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Neoproteoglycans in tissue engineering

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Abstract

Proteoglycans, comprised of a core protein to which glycosaminoglycan chains are covalently linked, are an important structural and functional family of macromolecules found in the extracellular matrix. Advances in our understanding of biological interactions have lead to a greater appreciation for the need to design tissue engineering scaffolds that incorporate mimetics of key extracellular matrix components. A variety of synthetic and semisynthetic molecules and polymers have been examined by tissue engineers that serve as structural, chemical and biological replacements for proteoglycans. These proteoglycan mimetics have been referred to as neoproteoglycans and serve as functional and therapeutic replacements for natural proteoglycans that are often unavailable for tissue engineering studies. Although neoproteoglycans have important limitations, such as limited signaling ability and biocompatibility, they have shown promise in replacing the natural activity of proteoglycans through cell and protein binding interactions. This review focuses on the recent *in vivo* and *in vitro* tissue engineering applications of three basic types of neoproteoglycan structures, protein–glycosaminoglycan conjugates, nano-glycosaminoglycan composites and polymer–glycosaminoglycan complexes.

Keywords

biomedical scaffolds; extracellular matrix; glycoconjugates; glycosaminoglycans; growth factors; nanocomposites; neoproteoglycans; proteoglycans; regenerative medicine; tissue engineering

Introduction

Tissue engineering, as its name implies, is a field of research focused on the engineering of tissues and functional tissue replacements for organs damaged by disease or injury [1–3]. Although recent advances in technology, such as printable 3D scaffolds and the

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reprogramming of adult cells to multipotent stem cells, are opening the field to the possibility of made-to-order designer organs, individually tailored to each patient, current limitations restrict the field to the *in vivo* repair and regeneration using a single, uniform tissue scaffold, typically in small volumes (~ 1 cm^3). There are three major focuses in advancing tissue engineering:(a) the design of biomaterials that can serve as implantable scaffolds that can be remodeled by the body's cells *in vivo*; (b) the design of scaffolds that can be implanted after *in vitro* remodeling; and (c) the culture and isolation of cells, such as multipotent stem cells, that can be used to repopulate a scaffold [4,5]. Scaffold design is one of the most crucial aspects of tissue engineering, as it must be constructed so that both the mechanical and chemical properties create an environment that supports cellular growth and remodeling.

In the body, the microenvironment that surrounds and supports the cells of each tissue is the extracellular matrix (ECM). The properties of the ECM vary from tissue to tissue, and are in a dynamic equilibrium with the tissue cells [6,7]. Designs for tissue engineering scaffolds are often based on the molecular and structural properties of the ECM present in the natural tissue that will be replaced [8,9]. Constructing a scaffold to mimic the mechanical and chemical properties of the ECM can be accomplished either by incorporating the same chemical compounds and macromolecules or by including synthetic constructs with the same chemical and mechanical properties. Often, these synthetic constructs are 'semisynthetic' molecules, natural compounds which have been chemically manipulated to create mimetics of species found in the ECM – for example, the use of electrospun collagen fibers to recreate the collagen fibrils found in most connective tissues [7,10–12].

One major class of natural compounds found in the ECM is a family of macromolecular glycoconjugates, the proteoglycans (PGs). PGs are comprise done or more long, linear, anionic polysaccharide chains, called glycosaminoglycans (GAGs), covalently linked through their reducing ends to the side chains of specific amino acid residues with in a core protein (Fig. 1) [13]. PGs play important structural and regulatory roles in the ECM and are involved in many important cellular signaling processes governing tissue growth and development [14–17]. Both ECM and cell-surface PGs are involved in cell signaling, proliferation, adhesion and motility [18,19]. Thus, their biological, mechanical and chemical properties make PGs a critical component of tissue engineering scaffolds [20].

The direct incorporation of natural PGs into synthetic scaffolds is often not feasible. A variety of synthetic and semisynthetic molecules and polymers have been constructed to serve as structural, chemical and biological replacements for PGs. These PG mimetics have been referred to as neoproteoglycans (neoPGs) and serve as functional therapeutic replacements for natural PGs in synthetic scaffolds. This review focuses on the recent developments in the design, synthesis and application of neoPGs in tissue engineering, and examines the role they fill in supporting tissue remodeling of synthetic scaffolds in tissue engineering applications (Table 1).

