Introduction

In 2009, chronic wounds had an incidence rate of 6.5 million cases annually in the USA, and $25 billion was expended on their treatment.1 Audits of community healthcare clinics in the UK in 2005–2007 showed wound care costs ranging from £2.5 to 3.1 million per 100,000 patients or 2%–3% of local healthcare budget expenditure.2 Unfortunately, almost half of these wounds are nonresponsive to current treatment strategies and they represent a challenging clinical problem.3 Early and late complications are frequent causes of morbidity and mortality in the clinical treatment of wounds.4,5 Chronic wounds have an incidence rate of 120 per 100,000 people aged over 60 years.6,7 Population dynamics calculated by The World Bank indicates that this latter age group is steadily increasing on a global scale.8 The cost of treating chronic wounds is escalating due to such aging trends and increasing rates of diseases such as diabetes and obesity, which prolong the treatment of chronic wounds.9 There is a clear need to better understand the underlying pathobiology of chronic wounds in order to formulate more effective means of treating these conditions.8 Without such basic studies and their application in the clinic, no meaningful progress will be made in the treatment of wounds.

Definition of wound healing. Mason and Dunhill29 proposed that the healing response should be considered as a replacement or regeneration of human cells, tissue, or organs to restore normal tissue function.30 The use of the term healing in the context of wound repair, however, is actually a misnomer. As already stated, healing infers the replacement of damaged tissue with one equivalent in composition, structure, and function to that of the original tissue. By this definition, the only tissue that actually heals in the human body in the strictest sense is bone, and all other wounds heal by variable degrees of collagenous scar formation, which undergo maturational changes to provide a repaired tissue; however, the collagen fibrillar arrangements within it never return to their original structural orientations, and consequently, the functional properties of the repaired tissue only approximate those of the original tissue. Fetal wounds that heal seamlessly without scar tissue formation are an exception. Hyaluronan (HA) is a major glycosaminoglycan (GAG) in fetal wounds.
Polymeric HA inhibits platelet degranulation and downregulates inflammation and excessive collagen deposition, which contribute to scarless healing. In contrast, in adult wound healing, chondroitin sulfate (CS) is the major GAG, and platelet degranulation, inflammation, and collagen deposition at the wound site are essential components of the adult wound healing process but contribute to scar tissue formation.

GAGs and wound healing. Four classes of GAGs have been identified, including HA, CS, keratan sulfate (KS), and heparin sulfate (HS). These are composed of characteristic repeat disaccharides, with specific monosaccharides sulfated at C-2, -3, -4, or -6 (Fig. 1). All of these GAGs are widely distributed in connective tissues. An additional carbohydrate recognition motif human natural killer-1 (HNK-1) has a relatively restricted distribution mainly in the peripheral nervous system (PNS)/central nervous system (CNS). HNK-1 has a widespread distribution in neural tissues and is a component of proteoglycans (PGs), cell adhesion molecules, bioactive lipids, structural glycoproteins, and key synaptic enzymes (Fig. 2).

The phases of wound healing. Wound healing is a dynamic process with some complexity where devitalized and degraded tissue is replaced with new functional tissue. GAGs have roles to play in all phases of wound healing.

Phase 1. The initial stage of wound repair lasts one to two hours with formation of a transitional fibrin matrix through activation of the coagulation cascade, cleavage of fibrinogen by thrombin, and formation of a fibrin clot (Fig. 3). HA synergizes with thrombin and promotes clot formation. Soluble fibronectin is cross-linked into the clot, and platelets, immune cells, and mast cells are attracted into this tissue. Clot formation is countered by enzymes of the fibrinolytic cascade, which ensure that excessive thrombus formation does not occlude blood flow. PDGF and TGF-β released from the platelet α-granules and other mediators released at the wound site such as epidermal growth factor (EGF) and IGF-I act as chemoattractants for neutrophils, smooth muscle cells, and fibroblasts and induce cellular proliferation, differentiation, and matrix synthesis. Serpins ensure that excessive fibrinolytic activity does not occur. GAGs have specific roles to play in the regulation of serpins (Table 1). Heparin and HS promote the inhibitory capability of many of the serpins. Antithrombin (AT) (SERPINC1), heparin cofactor II (HCII) (SERPIND1), plasminogen activator inhibitor-1 (PAI-1), protein C inhibitor (PCI) (SERPINA5), and protease nexin-1 (PN-1) interact with heparin and HS, leading to conformational rearrangements, which improve inhibitory performance. The antithrombotic activity of AT stems from its ability to inactivate thrombin, Factor Xa, and Factor IXa (Fig. 3). In the presence of heparin, the rate of inhibition of thrombin by AT is increased 2000- to 4000-fold, the rate of inhibition of Factor Xa is increased 500- to 1000-fold, and the rate of inhibition of Factor IXa is increased 1 million fold. Kallistatin, a kallikrein inhibitory protein of endothelial cells and SMCs, and α₁-proteinase inhibitor also interact with GAGs. Proteases (chymase, trypsin, leukocyte elastase, and cathepsin G) are complexed with granule PGs (serglycin) in mast cells and leukocytes, and degranulation releases these active proteases, which degrade the extracellular matrix (ECM), and inactivates microbial infection at the wound site. Macrophages attracted into the wound site mop up the cellular and ECM debris.

Phase 2. The next phase of wound repair is an inflammatory phase acting over the next 24 hours involving members of the kallikrein protease family, which release kinins at the wound site and other vasodilatory compounds (histamine, prostaglandins, and leukotrienes), increasing vascular permeability. Leukocytes (neutrophils and monocytes), mast cells, neutrophils, and monocytes gain access to the wound site. Neutrophils release IL-1 and TNF-α, inducing inflammation. Neutrophil collagenase and elastase remove damaged tissue from the provisional matrix of the wound site. Mast cells also degranulate at the wound site releasing chymase and tryptase. Monocytes transform into macrophages and phagocyte fragments of denatured ECM debriding the wound site and inactivating any source of microbial infection. They also secrete TGF-β, PDGF, FGF-2, IL-1, and TNF-α, which modulate collagen deposition by fibroblasts and penetration of new blood vessels into the wound site. T-lymphocyte migration into the wound site delivers lymphokines, which modulate fibroblast and endothelial cell activity; fibroblast-activating factor is a stimulatory factor, while IFN-γ is a negative regulator.

Phase 3. The laying down of new matrix by fibroblasts over the next two to three days restores tissue at the wound site. Matrikines and matriçryptins generated at the wound site act as chemotactants, while other matriçryptins act as angiogenesis inhibitors or stimulate collagen synthesis. Fibroblasts, endothelial cells, and keratinocytes produce IGF-I, FGF-2, TGF-β, PDGF, and vascular endothelial cell growth factor (VEGF), promoting cellular migration, proliferation, matrix synthesis, and angiogenesis. A number of PGs are laid down in the wound repair site, and their GAG side chains have roles in the sequestration, stabilization, and activation of these growth factors. The PGs act as scaffolding material in the wound site providing architectural form and stability and a matrix for cellular attachment. Some PGs (hyalurons) form ternary complexes with HA hydrating the tissue promoting cell survival and migration into the repair site.

