[167] The degree of sulfation of GAGs incorporated within collagen-GAG scaffolds has an effect on the binding of growth factors, with highly sulfated HS and moderately sulfated CS sequestering much higher amounts of growth factors than the nonsulfated HA.[124] Furthermore, collagen-HS scaffolds induced the greatest tenocyte metabolic activity even in metabolically limited culture conditions. Thus, manipulation of the differing levels of sulfation of GAGs can regulate binding of growth factors and reduce the dose required for the enhancement of bioactivity of tenocytes within scaffolds.[124,167] As more and more studies highlight limitations of surgery and repair, it is becoming more apparent that, particularly for complicated tissues such as tendon, multimodal approaches are necessary to combat all the requirements for healthy healing. Recently, a study was performed using a cocktail of materials to repair flexor tendon injury in dogs;[168] a heparin/fibrin gel was layered with an electrospun nanofiber poly(lactic-*co*-glycolic acid) (PLGA) mat. The heparin and fibrin combined provided a delivery platform for growth factors and cells, while the PLGA allowed for ease of surgical handling and manipulation of scaffold. Heparin was successful in its role of sustained growth factor release, even within the woven mat, having the opposite effect expected, with heparin within the mat releasing the attached growth factor faster than heparin alone. The degradation products of sulfated GAGs also play a vital role in the repair of injured tissue, with studies demonstrating that these oligosaccharides can modulate chemotaxis for migratory stem and progenitor cells following injury.[169] It may therefore be worthwhile to evaluate collagen-CS-HS scaffold combinations versus collagen-CS.

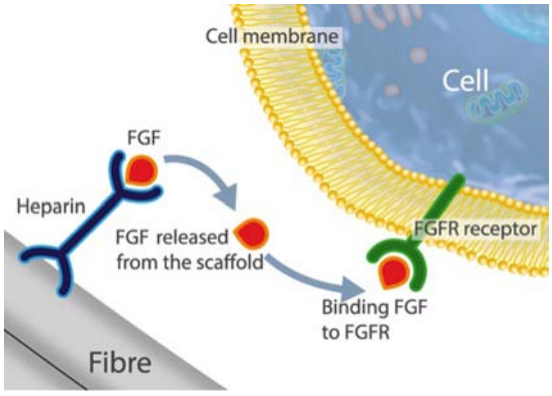

Figure 2. Functionalization of scaffold with GAGs and mechanism of growth factor sequestration.

In the case of tendon repair and regeneration, in the literature there is a lack of investigation into the use of KS and DS alone or within scaffolds. As KS is more commonly associated with corneal tissue, this is to be expected; however, DS is the main GAG present within tendon and warrants further exploration therapeutically.

Nonsulfated GAGs in Tendon Repair and Regeneration. HA has been demonstrated as a highly biocompatible material as it does not evoke an immune response in vivo due to its ability to alter immune cell behavior.[170] HA has several important roles during tendon healing and rehabilitation, including migration, proliferation, and differentiation of various cells, as well as adhesion prevention.[171−174] A commonly occurring postoperative complication, particularly debilitating in flexor tendon rehabilitation, is the formation of fibrotic adhesions between the tendon and surrounding tissues during repair.[175−178] These adhesions reduce tendon gliding capacity and, therefore, restrict range of motion in the affected body part.[179] Tendons coated or soaked in HA and later subjected to biomechanical testing have demonstrated reduced gliding resistance and thus may act to limit adhesion formation.[180−182] HA can prevent the formation of these adhesions by inhibiting mononuclear phagocyte and lymphocyte activity.[183] Prevention may also be attributed to the viscoelasticity of HA, allowing a scaffold to be formed around the surgical site.[176−178] HA triggers efficient healing by means of regeneration and growth, as opposed to the scarring and fibrosis associated with the natural tendon repair process.[184] Thus, HA is forming a physical barrier between the tendon and the surrounding environment. Many scaffolds have been developed in order to perform this function in the prevention of adhesion formation; however, many have caused an aggravated inflammatory response[142] or do not have the flexibility and strength required to facilitate tendon.[185] However, some groups have incorporated HA onto the surface of synthetic scaffolds in order to increase flexibility of their meshes.[146,148] Electrospun nanofiber poly(ε-caprolactone) (PCL) films were surface functionalized with HA; improved tensile strength[146,148] and increased elongation at break of up to 87% were measured.[179] HA grafted onto PCL reduces fibroblast attachment and migration compared to PCL alone, while having no effect on proliferation which is important for tissue repair,[148] and loading of HA into the highly porous mesh allowed for controlled release for further promotion of adhesion formation and release.[146] Proliferation and adhesion are improved on collagen membranes with the presence of HA, and thus can be used to improve culture conditions for cells on scaffolds in vitro.[186]