Proteoglycan structure

NeoPGs serve to mimic the structure and function of PGs, proteins with one or more covalently bound GAG chains, in tissue engineering scaffolds. Design of a successful neoPG construct requires a clear understanding of the structure and functional properties of natural PGs, which are influenced by the complex structures of both the GAG side chains and protein core (Fig. 1).

GAGs are long linear chains (with branching or bisecting sugars occasionally observed) of repeating disaccharide units, which define their classification or family (Fig. 1) [21,22]. These repeating disaccharide units (composed primarily of hexosamine and uronic acid saccharide structures)are often further modified through the addition of negatively charged sulfo groups and/or the epimerization of the uronic acid residue, itself containing a negatively charged carboxyl group. The extensive cellular signaling properties of GAGs are governed by their structure; the structure of the saccharide backbone along with the placement and patterning of these negatively charged sulfo and carboxyl groups are believed to be critical in governing their interactions [23]. These negatively charged groups are often involved in ion-pairing or hydrogen-bonding interactions with positively charged, basic residues within the GAG-binding site of its protein binding partner [24,25]. The highly negative nature of GAG chains – heparin has the highest negative charge density of any biological macromolecule –influences not only their ability to interact with proteins, but also the properties of the ECM [23,26]. Highly negatively charged PGs enable the sequestration of diverse compounds in the ECM, such as growth factors and chemokines, and cations, such as sodium, helping to regulate the osmotic pressure of this cellular scaffold [6,23].

Although the structure of GAG chains governs their signaling properties, the structural complexity and polydispersity of GAGs confound their structural analysis, and limit the analysis of PGs [27]. Analysis of GAG chain composition, often 20–100 saccharides long, is typically limited to disaccharide compositional analysis, oligosaccharide mapping (4–8 saccharides) and occasionally domain analysis, although advances in mass spectroscopy are expanding these limitations [28,29]. Recent technological advances in Fourier transform mass spectrometry have enabled the full sequence analysis of the simplest PG, bikunin having a single small GAG chain [30]. This structural analysis revealed that the bikunin GAG chain had a defined sequence, strongly suggesting that some other GAGs may have defined structures as well. Although complete knowledge of the exact GAG chain structure used in the design of neoPGs is usually not possible, commercial GAGs are reproducible, pure (a mixture of chains from a single GAG family), with well-defined polydispersities, comprised of defined disaccharide structures, and with known signaling properties.

The core proteins of PGs serve important structural and signaling functions. Core proteins direct the tissue localization of PGs, which limits and controls PG interactions. PGs can reside inside cells, on the cell membrane and in the ECM. Although all PGs are biosynthesized within the cell in the endoplasmic reticulum and Golgi, with few exceptions, they are typically transported to the outer cell membrane or into the EMC [23]. Membrane PGs are bound to the cell membrane either through a glycophosphatidylinositol-anchor (e.g. glypican) or as an integral transmembrane protein (e.g. syndecan) [31,32]. PGs found in the

ECM include temporary residents such as ones shed from the cell membrane or permanent residents of the ECM (e.g. decorin, aggrecan, perlecan) [17]. The PG core proteins can also play a role in the adhesion and signaling functions of PGs, like the decorin core protein, which binds to collagen fibrils, helping to establish their spacing in the ECM [33–35]. PGs can also act simply as a scaffold for the aggregation and support of GAG chains. The intracellular PG serglycin, carrying heparin and/or chondroitin sulfate chains, resides in the granules of mast cells until its extracellular release is stimulated in an allergic response [36]. Serglycin is used in these intercellular compartments to bind and store positively charged proteases and smaller molecules, such as immune reactive histamine, along the negatively charged GAG chains [37]. Thus, the core proteins can play a variety of roles from serving as a scaffold on which to biosynthesize the GAG chains of serglycin, as a transmembrane protein to anchor syndecan GAG chains to the cell membrane and appropriately display its chains as coreceptors for signal transduction, or as the primary signaling or protein binding ligand in decorin.