Phase 4. The final stage in wound repair is a maturational remodeling stage where recovery of normal tissue form and function occurs. This may be over a protracted period of time up to one year after the initial wounding. New collagen fibrils laid down in the repair zone undergo cross-linking to stabilize collagen fiber bundles and MMPs remodel this repair tissue.

The role of HA in wound healing. HA is abundant in cutaneous wounds and promotes the migration of keratinocytes into the wound site to undertake reepithelialization (Fig. 4). HA (>30 kDa) is a component of the fibrin clot in wound sites synergizing with thrombin during fibrin clot
Figure 1. Structures of glycosaminoglycans.
Figure 2. GAG organization in selected neural biomolecules.
Table 1. Proteins and physiological processes regulated by GAGs.

<table>
<thead>
<tr>
<th>MATRIX ASSEMBLY</th>
<th>MATRIX REMODELING</th>
<th>CELL REGULATION</th>
<th>PROTEINASE INHIBITION</th>
</tr>
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<tbody>
<tr>
<td>Laminin</td>
<td>Cathepsin D</td>
<td>FGFs</td>
<td>Kallistatin</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Cathepsin K</td>
<td>HGF</td>
<td>ITI/bikunin</td>
</tr>
<tr>
<td>Collagen type I</td>
<td>Thrombin</td>
<td>VEGF</td>
<td>SLPI</td>
</tr>
<tr>
<td>III, IV, V, VI,</td>
<td>ADAMTS4</td>
<td>PDGF</td>
<td>TFPi</td>
</tr>
<tr>
<td>IX, XV, XVIII</td>
<td>ADAMTS5</td>
<td>IL-2, IL-7, IL-8</td>
<td>α1-PI</td>
</tr>
<tr>
<td>Fibrillin-1</td>
<td>Mast cell Chymase</td>
<td>TGF-β/Wnt</td>
<td>TPA</td>
</tr>
<tr>
<td>Elastin</td>
<td>Mast cell Tryptase</td>
<td>BMP-2, 4, 7</td>
<td>PAI-1</td>
</tr>
<tr>
<td>Decorin</td>
<td>Leucocyte elastase</td>
<td>Heparanase</td>
<td>AT-III</td>
</tr>
<tr>
<td>Biglycan</td>
<td>Leucocyte Cathepsin G</td>
<td>SHH</td>
<td>HCII</td>
</tr>
<tr>
<td>Fibromodulin</td>
<td>Kallikrein</td>
<td>IHH</td>
<td>PC-1</td>
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<tr>
<td>LTBP1, LTBP2</td>
<td></td>
<td></td>
<td>PN-1</td>
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</tbody>
</table>

Abbreviations: LTBP1/2, latent transforming growth factor-β binding proteins 1 and 2; ITI, inter-α-typsin inhibitor; ATIII, antithrombin III; TFPi, tissue factor proteinase inhibitor; TPA, tissue plasminogen activator; PAI-1, plasminogen activator inhibitor-1; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; VEGF, vascular endothelial cell growth factor; PDGF, platelet-derived growth factor; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; α1-PI, α1-proteinase inhibitor; SHH, sonic hedgehog; IHH, Indian hedgehog; PN-1, protease nexin-1; PC-1, proteinase C inhibitor; HCII, heparin cofactor II.

Figure 3. Accumulation of HA in cutaneous wounds. Induction of HA synthesis in the wound site and adjacent areas, which serve as a source of keratinocytes that migrate into the wound site to speed up the re-epithelialization. (A) Normal mouse skin distant to the wound site. (B) Skin adjacent to the wound. (C) The wound site three days after wounding. Normal mouse epidermis has very little HA, but it increases adjacent to the wound site and is associated with epidermal thickening (increased cellular proliferation). Dermal tissue is always rich in HA. Figure supplied courtesy of Professor R.H. Tammi and Professor M. Tammi, Department of Biomedicine and Anatomy, University of Eastern Finland, Kuopio, Finland.
not increase TGF-β1 content in the wound or tissue fibrosis. High molecular weight is anti-inflammatory, whereas low molecular weight (≤50 kDa) is proinflammatory. Low molecular weight HA is proangiogenic and synergizes with VEGF to promote wound repair. High molecular weight HA promotes the healing of diabetic foot ulcers through the action of IL-10 with adult fibroblasts, a potential mean of improving scarless wound healing. HA inhibits fetal platelet aggregation and the release of TGF-β at wound sites contributing to scarless healing and formation of the fibrin clot, which provides hemostasis during the early stages of wound repair (Fig. 4).

**GAG complexity and biodiversity and their roles in wound healing.** Four classes of GAGs, including CS, KS,
### Table 2. Proteoglycans involved in hard and soft tissue repair.

<table>
<thead>
<tr>
<th>PROTEOGLYCAN</th>
<th>GAG COMPONENTS</th>
<th>FUNCTIONAL PROPERTIES</th>
</tr>
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<tbody>
<tr>
<td>Aggrecan</td>
<td>~100 CS chains</td>
<td>HA-aggrecan/versican link ternary complexes hydrate tissues and provide ECM stabilisation and a hydrated matrix conducive to cell attachment and migration</td>
</tr>
<tr>
<td>Versican</td>
<td>20–30 KS chains</td>
<td></td>
</tr>
<tr>
<td>HA*</td>
<td>HA non sulphated GAG</td>
<td>Tissue hydration, regulation of cell signalling, cell proliferation/migration, properties are size dependent, large Mw HA is anti-inflammatory, low Mw HA is pro-inflammatory</td>
</tr>
<tr>
<td>Decorin</td>
<td>1 CS/DS chains</td>
<td>Collagen fibrillogenesis, ECM organisation, interaction with growth factors/cytokines, plasma membrane receptors-cellular regulation, interaction with focal adhesion and cytoskeletal proteins modulate cell shape and migration, roles in biomineralisation</td>
</tr>
<tr>
<td>Biglycan</td>
<td>2 CS/DS chains</td>
<td></td>
</tr>
<tr>
<td>Fibromodulin</td>
<td>Up to 3 KS chains</td>
<td></td>
</tr>
<tr>
<td>Perlecan</td>
<td>3 HS chains (endothelial perlecan), some perlecans are hybrid CS/HS proteoglycans (cartilage, SMC) epithelial perlecan contains HS/CS/KS (keratinocyte)</td>
<td>HS growth factor interactions with FGF, VEGF, PDGF promote angiogenesis and wound repair, interactions with BMPs/morphogens regulate tissue development, matrix stabilisation in avascular tissues (cartilage), 4,6-di-sulphated CS in growth plate perlecan regulates collagen fibrillogenesis, major base ment membrane and vascular (endothelial) proteoglycan</td>
</tr>
<tr>
<td>Syndecan</td>
<td>1–2 CS or HS chains</td>
<td>Links cytoskeleton to ECM, roles in cell signalling and tissue morphogenesis</td>
</tr>
<tr>
<td>Thrombomodulin</td>
<td>1 CS (HNK epitope)</td>
<td>Regulates activation of Protein C, Protein S which act as anticoagulants inactivating Factors Va and Vill</td>
</tr>
<tr>
<td>CD44</td>
<td>CS (HS, epicand CD44 variant)</td>
<td>Widely distributed cellular HA receptor with a cytoplasmic tail which interacts with the cytoskeleton and effects cell signalling, modulates cell adhesion</td>
</tr>
<tr>
<td>Serglycin</td>
<td>CS, DS, heparin, HS GAG chains vary with cell type (macrophages, mast cells, leucocytes)</td>
<td>Intracellular proteoglycan, storage of granule protease activity (tryptase, chymase, cathepsin G, leucocyte elastase), regulation of TNFα in macrophages, release of immune and inflammatory mediators (histamine, prostaglandin, leukotrienes), regulation of immune response and inflammation focally in tissues</td>
</tr>
</tbody>
</table>