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Stimuli (e.g., temperature, pH, ionic strength, light, and chemical, biological, electric, or magnetic signals) responsive materials are under intense investigation for various tissue engineering and regenerative medicine applications.[187−196] Temperature-responsive and degradable HA/Pluronic hydrogels exhibited a sustained release of human growth hormone, which followed a mass erosion pattern.[197] Poly(*N*-isopropylacrylamide)-HA hydrogels have been shown to act as drug release,[198,199] tissue adhesion prevention,[200] and adipose tissue engineering[201] devices. Further, HA has been extensively investigated in order to tailor optimal microenvironments for cell-based therapies.[202,203] For example, a fibrinogen−HA conjugate hydrogel has been recently formulated as a unique minimally invasive injectable that can effectively transport viable cells or bioactive molecules into an ex vivo organ system.[204] Another novel injectable hydrogel system combines a temperature-responsive form of HA with Arg-Gly-Asp-Ser (RGDS) functionalized dendrimers to deliver bioactive peptides.[205]

HA has demonstrated the ability to suppress granulation and scar formation during healing of the tendon, preventing adhesions from occurring during the repair process in a variety of species in vivo including chicken, rabbit, and canine.[206−211] Specifically, electrospun PCL nanofibrous scaffolds coated with HA were investigated in rabbits and found to be superior to the FDA approved Seprafilm for adhesion prevention.[148] While Seprafilm is composed of hyaluronan and methylcellulose, it is degraded within a week in vivo, while the HA-PCL films resided for longer due to the slower degradation time of PCL and thus have more time to have a beneficial effect. HA can also be used as an injectable system and improves the mechanical properties of injured tendon via early termination of the inflammatory phase of tissue damage.[170] In a canine model, HA treatment of grafts for tendon repair demonstrates an improved clinical outcome due to the lubrication that HA provides facilitating reinforced tendon repair.[208] In order to maintain sufficient HA levels during the critical healing period and to reduce peritendinous adhesions forming, within the first 2 weeks of repair subsequent injections of HA are often required. In flexor tendon repair studies, it has been demonstrated that HA is eliminated in 7 days and therefore HA delivered via injection would require weekly administration.[212,213] However, HA derivatives may be appropriate alternatives, as they reside for longer time in vivo, maintain the same beneficial effect of adhesion formation reductions,[214,215] and have even been found to accelerate the overall healing time in rabbit tendon flexor models.[211,214] In a recent study, a combination of tenocytes and HA were delivered via injection into the rupture site of tendon in a rat model, resulting in improvements in tendon stiffness and mobility compared to HA alone or the saline group.[170] The addition of tenocytes led to a faster rate of recovery with a reduced inflammatory stage; this is thought to have led to the superior mechanical function versus the other groups.

Although there has been an abundance of favorable results regarding HA treatment for tendon repair and regeneration from preclinical studies, few so far have made it to the clinical stage of testing. Most clinical trials performed have been for the management of tendinopathies,[216−220] rotator cuff tears,[221−223] and a handful for the prevention of peritendinous adhesions.[224,225] In tendinopathies and overuse injuries, in which tendons have undergone degenerative changes,[226,227] treatment management is often conservative and can involve corticosteroid injections, nonsteroidal anti-inflammatory drugs (NSAID), and physiotherapy,[228] all of which have limitations and drawbacks. In the last ten years, several clinical studies have demonstrated favorable results following HA injection therapy and may eventually replace the traditional conservative actions in place today. For example, in one study a series of ultrasound guided HA injections were compared to physiotherapy; both improved pain scores initially, but the injections outperformed the physiotherapy group over time during treatment.[228] A retrospective study was performed on a range of athletes suffering with patellar tendinopathy,[217] while pain was seen to be reduced; there was no control group, and as such the efficacy of HA therapy in athletes should be investigated further in a randomized placebo controlled study. However, patients with tennis elbow and their clinicians found HA injections to be highly satisfactory in pain management and function, with improvement persisting up to a year after treatment.[229] Overall, HA therapy has provided general improvement in pain and function in tendinopathy cases, even after as little as a single injection,[216] and thus may be used in conjunction with physiotherapy or as an alternative treatment.[219]

A number of clinical trials have been conducted for the conservative treatment of rotator cuff injuries employing injectable HA, which is capable of increasing range of motion in patients, the majority being elderly with reduced capacity for regeneration.[222] Sodium hyaluronate injections were investigated as a conservative treatment for rotator cuff tears and were found to be an effective alternative to steroid injections.[221] Subacromial injection of sodium hyaluronate has also been examined in patients with rotator cuff lesions, but no actual tears, resulting in improvement of symptoms and a reduction in disability.[223] Functional and pain scores were better 6 weeks after treatment. Furthermore, a follow up study, almost three years later, determined that over 90% of patients had satisfactory results. Overall, injectable HA is a viable option for conservative treatment in tendon tears/ruptures where there is no possibility of surgery. However, it seems to be more effective in improving function outcome where no tears have yet formed and only lesions are present, and thus may be more appropriate for prophylaxis.