Incorporating the structural and chemical properties of PGs into tissue engineering constructs of the ECM requires either the direct use of PGs or the use of neo- PGs. Because isolating natural PGs from tissues is often highly impractical for any purpose other than analysis, recombinant PGs are often prepared through genetic engineering to obtain PGs for biomedical applications. A number of ECM PGs have been prepared as recombinant proteins, including perlecan [38–40], aggrecan and versican [41], neurocan [42], decorin [43] and biglycan [44]. Recombinant PGs have even been explored for use in tissue engineering. Recombinant PGs have been used to help reprogram fibroblasts into multipotent cells, which are thought to be key to creating successful tissue engineering constructs [45]. Recombinant PGs, specifically the heparan sulfate PG perlecan, have been used in *in vitro* studies of cell growth in tissue engineering scaffolds. Recombinant perlecan has been electrospun with collagen fibrils to create tissue engineering scaffolds with better properties for fostering cellular growth [11] and recombinant perlecan has also been used to modify collagen [46] and poly(lactic acid) scaffolds [47], which showed increased ability to support cellular growth and support chondrogenic differentiation to cartilage cells, respectively.

Although these recombinant PGs have shown promise, several limitations including expense, scalability and control of the GAG structure limit their use in tissue engineering. Some GAG chain structures require specialized enzymes or Golgi components and control found only in mammals, and are absent from the single- celled organisms used to make recombinant PGs. Thus, recombinant PGs are useful, but imperfect components for tissue engineering. Although neoPGs have their own set of limitations, their typical ease of preparation and ease of structural (and hence chemical) control often makes them low-cost, useful alternatives to natural and recombinant PGs in preparing tissue engineering scaffolds.

Neoproteoglycan structures

NeoPGs, PG biomimetics, should ideally replace the functions associated with both a PG's GAG chains and its core proteins in a tissue engineering scaffold. As discussed above, the PG core protein can have direct binding and signaling properties, can be used to direct the

tissue localization or can simply serve as backbones to carry and display the bound GAG chains. In many current tissue engineering scaffolds, localization is not an important design characteristic, as these scaffolds are uniform in nature, with neoPGs evenly distributed throughout the scaffold. In most cases, the direct chemical properties of the protein core are also ignored, and neoPGs protein core mimetics are utilized only to anchor the GAG chains and properly display them. These protein core mimetics fall into three basic classes, proteins (including peptides), nanomaterials and polymers (Fig. 2).

NeoPG protein core mimetics are not without limitations, and each core mimetic class has its own specific drawbacks. Core mimetics made of nanomaterial scan have potential toxic side effects, depending on biocompatibility, degradation and clearance, oxidative potential and distribution of the nano core material [48,49]. Core mimetics made of proteins and peptides – especially those protein sequences, like avidin, which are foreign to the human body – have the potential to illicit an immune response, and lack the original signaling properties of the natural PG core [50]. Finally, even biologically compatible polymers have the potential to illicit a foreign body reaction, triggering the body to wall off and encapsulate the implanted material in a collagenous bag [51].

Careful engineering can mitigate many of these limitations. Nanomaterials can be designed to optimize their biocompatibility, biodegradabilty and efficacy [52]. Proteins and peptide sequences can be designed to be immunologically silent, and even to restore the original chemical binding interactions of the natural PG [53]. Polymer scaffolds can be found that are ‘stealth’ or biologically indistinguishable from the body’s own systems; one of the major chemical adaptations to designing stealth scaffolds is the incorporation of the natural molecules of the ECM – such as PGs – on the surface of the scaffold [51].

Depending on their design and utilization, the GAG chains incorporated into the neoPG will also have limitations in mimicking the natural PG properties. The chain structure of the commercial or synthetic GAG used, although often similar to the natural GAG present, is isolated from non-human sources and will inevitably contain small structural differences in the overall GAG sequence, which may lead to signaling differences. Natural PGs also may contain more than one type of GAG side chain – for example, aggrecan contains both chondroitin sulfate and keratan sulfate chains, and forms macromolecular complexes with hyaluronic acid (Fig. 1) – whereas current neoPGs typically only utilize a singular GAG. Finally, depending on the method of attachment used, the attached neoPG GAG chain orientation may vary from that of the natural side chain orientation (Fig. 2) [54].