**Note:** HA is a macromolecular GAG and not a proteoglycan by definition but displays interactive with and has similar properties to proteoglycans in tissues. **Abbreviations:** GAG, glycosaminoglycan; HA, hyaluronan; CS, chondroitin sulfate; HS, heparan sulfate; KS, keratin sulfate; DS, dermatan sulfate.

dermatan sulfate (DS), and heparan sulfate (HS)/heparin, have been identified (Fig. 1). A further GAG, HA, occurs devoid of a core protein and is synthesized at the cell surface and extruded out of the cell into the ECM. All other GAG members are synthesized by a complex series of biosynthetic steps in the endoplasmic reticulum with 20+ enzymes involved in HS biosynthesis, while CS requires ~10 biosynthetic enzymes. GAGs have diverse regulatory roles in ECM assembly, proteinase regulation, cell regulation through the action of growth factors, cytokines, and transcription factors, and proteinase inhibition in the coagulation/fibrinolytic systems relevant to wound repair (Table 1).

GAGs display a considerable degree of structural diversity and complexity encoding a sophisticated information system, which directs cellular behavior. Potentially, 1008 different pentasaccharide sequences are possible in CS/DS and 2916 pentasaccharide sequences are possible in HS.\(^{22}\) GAGs can explore a varied number of interactive spatial orientations. Their structural form has persisted virtually unchanged throughout vertebrate and invertebrate evolutions.\(^{23}\) GAGs have essential life-preserving roles to play in a diverse range of physiological processes involving chemokines, morphogens, and growth factors. GAGs are composed of characteristic repeating disaccharides, linear sequences of five to six monosaccharides, typically provide interactive properties for a specific ligand. GAGs are assembled from a limited number of monosaccharides and amino sugars into characteristic disaccharide repeat units (Figs. 1 and 2). Modifications such as N-deacetylation, N-sulfation, epimerization of glucuronic acid (GlcA) at C5 to iduronic acid (IdoA), O-sulfation at C2 of IdoA and GlcA, C6 of GlcNAc, and C6 of galactose provide a further level of complexity. It is estimated that 3000 GAG determinants are present in the human genome and an additional 4000 theoretical pentasaccharide sequences are possible.\(^{24}\) Heparin is the most heterogeneous GAG and contains the highest levels of structural modification, and HS has a related structure but contains areas of high modification interspersed with areas of low modification. HA is the simplest GAG, but unlike all other GAGs, it is not attached to a PG core protein and is nonsulfated. HA is composed of β1-3- and β1-4-linked GlcN and GlcA disaccharides with as many as 10,000 disaccharides assembled to form HA of a molecular size in excess of 5 MDa. HA has a widespread distribution.\(^{25}\) Despite its relative simplicity of structure, HA is interactive with a diverse range of ECM and cellular proteins, which impact on a number of important physiological processes including wound repair. HA also has high water regain properties and is important in the hydration of tissues providing a highly interactive and hydrated matrix conducive to cell survival and cellular migration. HA is a prominent component of skin and one of its most abundant components with major roles in skin repair (Fig. 4).

**Bone repair.** After bone fracture, coagulation and inflammatory phases occur in bone similar to those seen in...
the repair of subcutaneous wounds. A hematoma is initially formed in the fracture site, and fibroblasts and platelets are attracted into the fibrin clot and lay down a transient soft cartilaginous callus with collagen and PGs providing structural support. Biglycan, lumigan, aggrecan, perlecan, syndecan-2, and syndecan-4 have been identified in the cartilaginous fracture callus. HA also has roles to play in all stages of fracture repair, particularly in the initial hematoma formation and in the regulation of inflammatory conditions and the influx of cells into the fracture site. Thus, HA, HS, CS, and DS have roles to play in fracture repair, their roles in fracture repair are outlined below. Fracture healing is a complex physiologic process that involves the coordinated participation of several cell types. Fracture healing can be categorized into direct healing of the fracture site through internal remodeling processes or indirect healing through formation of a transitional callus matrix at the fracture site, which undergoes progressive stages of remodeling to become bone. Like cutaneous wound healing, fracture repair also occurs through a number of identifiable stages. GAGs have roles to play in all stages of the fracture repair process. 

Stage 1: hematoma formation. The first stage is the formation of a hematoma at the fracture site to effect hemostasis, inflammation at the fracture site, followed by the laying down of a soft callus and then a hard callus and remodeling of the fracture repair tissue to produce bone. These stages are represented schematically in Figure 5.

Transformation of the hematoma into granulation tissue. After initial hematoma formation over a few hours, inflammation occurs at the fracture site over the ensuing one to seven days. Degranulation of platelets and an influx of inflammatory cells into the fracture repair site occur in a similar manner to that described for cutaneous wound healing. Granulation tissue eventually replaces the hematoma, and osteoclasts remove necrotic bone fragments. Intramembranous

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**Figure 5.** Composite figure depicting the complexity of the coagulation cascade reactions, which regulate blood clot formation in wounds. The interrelationship between the intrinsic pathway (A), extrinsic pathway (B), and events in the endothelium (C) and in fibrin network assembly (D) are shown. Thrombomodulin is a GAG-substituted protein, which has important roles in the regulation of the procoagulant proteins, protein C and protein S. Thrombin and Factor Va have central roles in fibrin network assembly (D). The reactions surrounded by the central dotted pink area in this figure are those which occur on the platelet surface. Key steps in the generation of Xa, Va, and VIIIa in the cascade are modulated by the GAG-regulated serine proteinase inhibitory protein ATIII (AT). Kallistatin is another tissue serine proteinase inhibitory protein regulating the formation of kallikrein, which feeds into the coagulation cascade in early stages of the intrinsic pathway and also regulates the generation of kinins from kinogen, which regulates vasodilation in early stages of wound repair. The APC and protein S are anticoagulant proteins, which regulate Factors Va and VIIIa. Hyaluronan also regulates fibrin assembly in D. ATIII and APC–protein S are highlighted by a yellow flash symbol to highlight their important contributions to the regulation of the coagulation cascade and the key roles they play in wound repair.
ossification then occurs by progenitor cells from the periosteum/endosteum, which differentiates to become osteoblasts forming stabilizing bone collars around the fracture site and filling the medullary canal.