Hyaloglide, a biodegradable gel acting as a physical barrier for the prevention of the formation peritendinous adhesions between tendon and surrounding tissue, was evaluated for the prevention of adhesion formation following flexor tendon tenolysis.[224] It was found that Hyaloglide allowed patients to return to work and daily activities earlier than those in the control group and had superior recovery of finger motion. In a smaller study, 11 patients received three HA injections following flexor tendon repair.[225] In the short term, 3 weeks following treatment, there was no difference in functional outcome between the treatment and placebo groups; however, at three months and in the longer-term patients receiving injections had better improved function. Although investigation needs to be performed in much larger patient populations; as it stands, Hyaloglide is a much more promising approach in prevention of adhesion formation and may drastically improve postoperative outcomes in the future.

■ ALTERNATIVE GAG MIMETICS

Given that GAGs are animal extracted proteins, batch-to-batch variability, low yield, and ethical issues have stimulated investigation into alternative molecules with similar properties. Specific structural domains in GAGs are responsible for their

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Table 5. Biological Properties of GAG Alternatives

molecule	biological properties	ref
RGTA − Sulfated	increases proliferation; stimulates differentiation; enhances cell adhesion, migration and self-renewal; anti-inflammatory effect; promotes angiogenesis; enhances tissue repair and regeneration; prevents collagen III overexpression associated with fibrosis; reduces ischemia-induced necrosis	233,235−239
DxS − Sulfated	anti-inflammatory effect; binds to heparin growth factors; enhances matrix deposition, when used as macromolecular crowding agent	240−242
HA mimetics − Nonsulfated	increase cell proliferation; support chondrogenic differentiation; reduce gliding; anti-inflammatory effect; reduce fibrosis; enhances tissue repair and regeneration; improve mechanical properties of scaffolds; improve tissue biomechanics; reduce tendon thickness and improve nuclear and fibrillar appearance; reduce fibrosis and edema	215,243−246
Ficoll − Nonsulfated	increases matrix deposition in vitro when it is used as macromolecular crowding agent; improves cell attachment and metabolic activity when it is used as functionalized molecule	247,248

biological activities; the sulfation pattern, for example, determines the binding specificity to heparin binding factors and enzymes.[230] Accordingly, compounds with analogous structural components and with the capacity to fulfill the same biological functions are under development and investigation as GAG mimetics (Table 5). Generally, the use of these mimetic compounds has resulted in faster and improved healing in several in vivo models.[231] Further, several GAG mimetics have been shown to induce organized angiogenesis at the site of implantation[232] and increased proliferation and differentiation in comparison to their natural analogues.[233,234] Evidence also suggests that GAG mimetics have the potential to recruit stem cells to injured areas during tissue remodeling as well as activating resident cells.[231]