Despite these limitations, the use of GAG chains in neoPG structures has shown much promise in aiding the structural and chemical properties of tissue engineering scaffolds. These GAG chains can serve a wide range of functions in tissue engineering scaffolds (Fig. 3). GAGs can be used modulate cellular signaling processes, supporting the growth and spread of cells in the new scaffold [45]. GAGs can be used as anchoring molecules, used to bind the scaffold into the new tissue or anchor cells to the scaffold [55]. The binding properties of GAGs can also be utilized to sequester important signaling molecules such as minerals, growth factors and chemokines. These ‘cargo loaded’ GAGs can then be used to

sequester the signaling molecules, allowing for slow, controlled release or even establish signaling gradients [23,56].

Although neoPGs have biological limitations they also still serve important tissue engineering applications, for example, in *in vitro* experimentation, where they are helping to establish the boundaries and functional properties of tissue engineering scaffolds. For example, a covalent conjugate of heparin/heparan sulfate– BSA has been used to examine the incorporation of growth factors on GAG chains in competition assays to better understand scaffold properties [57]. Knowledge and understanding gathered from the interactions of this first generation of neoPGs will also aid the design of the next neoPG generation, leading to the design of more sophisticated, responsive PG mimetics. Here, review of this first generation of *in vitro* and *in vivo* tissue engineering applications focuses on three basic neoPG structures: protein–GAG conjugates, nano-GAG composites and polymer–GAG complexes.

Protein– and peptide–GAG conjugates

Several different forms of protein–GAG conjugates have been prepared to serve as neoPGs. In the simplest case, the carboxyl groups in GAGs are activated and randomly conjugated to the amino groups on the surface of a simple protein scaffold such as albumin [54]. In such cases, the GAGs are coupled in an unnatural orientation (Fig. 2) through the center of the chains and their position and the number attached to the core protein are ill defined. In more sophisticated examples of neoPGs, GAG chains are biotinylated at their reducing ends and then bound in very strong noncovalent bonds to streptavidin protein to form GAG–streptavidin neoPGs [58]. GAG–streptavidin neoPGs allow for some control of the orientation of the GAG chain, the number of GAG chains and the position(s) of their attachment. Using a streptavidin–biotin noncovalent complex, glycopolymers containing synthetic chondroitin sulfate disaccharide constructs were prepared using end-functionalized ring-opening metathesis polymerization. These neoPGs could mimic the bioactivity of chondroitin sulfate PGs [59]. Such synthetic neoglycopolymers could also be incorporated into collagen scaffolds, showing promising material properties for tissue engineering [60]. It is even possible to covalently attach a correctly oriented glycan to a modified core protein backbone engineered to contain a single unnatural keto-containing amino acid [61].

Synthetic peptido-glycosaminoglycans (cross-linked dermatan sulfate–peptide constructs) have also been synthesized for tissue engineering purposes. These peptido-GAGs could replicate many of biological functions of decorin and showed much promise in a diverse array of biomedical applications. The peptido- GAGs modulated fibril formation and the stiffness of the resulting tissues [53,62], aided in cellular adhesion and prevented apoptosis [63], and helped to promote healing and minimize scaring in wound healing [64]. Similarly, aggrecan peptido-GAG mimics were used to enhance the scaffold properties in cartilage. These peptido-GAGs also showed reduced susceptibility to proteolysis, making their scaffolds promising for implantable tissue engineering scaffolds [65]. Despite these promising results, the functional activities of the core protein are often lost in protein–GAG conjugates. This limitation is a major drawback to the use of neo- PGs to replace natural

PGs, such as decorin, in which the core protein contributes a major portion of PG bioactivity.

Nano-GAG composites

Nano-GAG composites are nanoscale structures having attached GAG chains where a nanomaterial serves as a substitute for core protein. These composites have a size that is typically of the same order of magnitude as a PG and are capable of displaying similar mechanical and biochemical structural properties. Nano-GAG composites are, of course, limited in that the functional properties of the natural PG core proteins are completely absent. Several bioactive nano-GAG composites, with a wide range of tissue engineering applications, have been described in the literature.