Stage 2: soft callus formation. On days 7–14 of fracture repair, soft callus eventually replaces the hematoma, ingrowth of capillaries into the soft callus also occurs, and an increased vascular supply to the fracture repair site is also evident. This is an important aspect of the fracture repair process and, as in cutaneous wound repair, is regulated by a number of GAGs. Mesenchymal stem cells migrate into the soft callus and differentiate into fibroblasts and chondrocytes, which produce characteristic ECM components supporting the tissue repair. Thus, the soft callus is converted into a cartilaginous GAG and collagen-rich matrix. A number of PGs support this repair process including syndecan-2, syndecan-4, aggrecan, and biglycan. Syndecan-2 is upregulated in syndecan-4 knockout mice. Wnt-3a upregulates syndecan-2, while TNF-α elevates syndecan-4 levels in vitro. Deficient TNF-α signaling impairs intramembranous bone formation during fracture repair. Biglycan and decorin both interact with TNF-α and may have a regulatory role over this inflammatory cytokine in a similar manner to how they regulate TGF-β. Biglycan also sequesters endostatin promoting the vascularization of the callus repair tissue but also has roles in the formation of the hard callus. Biglycan knock-out mice display an osteoporotic phenotype, consistent with an increased osteoclast differentiation, a delayed osteogenesis, and an impairment in osteoblast activity demonstrating biglycans regulatory properties in bone formation, remodeling, and repair.

Stage 3: hard callus formation. After the soft callus tissue links the ends of the fractured bone, hard callus formation begins. Intramembranous bone continues to form in the bone collar, the soft callus undergoes endochondral ossification, and the soft callus is gradually converted into rigid calcified tissue. This begins peripherally at the margins of the fracture site where the tissue is under less strain and reduces the strain in a more central location, and this region also undergoes bony callus formation; thus, the fracture repair tissue is replaced by woven bone attached to the original cortical bone.

Stage 4: remodeling of the hard callus. Once the fracture site is replaced with solid woven bone, a slow replacement of this tissue commences with the laying down of lamellar bone, remodeling continues till the bone returns to its original morphology, and the medullary canal is also restored.

Animal models which have been developed to examine the stages of fracture repair and therapeutic interventions to improve repair processes. The rat and mouse are popular models for the examination of a number of aspects of fracture repair. A number of inbred mouse strains have been examined for this purpose as have a number of knockout mice for the examination of a range of biological agents to enhance fracture repair. Rodent models of nonunion have also been developed to examine biological strategies to improve fracture union and the identification of therapeutic targets.

Angiogenesis makes a major contribution to the healing of skin wounds but less so in the healing of relatively avascular tensional and weight-bearing connective tissues such as tendon, intervertebral disk, meniscus, and cartilage. These tissues also have high internal pressure, tough collagenous ECMs, and high PG contents refractory to the penetration of blood vessels, which might otherwise improve healing responses. When these tissues are damaged, the PGs undergo proteolytic processing, and a diminution in their density results in a reduction in the internal hydrostatic pressure and a matrix more conducive to the penetration of blood vessels. The GAG side chains of PGs have antiangiogenic activity, which prevents penetration of blood vessels, and are inhibitory for repair processes in neural tissue.

Cartilage repair. Unlike skin and bone, cartilage does not contain blood vessels. Oxygen and nutrients are delivered by diffusion limiting cartilage’s ability to grow and recover from traumatic damage. Small, full-thickness cartilage defects may undergo repair by the laying down of a fibrocartilaginous matrix in the defect site; however, this tissue does not reproduce the functional properties of cartilage and ultimately fails. The perichondrium surrounding the cartilage rudiments is also capable of reforming new cartilage through the action of resident progenitor cell populations. The perichondrium develops into the periosteum in the adult, which has strong bone regenerative capability.

Tendon repair. Tendons were once considered to be a comparable tissue to cartilage and inherently incapable of repair due to their avascular nature. However, it is now known that spontaneous healing can occur via proliferation of the epitenon and endotenon or, extrinsically, by invasion of cells from the synovium. Tendons (and bone) show improved healing with a limited level of stress and motion of the defect site during wound repair, emphasizing the stressful environment tenocytes require to maintain optimal function. Exercise-based rehabilitation programs are designed to accelerate tendon healing and maturational changes to improve the final strength of the repaired tendon. Application of stem cells in tendon repair is also a promising area as is the use of bioscaffolds.

Repair of neural tissue. In contrast to the aforementioned tissues where PGs support tissue repair, CS-PGs (lecticans) are refractory to repair in nervous tissues. The CNS and PNS have differing repair potentials. The PNS has an intrinsic capability to regenerate, while the CNS does not. Neurons are the principal functional cell type in the CNS and PNS and represent ~10% of the total cell population, and accessory glial cells (neuroglia, nerve glue) have a supportive role to play reinforcing, repairing, and insulating the nerve cells. Glial cells include astrocytes, star-shaped cells, which physically support the neurons, provide nutrition, and...
maintain its electrolyte balance, small mobile irregular shaped microglial cells have phagocytic roles during neural repair. Oligodendrocytes encapsulate nerves in the CNS in myelin and aid in their nutrition. Schwann cells in the PNS have an equivalent role to oligodendrocytes. Following injury, glial cells lay down a glial scar in the defect site as part of a repair response, neurocan, brevican, and versican are upregulated in the glial scar for up to two months following injury, and their CS side chains provide repulsive signals acting as a molecular barrier, which inhibits nerve ingrowth through the glial scar. The CS-PGs form aggregate perineural net structures through N- and C-terminal interactions with HA and tenascin-R, which cushion the neuron and are crucial for synaptic functional properties. Removal of CS chains from these PGs by chondroitinase ABC in models of axonal damage allows regrowth of severed axons and synaptic rearrangements, which restore neuronal function.72-78 The HNK-1 motif is widely distributed on glycolipids and glycoproteins in the CNS and PNS, and receptors for HNK-1 are distributed throughout the nervous system (Fig. 2).79-84 HNK-1 has roles in neuronal plasticity, development, and regeneration. Brain-derived neurotrophic factor regulates HNK-1 expression in regenerating motor neurons and promotes functional recovery but not sensory neurons.85 Schwann cells have indispensable roles to play in neuronal regeneration by increasing the expression of cell surface adhesion molecules such as NCAM, NgCAM, cadherins, and L2/HNK-1, which interact with laminin, fibronectin, and tenascin. Schwann cells also produce many of the neurotrophic factors and their receptors, which facilitate neuronal repair. A developmentally regulated and truncated form of the glial cell CS-PG DSD-1 PG/phosphacan consisting of the carbonic anhydrase and fibronectin type III-like repeat and spacer region of RPTP-beta (PTP-zeta) has roles in glial-neuronal interactions in neural development and regeneration.86 NG2 is also a glial PG associated with neurons undergoing repair and has a probable role in this process87-89 contrasting with other neural CS-PGs, which inhibit neuronal repair.90-92 The 3-O sulfate in HNK-1 is widely distributed in cell attachment molecules, structural glycoproteins, bioactive lipids, and PGs in the CNS/PNS with roles in neuron–oligodendrocyte and oligodendrocyte–oligodendrocyte communication (Fig. 2). Subpopulations of acetylcholinesterase and 5′-nucleotidase substituted with HNK-1 have roles in synaptic clustering and synaptic plasticity. Interactions between HNK-1 glycolipids and PG lectin domains mediate neuronal cell adhesion and neurite outgrowth.93,94 Some laminin isoforms bind specifically to sulfated glycolipids95 and are important in nerve development.94 A number of NCAMs contain HNK-1 including myelin-associated glycoprotein (MAG), myelin basic protein (MBP), NgCAM, contactin, P0, tenascin-C, and tenascin-R. The biosynthesis of HNK-1 is under strict spatial and temporal regulations on migrating neural crest cells, myelinating Schwann cells, and motor neurons but not on sensory neurons. HNK-1 sulfotransferase (HNK-1ST) catalyzes the transfer of sulfate to position 3 of terminal GlcA in protein and lipid N- and O-linked glycans in neural recognition molecules.96 Neural PGs bearing HNK-1 include α-TM, phosphacan, synaptic vesicle PG-2 (SV2), and aggrecan (Fig. 2). Thrombomodulin (TM) is an integral membrane pericyte or endothelial cell part-time PG substituted with an O-linked CS chain (β-TM) or HNK-1 (α-TM). Phosphacan occurs as a soluble PG and a variant cellular protein tyrosine phosphatase (PTP-zeta), and the HNK-1 motif in phosphacan is O-mannose linked through an Asn residue (Fig. 2). Notochordal and early rudiment cartilage cells synthesize developmentally regulated aggrecan substituted with HNK-1. HNK-1-substituted tenascin-R and -C contain multiple FNIII and EGF repeat and fibrinogen interactive domains,97,98 which form neuroprotective perineural nets with the C-type lectin domains of CNS/PNS CS-PGs.92,99