Sulfated GAG Mimetics in Repair and Regeneration. Although synthesis of sulfated GAG mimetics is difficult due to their diverse and complicated structure, three methods have arisen based on the sulfation system used. The first one is based on the sulfation reagent sulfur trioxide, which sulfates molecules in a homogeneous reaction; however, it initiates cleavage of glycosidic bonds, acid labile groups, and causes partial depolymerization.[249,250] The second uses chlorosulfonic acid in a heterogeneous reaction mixture; however, sulfated polysaccharides with sulfuric or chlorosulfonic acids yield impure and potentially toxic products due to severe depolymerization.[249,250] The third system overcomes these problems by employing a nitrogen base-free sulfation-free protocol, which utilizes a neutral acid scavenger 2-methyl-2-butene. A number of synthetic sulfated dextran polymers (structural mimics of heparin) have been synthesized using this method.[249] These polymers contain a polysaccharide backbone with similar carboxylate and sulfate group content to heparin and have been shown to enhance skin[239,251] and bone[252] regeneration. More recently, they have been shown to stimulate cell differentiation superior to that of heparin.[233,236] Given the diversity and vast range of functions that GAGs offer, a substantial amount of work is focused on adopting specific structural characteristics of GAGs to tailor the bioactivity and pharmacology of compounds for specific functions.[253] For example, the RGTA OTR4120 [Note: ReGeneraTing Agents (RGTAs) are a family of polymers engineered to protect and stabilize heparin-binding growth factors] promotes both cell migration and osteogenic differentiation, but not cell proliferation,[236] while RGTA11 has a strong proliferative effect of osteoblastic cells.[252] The antithrombotic properties of heparin have been attributed to the binding of antithrombin III (AT-III) to heparin. This interaction is modulated by an abundant polysaccharide chain with specific uronic and sulfation patterns that generates high affinity and specificity to AT III.[254] Arixtra is an FDA approved heparin mimetic drug that has been used to treat deep vein thrombosis.[254] Heparin mimetics are also valuable replacements of the natural GAG in medical devices due to their high resistance to glycanases, which are activated in injured tissue, resulting in the degradation and digestion of heparin/HS in vivo.[255] Thus, mimetics can enhance growth factor bioavailability at the site of injury and/or implantation of functionalized scaffolds. Several synthetic hexasaccharides have been proposed as potential replacements for HS; the degree of sulfation has been shown to influence their flexibility.[256] Low-molecular-weight dextran sulfate (DxS) has been found to be analogous to HS[257] and to exhibit a stronger anti-inflammatory response than HS, DS, and CS.[240] It has also been shown to inhibit dendritic cell activity, commonly associated with transplant tissue rejection, by preventing dendritic cells from reaching maturity and presenting attracter antigens to other cells associated with the immune system.[258] Furthermore, DxS has been shown to greatly inhibit the classical, alternative, and lectin complement pathways, protecting cells from rejection by the body post implantation.[209,210] However, despite all these promising results, the potential of such technologies in tendon repair and regeneration has still to be assessed. DxS and carrageenan have also been used as macromolecular crowding agents to enhance matrix deposition in cell culture,[241] with carrageenan to be more effective due to its inherent polydispersity and the associated more effective volume exclusion effect.[242]

Nonsulfated GAG Mimetics in Repair and Regeneration. Endogenous HA is limited by its short half-life and is degraded rapidly, meaning in vivo its beneficial effect on tendon healing may be limited, being eliminated before its full potential is achieved.[259] Thus, a range of HA derivatives have been developed to overcome the short half-life associated with HA, while retaining mechanical and hydration properties[260] for improved and prolonged therapeutic effect. Several injectable HA derivatives are commercially available at present: Synvisc is used for intra-articular repair, and Restylane, Juvederm, Teosyal, and Glytone are used as dermal fillers. Such derivatives are synthesized via chemical conjugation or cross-linking, both of which have three main target sites: the carboxylic acid group, the hydroxyl group, and the N-acetyl group.[260] The main types of reactions used to target the carboxylic acid groups include amidation, Ugi condensation, esterification, and oxidation; for the hydroxyl group, esterification and ether, carbamate, or hemiacetal formations are carried out, while targeting of the N-acetyl group involves deacetylation or amidation. Amidation of the carboxylic acid group of HA with carbodiimides is the most commonly used method of HA modification; Hylan G-F 20, an FDA approved HA compound (Synvisc) composed of chemically cross-linked Hylan polymers, was surface modified with carbodiimide and demonstrated improved tendon gliding ability over the unmodified form.[243] Carbodiimide modified HA is not as water-soluble as normal HA, and thus is retained

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within tissues for longer; this surface modification does not alter its actions to improve tendon gliding and hence function.[261−263] It may serve as an elimination-resistant alternative for the treatment of peritendinous adhesions in tendon repair and regeneration applications. Ficoll (a neutral, highly branched, high mass, hydrophilic polysaccharide formed by the copolymerization of sucrose and epichlorohydrin) has been suggested as a nonsulfated alternative of HA and has been shown to induce increased matrix deposition in vitro when it is used as a macromolecular crowding agent[247] and to improve cell attachment and metabolic activity when incorporated into collagen I films.[248]

CONCLUDING REMARKS

Sulfated GAG-loaded scaffolds have the capacity to favorably modulate cell behavior, enhance tendon-specific ECM synthesis, and improve growth factor delivery to injured tissues and residual cells, thus promoting tissue regeneration in tendons. HA has been shown to reduce peritendinous adhesions and trigger efficient healing. Research and development in GAG mimetics is slowly taking off, given that preliminary data suggest greater growth factor protection and function. Stimuli-responsive HA hydrogels are a promising method of injecting therapeutics and viable cell populations at the side of injury in a minimally invasive manner with very promising results in preclinical models and clinical setting. It is evidenced that GAGs and GAG-related moieties are at the forefront of scientific and technological innovation, given their great potential for tendon repair and regeneration. Better delivery and linking systems are under investigation to ensure sustained and localized delivery of these valuable molecules.

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Notes

The authors declare no competing financial interest.

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