Nano-GAG composites have been made that contain a number of different types of GAGs. Chondroitin- sulfate-bound nanofibers have been incorporated in poly(vinyl alcohol) scaffolds prepared for engineering of articular cartilage tissue. These chondroitin sulfate nanofibrils were found to enhance cartilaginous tissue formation, supporting chondrogenesis and type II collagen formation [66]. Chondroitin sulfate–collagen nanofibrils were electrospun to create novel scaffolds to support tissue regeneration, and were found to support increased cellular proliferation [10]. Collagen– chondroitin sulfate–calcium phosphate nanocomposite scaffolds have been investigated for tissue engineering of bone and cartilage [67–69]. Alginate–chitosan–dermatan sulfate complexes have been formed that provide sustained release of dermatan sulfate supporting cell proliferation in tissue regeneration [70].

Heparin and heparan sulfate nanocomposites have also been investigated, for both tissue engineering and biomedical applications [71]. The first nano-based neo- PGs described relied on carbon nanotubes wrapped with synthetic polymers to which heparin was attached (Fig. 4) [72]. Although these neoPGs were blood compatible, issues with carbon nanotube toxicity have largely precluded their development for *in vivo* tissue engineering applications and have limited their exploration to topical wound healing applications [73,74]. Heparin-bound gold and silver nanoparticles are bioactive and inhibit angiogenesis [75]. In addition, gold– heparin or silver–heparin nanoparticles injected into tissues exhibit local anti-inflammatory properties without any significant effect on systemic hemostasis [76]. Studies on silver–hyaluronan (an unsulfated GAG not found as a PG) nanoparticles showed that the silver hyaluronan neoPGs exhibited strong antimicrobial activity against Gram-positive *Staphylococcus*, suggesting their use in tissue engineering [77]. Heparan sulfate –chitosan nanoparticles support cell growth and chondrocyte function in a tissue engineering model of articular cartilage [78]. Heparan sulfate–chitosan nanoparticles also bind to and provide sustained vascular endothelial growth factor release in a decellularized tissue engineering scaffold, where they were found to stimulate endothelial cell proliferation *in vitro*, and support vascularization, production of the ECM and fibroblast infiltration *in vivo* [79].

Polymer–GAG complexes

Polymer–GAG complexes encompass a wide range of structures and compositions. Of all the neoPG types, these are often the least morphogenically similar to PGs. Polymer–GAG

complexes typically incorporate GAG chains uniformly throughout a scaffold, in contrast to protein- and nano-based neoPGs that typically link GAG chains to a single core structure which is then dispersed throughout a scaffold. However, the chemical properties of the incorporated GAG chains of these polymer–GAG complexes allow them to serve as biomimetics for PGs, providing the signaling and therapeutic support of natural PGs. Although the polymer provides the important anchoring and orientational structural features of the protein core, it is important to note that the functional properties of the core proteins are also completely absent in this type of neoPG.

A number of GAG–polymer complexes have been investigated. One study looked at the effects of sulfonation of a series of hyaluronan derivatives to support cellular proliferation and multipotent stem cell differentiation, finding that the hyaluronan–collagen scaffolds were able to support osteogenic differentiation, a promising finding for bone tissue engineering [80]. Heparin–poly(L-lysine)-coated poly (lactic-co-glycolic acid) microspheres were found to support cell adhesion and cellular growth as novel tissue regeneration supports [81]. Several studies have examined the used of heparin-bound star-poly(ethylene glycol) polymers as potential tissue engineering scaffolds, and their use in anchoring proteins and peptides that enable cell growth [82]. Heparin-bound star-poly(ethylene glycol) gels were found to stimulate nerve and nerve stem cell growth, for possible nervous system tissue regeneration [83]. Low molecular weight heparin-bound star-poly (ethylene glycol) gels were made that were found to bind and slowly release useful signaling proteins like basic fibroblast growth factor, an important feature for tissue engineering scaffolds and biomaterials [84]. Chondroitin sulfate–poly(ethylene glycol) hydrogels have been used as scaffolds to enhance healing and tissue regeneration in wound healing models [85–87]. Chondroitin sulfate–poly(ethylene glycol) hydrogels have also been used as a scaffold to support the differentiation of multipotent stem cells into chondrocytes in a model of cartilage tissue regeneration [55]. Synthetically modified glycans have also been explored, such as a chondroitin sulfate modified through its reducing end so that it could be polymerized to create biomimetics of aggrecan, a chondroitin sulfate brush-like extracellular PG [88]. This example reduces the unnatural polymer component to simply a cross-linker supporting GAG structure.