Neurotransmitter storage and release at the synaptic gap involve GAG interactions. Neurotransmitters are synthesized in the neuronal body transported to the Golgi and then to the synapse through a microtubular transport mechanism within the neuron. HS-PGs (agrin and perlecan) and CS-PGs (brevican) act as anionic intracellular storage media for the neurotransmitters prior to their transfer into synaptic vesicles by synaptic vesicle PG-2, a 12 span transmembrane KS PG with a transporter function, which also immobilizes the neurotransmitters within the vesicles.100 Small neurotransmitters are stored in small translucent vesicles, while neuropeptides are stored in larger dense vesicles. Neurotransmitters are stored in separate vesicles and not as a mixed population; however, how this selectivity is undertaken awaits clarification but may involve some sorting mechanism by the intracellular CS- and HS-PGs. Neuropeptide transmitters are synthesized as propeptide forms, which are trimmed of a signal peptide prior to transport into the dense synaptic vesicles. The neurotransmitter vesicles are stored in the synaptic terminal until release across the synaptic gap to their respective receptors. This occurs when an action potential depolarizes the presynaptic nerve terminal, voltage-gated Ca2+ (calcium) channels located in the presynaptic terminal membrane open, and Ca2+ ions influx into the synaptic terminal permeability increases. This causes the membrane of the vesicles to fuse with the presynaptic membrane at the active zone and release the neurotransmitter into the synaptic gap to travel to their respective receptors. HS-PGs also have some role to play in the regulation of the generation of this action potential in the neuron and in the coordination of electrical signals in neuronal networks.101 A deficit of HS leads to an autistic phenotype in mice.102 Chronic heparinase treatment reduces the mean firing rates of neurons and drastically affects functional maturation of neuronal networks. The action of Sulf1 and Sulf2 in situ may regulate this activity displayed by HS.100 Reduced levels of agrin and perlecan in the NMJ also detrimentally affect the organization of the NMJ and neuromuscular function.103-105 Brevican also has roles in promoting fast

Melrose
Glycosaminoglycans in wound healing

Roles of GAGs in matrix assembly processes relevant to wound repair. GAGs have many and varied roles to play in all stages of wound repair. HA oligosaccharides promote angiogenesis and can synergize with VEGF/PDGF. The HS side chains of perlecan interact with the FGF family, VEGF, PDGF, BMPs, Wnt family, and hedgehog proteins, which promote wound repair. Perlecan sequesters and stabilizes FGF-2 and other members of the FGF family in tissues promoting cellular proliferation, differentiation, ECM synthesis, and tissue development. This is clearly evident in perlecan knockout mice, which have severely impaired large vessel and nerve development. Malformed skull bones and distorted long bones, and severely distorted growth plate cartilages and a short stature. Another model has been developed with ablation of a 20 kDa N-terminal portion of the perlecan domain-1 HS attachment sites in Hpg2 exon 3 null mice. Mutant perlecan cannot interact with FGF-2 and promote angiogenic repair of wounds and also cannot participate in HS-dependent interactions with structural matrix glycoproteins, which organize and stabilize the pericellular and ECM. This becomes apparent in a reduced capacity of the mutant tissue to undergo repair after challenged with a biomechanical insult, which results in tissue failure. In this case, the mutant perlecan or other redundant HS-PGs are incapable of coordinating the assembly of repair tissue with a resultant impairment in tissue function. Deposition of TGF-β in skin is also severely impaired in Hpg2 exon 3 null mice contributing to poor skin healing. Perlecan exon 3 null mice do not develop severe OA changes in a post-traumatic OA model of joint instability, and synovitis and osteophyte size are also reduced. This indicates that the GAG chains of perlecan have roles in ECM remodeling and in tissue repair recapitulating tissue development. Endothelial cell perlecan is an HS-PG, and perlecan isolated from articular cartilage, intervertebral disc, meniscus, and SMC perlecan are HS–CS hybrid PGs. Keratinocyte perlecan is substituted with HS, CS, and KS. Irrespective of their GAG substitution pattern, perlecan is a participant in tissue remodeling processes, including regulation of cellular proliferation, adhesion, migration, and differentiation and in ECM stabilization. Heparanase-1 and -2 have emerging roles in wound repair through their ability to release HS and associated growth factors from tissue HS-PGs and are of importance in inflammation, wound healing, and angiogenesis.

ECM PGs and wound repair. PGs provide architectural support and hydration to tissues conducive to cellular migration into the wound site to promote repair processes (Table 2). Many PGs have a modular design containing functional subdomains important in tissue function. The GAG side chains of PGs have key roles to play in various stages of the wound healing process, and HS interacts and sequesters a number of growth factors at the wound site including FGF family members, as well as BMP, VEGF, and PDGF. These growth factors promote cellular migration, proliferation, differentiation, and ECM synthesis essential for wound healing. The ECM PGs also provide surfaces for cellular attachment and are interactive with a varied number of structural glycoproteins, collagens, and integrins, which stabilize the newly formed wound repair tissue. The lectican PG family forms perineural net structures with HA and tenascin-R in the CNS/PNS. If neural tissues are damaged, repulsive signals from the lectican PGs prevent neural outgrowth and repair processes. Chondroitinase ABC has been used in models, which demonstrate axonal regeneration, identifying the CS side chains as the source of repulsive signals, which inhibit repair. Table 2 summarizes the major PGs in tensional and weight-bearing connective tissues and their roles in ECM assembly and function. The reader is referred to recent reviews in this subject area for further PG information.