Hyaluronan hydrogels are one of the most studied and most used scaffolds in tissue engineering. Because hyaluronan is a GAG not a PG, the reader is directed to previous reviews of hyaluronan in tissue engineering [89,90]. Hyaluronan scaffolds, however, can be used in concert with other GAGs and proteins. Alginate gels with chondroitin sulfate and hyaluronan additives support the growth of chondrocytes in a cartilage engineering scaffold [91]. Heparin-modified hyaluronan scaffolds have been used to enable the slow release of bone morphogenic protein in bone engineering scaffolds [92,93]. Gelatin–chondroitin–hyaluronan scaffolds have also been seeded with dental bud cells and used to grow tooth progenitors in *in vivo* models [94] and for *in vivo* studies of cartilage tissue repair [95,96].

Conclusion

PGs are known to be important components of the ECM, supporting the growth and development of cells through their signaling and structural properties. As discussed in this

review, neoPGs, artificial constructs designed to mimic the signaling and therapeutic properties of PGs, are becoming common tools for bioengineering scaffolds for tissue regeneration. These neoPGs can take many forms, most prominently as protein–GAG conjugates, nano-GAG composites and polymer–GAG complexes. Although the limitations of different core protein mimetics in various neoPGs, such as toxicity, potential for immunological responses and lack of native core protein function, limit the efficacy of neoPGs, advances in biotechnology are rapidly enabling new engineering solutions that overcome these functional drawbacks.

NeoPGs enhance the properties of tissue engineering scaffolds by not only augmenting the structural properties of the scaffold, but also providing chemical signaling support for the cells that repopulate the scaffold. Although there are many limitations of neoPGs, they also offer advantages over natural PGs, such as greater stability, signaling and structural control. They are often protease resistant, and hence more stable than natural PGs. Commonly formed through the attachment of commercial GAGs to a synthetic backbone, their structures can be easily adjusted, allowing for a diverse range of tissue engineering scaffolds with design-specific properties. Currently, most studies focus on controlling the structure of the GAG side chain, either for use in the binding and controlled release of growth factors or for controlling structural morphology. However, as our understanding of the structure–function relationships of GAGs improves and our ability to chemically and enzymatically synthesize specific GAG structures advances, more opportunities for the control of tissue engineering scaffolding properties through the design of novel neo- PGs will certainly arise.

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Abbreviations

ECM	extracellular matrix
GAG	glycosaminoglycan
neoPG	neoproteoglycan
PG	proteoglycans

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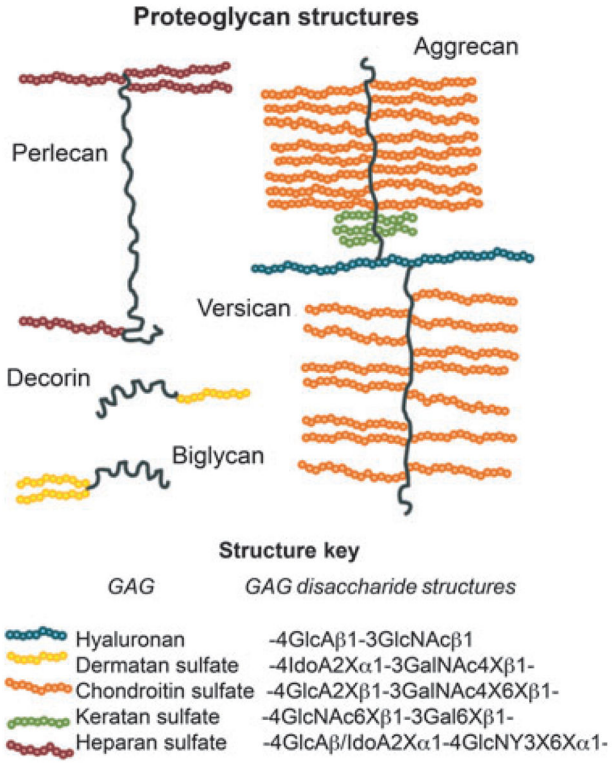

Fig. 1.

Structures of common PGs found in the ECM. Protein backbones are drawn in black, with each different family of GAG chains shown in different colors. Structures of the disaccharide repeating unit of each GAG chain are shown in the Structure Key. Gal, galactose; GlcA, glucuronic acid; IdoA, iduronic acid; GlcNAc, glucosamine; GalNAc, galactosamine. Bold text indicates the locations of possible sulfate groups; X = H or SO_3^-, Y = $COCH_3$ or SO_3^-

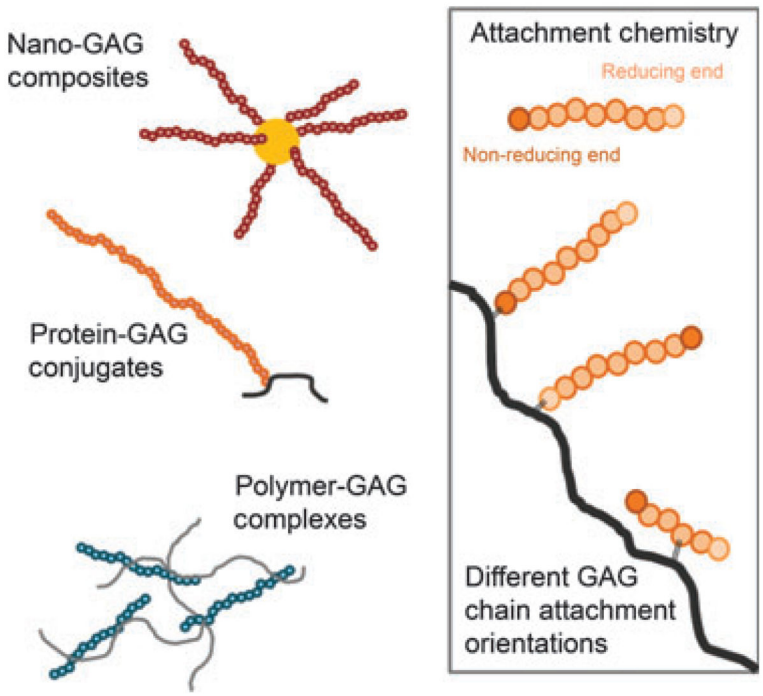

Fig. 2.

Structure of the three basic neoPG structures and GAG chain attachment orientations. From top to bottom, nano-GAG composites, protein–GAG conjugates, and polymer–GAG complexes. The GAG chain attachment orientation illustrates the asymmetry of GAG chains.GAG chains may be attached through their reducing end (natural orientation on native PGs), their nonreducing end or various attachment points along the chain itself.

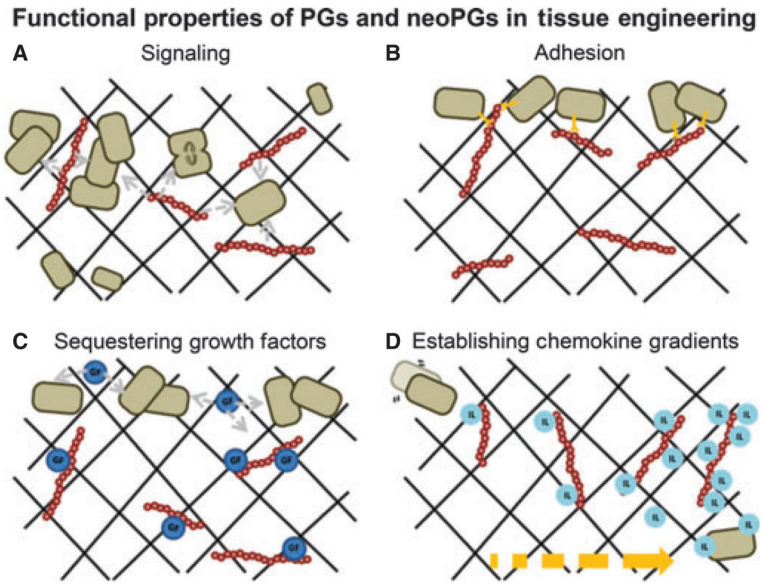

Fig. 3.