What role do the CS chains on TM play in its anticoagulant activities? TM acts as a thrombin receptor on the endothelial cell surface. The presence of CS on TM-β decreases the Kd for thrombin binding, significantly accelerating the inhibition of thrombin by AT, and has profound effects on TM’s anticoagulant properties. The C-4-S chains on TM are relatively small (10–12 kDa) but are essential for its anticoagulant activities. TM-α does not contain a CS chain but contains the HNK-1 linkage saccharide only and is devoid of anticoagulant activity (Fig. 6). In addition to enhancing its cofactor activity for protein C activation, the CS chain on TM-β also affects the inhibition of thrombin-induced fibrinogen clotting, accelerating the inactivation of thrombin by AT. The CS chain on TM-β binds at least two molecules of thrombin (Fig. 6).

GAG interactions with serpins improve their inhibitory properties. GAGs act as cofactors for many of the serpins enhancing their inhibitory properties. The GAG-binding serpins include AT, HCII, and PCI. Heparin and HS bind to AT, HCII, and PCI, and HCII also uses DS as a cofactor. Other serpins such as PAI-1, kallistatin, and α1-antitrypsin also interact with GAGs, accelerating protease inhibition. GAG binding to the serpins, generally occurs to a conserved region on the serpin leading to a conformational change resulting in increased or tighter protease binding accelerating the rates of inhibition up to 10,000-fold compared to the unbound native serpin. Several different AT activities were originally reported in plasma (AT1–IV); however, the major activity was subsequently shown to be due to one molecule, ATIII. The International Society on Thrombosis and Haemostasis in 1993 therefore recommended that AT should be used.
as the standard abbreviation for AT activity. AT is a heparin cofactor and a member of the serine protease inhibitor family (serpin). AT is an important protease inhibitor of thrombin and Factor Xa but can also inhibit IXa, XIa, XIIa, kallikrein, and plasmin and is thus one of the major naturally occurring inhibitors of coagulation.\(^\text{149}\)

AT is unique among the serpins in that it circulates in plasma in a native inactive conformation. Activation of AT occurs upon binding of domain 1 to a specific pentasaccharide sequence in heparin/HS in the vasculature (Fig. 7).\(^\text{150}\) This results in a rearrangement of the reactive center loop of AT in domains 3 and 4 facilitating productive associations with the coagulation proteases. A 3-O sulfate motif in N-acetyl glucosamine in this HS pentasaccharide has particularly important roles in this activation mechanism.\(^\text{151}\)

Heparin also acts as a tight binding competitive inhibitor of human neutrophil elastase (HNE) and also inhibits cathepsin G and mast cell chymase, this is strongly dependent on heparin chain length and charge density.\(^\text{152}\) α1-PI, α2-macroglobulin, SLPI, and clafin are also potent inhibitors of HNE, and their inhibitory capacity for the inhibition of HNE is enhanced by HS.\(^\text{153–155}\) Several serpin members (HCII, PN-1, PAI-1, and PCI) are heparin-binding proteins, and this improves their inhibitory properties.\(^\text{156}\) Squamous cell carcinoma antigens (SCCA-1 and SCCA-2) inhibit cathepsin-L through heparin- and HS-mediated interactions, but other GAGs are ineffective.\(^\text{157}\) Heparin binding also accelerates the inhibitory properties of tissue inhibitor of metalloproteinase (TIMP)-3, which have a broad inhibitory spectra including interstitial and cell membrane MMPs, and key members of the ADAMs and a disintegrin and metallo-proteinase with thrombospondin motifs (ADAMTS) metalloprotease families.\(^\text{158}\) TIMP-3 inhibits MMP-17, an ADAM sheddase.\(^\text{159}\) Unlike other members of the TIMP family, TIMP-3 displays limited solubility in free solution and associates with GAG-substituted cell adhesion proteins and PGs localizing TIMP-3 pericellularly in the vicinity of MMPs and related proteinases. TIMP-3 also associates with many proMMP enzyme forms in the PCM, which may improve its inhibitory properties and the protection of the ECM and PCM from excessive proteolysis.\(^\text{160}\)

**Proteinase activation and regulation by GAGs.** GAGs have multiple functional properties over proteinases relevant to wound repair (Table 3). Heparin promotes the binding of thrombin to fibrin\(^\text{161}\) and also stimulates uPA activity.\(^\text{162}\) Heparin also acts as a tight binding competitive inhibitor of HNE and also inhibits cathepsin G and mast cell chymase, and this is strongly dependent on heparin chain length and charge density.\(^\text{152}\) Specific cell surface interactions with PGs, integrins, and GAGs have been observed localizing MMPs at the cell surface.\(^\text{163}\) These include MMP-2-integrin αvβ3,\(^\text{164}\) MMP-9-integrin α4β1,\(^\text{165}\) and MMP-1-integrin α2β1.\(^\text{166}\)

Membrane-type (MT)-MMPs (MMP-14, -15, -16, -17, -24, and -25) are single-pass transmembrane proteins that are active at the cell surface may also act as docking modules for other MMPs and may be actively involved in their activation, e.g, MMP-14 and proMMP-2.\(^\text{167}\) TIMP-2 also has a
specialized role in the activation of proMMP-2 by MMP-14. The N-terminal domain of TIMP-2 forms an inhibitory complex with the active site of MMP-14, while the C-terminal domain interacts with the hemopexin domain of MMP-2 forming a trimeric activation complex. Cell surface HS-PGs may also act as docking sites for MMPs at the cell surface. Syndecan-2 acts as a docking receptor for proMMP-7 interacting directly with proMMP-7 at the plasma membrane, enhancing its processing into active MMP-7. The major cell surface HA receptor, CD44, may also dock MMP-7 and MMP-9 to the cell surface. The localization of MMPs at the cell surface confers resistance to TIMP inhibition and promotes their activation in the cell-surface pericellular matrix. ADAMTS and a subset of MMPs, including the MT-MMPs, contain a furin recognition sequence between their propeptide and catalytic domains, and furin convertase enzymes cleave at this site in the Golgi apparatus. Prodomain removal from MMPs can also be achieved by the action of other MMPs, such as the MMP-14-mediated activation of proMMP-2, or by activation cascades involving coactivator enzymes such as plasmin. Sulfated GAGs also play important roles in controlling MMP activation at the cell surface. MMP-2 and -7 and ADAMTS-5 are shown in Figure 8 as representative examples. HS regulates ADAM 12 through a molecular switch mechanism. The noncovalently associated prodomain in concert with the catalytic domain of ADAM 12 form a novel molecular switch critical for the regulation of the ADAM 12 proteolytic activity by HS-PGs. Via direct interaction with proMMP-7, sulfated GAGs such as chondroitin-4,6-sulfate (CS-E) act as allosteric modulators promoting the autolytic activation of the proteinase. Once activated, GAGs may facilitate proteolysis of certain substrates by interacting with the substrate, the enzyme, or both. Activation of proMMP-2 by MMP-16 is also significantly enhanced in the presence of excess chondroitin 4-sulfate (C4S), whereas chondroitin 6-sulfate or low molecular mass HA is ineffective. ADAMTS-5 is the major aggrecanase in mice with an activity at least 1000-fold greater than that of ADAMTS-4 under physiological conditions. ADAMTS-4 activation involves the coordinated activity of both glycosylphosphatidylinositol-anchored MMP-17 and syndecan-1 on the cell surface. Syndecan-4, controls ADAMTS-5 activation through direct interaction with this proteinase. Syndecan-4 is also crucial in the regulation of MMP-3 through the activation of ERK1/2 and by targeting ADAMTS-5 to the cell surface. Thus, loss of syndecan-4 results in a reduced expression of both MMP-3 and aggrecanase activity. This process involves the HS chains of syndecan-2, and treatment of low metastatic tumor cells with heparitinase-I promotes MMP-2 activation. Another soluble PG, testican, inhibits proMMP-2 processing by MT-MMPs.

Table 3. Subcellular/ECM localization of proteinases and their regulation by GAGs.

<table>
<thead>
<tr>
<th>SUBCELLULAR LOCALISATION</th>
<th>PROTEINASE</th>
<th>CATALYTIC CLASS</th>
<th>EXTRACELLULAR</th>
<th>PLASMA MEMBRANE</th>
<th>LYSOSOME</th>
<th>GAG REGULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsin D</td>
<td>Aspartyl</td>
<td>+</td>
<td>–</td>
<td>+++</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>BACE 1</td>
<td>Aspartyl</td>
<td>+</td>
<td>–</td>
<td>+++</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>MMP1–13, 19–21, 26–28</td>
<td>Metallo</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>MT-MMPs (MMP14–17, 23–25)</td>
<td>Metallo</td>
<td>+</td>
<td>+++</td>
<td>–</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>ADAMs</td>
<td>Metallo</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>ADAMTSs</td>
<td>Metallo</td>
<td>+</td>
<td>+++</td>
<td>–</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>Cathepsin B, L, S, K</td>
<td>Cysteine</td>
<td>–</td>
<td>+</td>
<td>+++</td>
<td></td>
<td>↑</td>
</tr>
<tr>
<td>Neutrophil elastase</td>
<td>Serine</td>
<td>–</td>
<td>+</td>
<td>+++</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Cathepsin-G</td>
<td>Serine</td>
<td>–</td>
<td>+</td>
<td>+++</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Thrombin</td>
<td>Serine</td>
<td>+++</td>
<td>–</td>
<td>–</td>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Tryptase</td>
<td>Serine</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>(mast cell granules)</td>
<td>↓</td>
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<tr>
<td>Chymase</td>
<td>Serine</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>(mast cell granules)</td>
<td>↓</td>
</tr>
<tr>
<td>Proteinase-3 (myeloblastin)</td>
<td>Serine</td>
<td>–</td>
<td>+</td>
<td>+++</td>
<td>Neutrophil azurophil granules,</td>
<td>↓</td>
</tr>
</tbody>
</table>

Notes: Depending on cellular context, GAGs can activate selected MMPs or inhibit their activation on the cell surface (see Fig. 8 for details).

- Chondroitin-4 sulfate, MMP-14, and TIMP-2 enzymatically activate proMMP-2 on the cell surface, while the HS chains of syndecan-2 or GAG chains of testican-2 can inhibit this process.
- The chondroitin 4,6 sulfate (chondroitin D) chains on cell surface proteoglycans or HS chains of syndecan-2 can spontaneously activate proMMP-7.
- The HS/CS chains of syndecan-4 can also spontaneously activate proADAMTS-5 on the cell surface.
MMP cascades involving cathepsins (lysosomal cysteine proteinases), which release uPA turn, generate plasmin from plasminogen and the plasmin feeds back into the activation of MMPs illustrating some of the complexities of proteinase activation in situ. Endopeptidases, such as cathepsins B, L, S, and K, can also be activated autocatalytically or by other proteinases such as cathepsin D and pepsin. Lysosomal GAGs interact with and regulate the activities of lysosomal cathepsins. Autocatalytic activation of cysteine cathepsins is substantially accelerated in the presence of GAGs, and these facilitate procathepsin B activation. Furthermore, trafficking of the cathepsins to the cell surface may also involve the action of cell surface HS-PGs. Cathepsin D, an aspartyl lysosomal cathepsin, is also regulated by GAGs. GAGs increase cathepsin D activity in vitro, and heparin increases the activation of cathepsin D from its Pro form. GAGs induce the activation of the β-site amyloid-cleaving enzyme (BACE1), a membrane-anchored enzyme that catalyzes the production of β-amyloid, and its accumulation in the brain of patients with Alzheimer’s disease.

Heparin and HS stabilize and increase the activity of lysosomal cysteine cathepsins. The mature form of cathepsin B

Figure 8. Regulation of MMP activation at the cell surface by GAGs (1–4). MMP-2 and MMP-7 are presented as illustrative examples (A). Heparin promotes autocatalytic conversion of proMMP-2 to active MMP-2. MMP-14 also activates proMMP2 attached to C4S proteoglycan at the cell surface. The HS chains of syndecan-2 and testican inhibit this activation process. The heparan sulphate chains of syndecan-2 and chondroitin-4 and 6-sulphate side chains of cell surface proteoglycans promote autolytic conversion of proMMP7 into active MMP7. The HS chains of syndecan-4 promote the activation of ADAMTS-5.
is rapidly inactivated at neutral or alkaline pH and by its endogenous cystein proteinase inhibitor family, but membrane-bound cathepsin B is very resistant to inactivation at neutral pH possibly through steric effects that prevent the access of TIMPs. Cathepsin K is the principal cysteine proteinase responsible for the degradation of bone. At acidic pH, GAGs expressed in bone and cartilage, such as CS and KS, enhance the collag enolytic and bone resorptive activity of cathepsin K, whereas DS, HS, and HP selectively inhibit its activity; thus, GAGs can participate in the regulation of bone resorption. GAGs act as allosteric modifiers of cathepsin K by influencing its conformational state either enhancing or inhibiting its enzymatic degradative properties.

Regulatory molecules in the coagulation cascade and GAGs. GAGs have regulatory roles to play in a number of coagulation proteins, such as TM, protein C, protein S, endothelial protein C receptor (EPCR), and protease-activated receptors 1 and 2 (PAR-1 and 2). Protein S shares homology with other vitamin K-dependent coagulation proteins and acts as a cofactor to protein C in the inactivation of Factors Va and VIIIa. Protein S contains four EGF, one γ-carboxyglutamatic acid, and two laminin-G-like domains. Protein C belongs to the peptidase S1 family. It contains two EGF, one glutamatic acid, and one peptidase S1 domain. TM (CD141) is a 74–105 kDa cell-surface PG mediator of endothelial anticoagulant activity and activator of protein C. TM contains one N-terminal C-type lectin and six EGF-like domains. It occurs as a part-time CS-PG (B-TM) containing the CS disaccharides 4GlcUAβ1–3GalNAcβ1– sulfated at the C-4 and C-6 positions and a form containing a truncated side chain containing the HNK-1 (HSO(3)–3GlcAβ1–3Galβ1–4GlcNAc–) linkage tetrasaccharide, which is terminated in a 3-O sulfate motif (α-TM). This latter variant form of TM does not display anticoagulant activity. TM is synthesized by endothelial cells, mesothelial cells, monocytes, and a sub-set of dendritic cells. Mutant cells defective in CS elongation do not exhibit anticoagulant activity. The N-terminal lectin-like D1 domain of TM suppresses vascular inflammation by inhibiting leukocyte recruitment to the endothelium by attenuating Lewis Y (Ley)-mediated adhesion. The cytoplasmic domain of TM is bound directly to the N-terminal domain of the cytoskeletal protein ezrin and colocalizes with actin filaments, EGF acting upstream of ezrin activation stimulates the interaction between ezrin and TM maintaining epithelial morphology and cell migration during wound healing.

Multifunctional properties of TM impacting on the coagulation cascade and wound healing. TM is a widely expressed multifunctional cell surface protein, which mediates the activation of protein C and the inhibition of thrombin, generates thrombin-activatable fibrinolysis inhibitor (TAFI), inactivates Va and VIIIa, and has anti-inflammatory activity mediated by its lectin-like D1 domain (Fig. 6). TAFIα is a carboxypeptidase-B enzyme that suppresses fibrinolysis. Binding of the lectin domain of TM to the cytokine, high mobility group box protein 1, and Lewis Y cell surface tetrasaccharide on endothelial cells provides anti-inflammatory and antiangiogenic activities. Binding of thrombin to TM on the endothelial cell surface prevents the activation of Va by thrombin. Thus, TM acts as an anticoagulant protein through its actions on thrombin and by generation of activated protein C (APC). Once APC is formed, it binds to protein S on the cell surface, and the APC–protein S complex inactivates Va and VIIIa. APC can also act in free solution in the presence of Ca++ but its action is significantly improved by the presence of phospholipid vesicles. APC also activates MMP-2 and MMP-9 produced by endothelial cells and stimulates proliferation, migration, and wound closure in cutaneous wounds and a healing phenotype in cultured tenocytes. APC also activates MMP-2, -9, and -13 produced by chondrocytes in rheumatoid arthritic and osteoarthritic articular cartilages. Thus, APC modulates ECM remodeling in disease and during repair. TM’s multidiomain structure and multicompartment interactions with thrombin, protein C, TAFI, complement, Lewis Y antigen, and high mobility group box protein 1, a chromosomal protein regulating transcription, replication, recombination, and DNA repair, facilitates TM’s physiologically significant anti-inflammatory, anticoagulant, and antifibrinolytic properties.

APC and wound healing. APC has emerging roles as a new biotherapeutic in wound repair applications. APC is the central enzyme in the natural anticoagulant pathway inactivating nonactive and active Factors V and VIII, resulting in an attenuation of thrombin formation and downregulation of coagulation (Fig. 4). The presence of the APC cofactor, protein S, TM, EPCR, and a phospholipid surface contributes to the expression of APC-mediated anticoagulant activity. APC also binds to EPCR in lipid rafts to activate PAR-1 and anti-inflammatory and cytoprotective signaling responses in endothelial cells. APC also promotes wound healing in chronic ulcers and in bone. Low circulating levels of protein C are associated with lower healing responses in leg ulcers in patients with diabetes. APC increases bone anabolism via a PAR-1/2-dependent mechanism and may synergize with rhBMP-2 for bone repair. APC differentially regulates the viability and differentiation of osteoblasts mediated by biphosphonates and may be useful in combination therapy for bone regeneration. APC signals through PAR-1 and PAR-2 to activate Akt (RAC-alpha serine/threonine-protein kinase) and increase keratinocyte proliferation and skin wound healing.

Therapeutic application of GAGs and GAG-regulated proteins in wound repair. From studies on the pathobiology of the wound repair process and a greater understanding of GAGs in these processes, it has been possible to formulate therapeutic approaches, which improve wound healing. A survey of therapeutic approaches with GAGs, GAG derivatives, or GAG mimetics is presented in Table 4. Peptides have...
Table 4. GAG therapeutic agents applied in wound repair strategies.

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been developed, which mimic HNK-1 to stimulate motor neuron repair. A 93% recovery in quadriceps muscle function was achieved in a three-month recuperative period using this procedure. An HA–VEGF gene sheath has also been developed, which can be placed over damaged axons for peripheral nerve regeneration with minimal scar formation. Heparin and HS are the most extensively modified GAGs and most heterogeneous. Affinity procedures have been developed to isolate HS subfractions for specific therapeutic applications in bone and vascular repairs and in the expansion of progenitor MSCs for regenerative procedures. These include BMP-2 interactive HS, RGTA biomimetic HS, affinity-purified HS, and engineered HS samples for enhanced bone repair. HA-GDF-5 hydrogels have also been developed for bone repair. FGF-2 affinity-purified HS for enhanced expansion of stem cells, a trisulfated HS RANTES antagonist, IFN-γ-binding HS, AT-binding HS pentasaccharides, and VEGF affinity-purified HS for an improved vascular repair. HA biomimetics based on 6-deoxy-α-talose, OTR4120 mimetic HS for an improved cutaneous wound repair, and HS biomimetic clusters have been developed. The heparinoid pentosan polysulfate has found application in IVD and cartilage repair and in combination with MSCs and spinal interbody cages for spinal fusions. Engineered aggrecan and perlecan domain-1 growth factor delivery vehicles have been developed, and GAG-copolymer hydrogels for wound repair include HA-type II collagen, collagen–GAG, high-charged density sulfonate analog GAG biomimetic for IVD restoration, and alginate–HA composite hydrogels. A diverse range of HA formulations and products have been developed to treat cutaneous wounds including Healoderm for the treatment of diabetic ulcers, HA soft tissue fillers, decellularized scaffolds derivatized with HA/EGF and HA/FGF-2 for the treatment of deep large skin wounds. Resveratrol–HA microparticles, HA nanofiber dressings, HA ± vitamin C–EGF spongy dressings supplemented with dermal fibrin–HA sheet dressings have been applied for the treatment of acute and chronic skin wounds. Chitosan–HA/slow release nanosilver composite sponges and HA–iodine preparations have been used for the treatment of infected diabetic wounds. Application of APC in the treatment of diabetic wounds offers a particularly novel treatment in a clinically demanding area of chronic skin wound repair. APC may also prove useful in combination therapy with bisphosphonates or BMP-2 in the treatment of bone lesions.

Conclusion

A greater understanding of the roles that GAGs play in tissue homeostasis and wound repair has fuelled significant advances in treatments for the repair of clinically demanding wounds in neural tissues and diabetic ulcers. The application of MSCs for tissue repair in tensional and weight-bearing tissues has also yielded promising results, and GAGs have essential regulatory roles in the proliferation and differentiation of progenitor cell populations, which undertake wound repair. The sophistication of GAGs as an information delivery system to cells is significant and capable of directing many aspects of cellular behavior, which control tissue homeostasis and wound repair. A greater understanding of the glycoside provided by GAGs will further advance the treatment of chronic and acute wounds.

Abbreviations

TGF-β, transforming growth factor beta; IGF-I, insulin-like growth factor-I; IL-1, interleukin-1; TNF-α, tumor necrosis factor alpha; PDGF, platelet derived growth factor; FGF-2, fibroblast growth factor-2; IFN-γ, interferon gamma; MMPs, matrix metalloproteinases; NCAM, neural cell adhesion molecule; NgCAM, neuroglia cell adhesion molecule; NMJ, neuromuscular junction; MSCs, mesenchymal stem cells; ADAMS, A disintegrin and metalloproteinase; ADAMTSs, A disintegrin and metalloproteinase with thrombospondin motifs.

Author Contributions

JM Conceived the study, analyzed the data, and wrote the manuscript.

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