Various functional properties of PGs and neoPGs in native and synthetic scaffolds.PGs and neoPGs may act as (A) signaling molecules, shown here supporting cellular growth; (B) cell adhesion molecules, shown here anchoring cells to the scaffold matrix; (C) sequestering signaling molecules for cellular support, shown here binding growth factors (GF) for slow release and cellular growth; or (D) binding molecules to create gradients and drive cellular responses, shown here establishing a chemokine (interleukin, IL) gradient driving cellular infiltration of the scaffold.

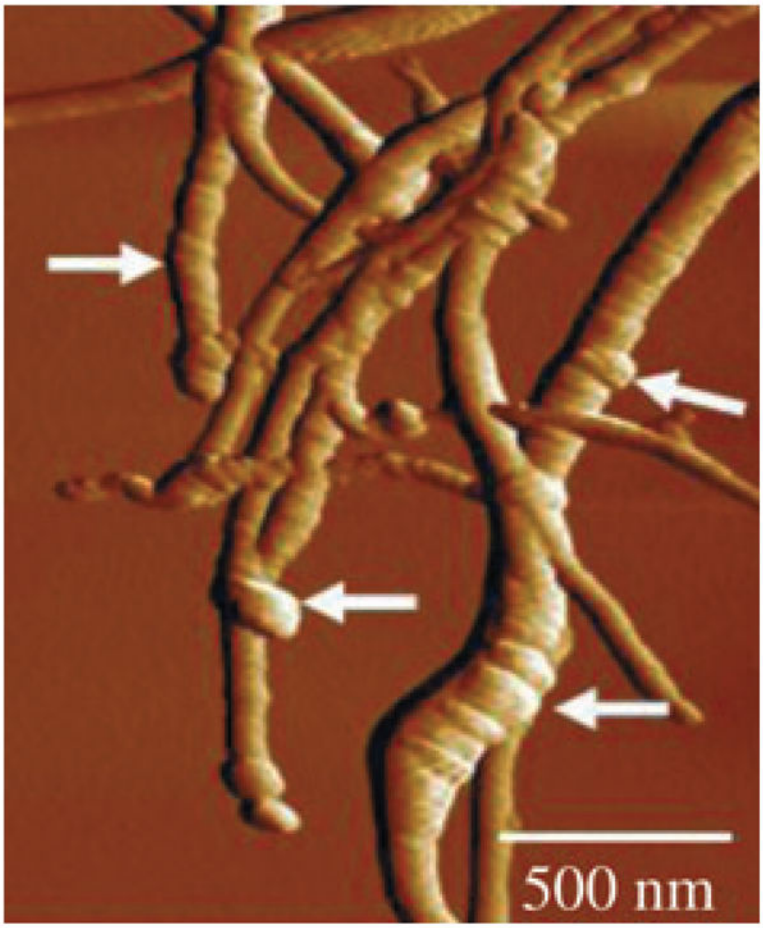

Fig. 4.

Example of nanocomposites containing neoPG. Multiwalled carbon nanotubes wrapped with poly(ethyleneimine) to which heparin was covalently attached [72]. Tapping mode-atomic force microscopy phase image of poly(ethyleneimine)-coated and heparinized multiwall nanotube neoPGs. The surface bulges indicated by arrows correspond to heparin.

Table 1

List of tissue engineering applications for different neoproteoglycan formulations. CS, chondroitin sulfate; DS, dermatan sulfate; HA, hyaluronan; LMWH, low molecular weight heparin; PLA, poly (lactic acid); PLGA, poly (lactic-co-glycolic acid); PVA, poly (vinyl alcohol).

	Neoproteoglycanformulation	Tissue engineering application	References
Protein–GAG conjugates	Recombinant keratan sulfate PG	Used to reprogram fibroblasts to multipotent stem cells	45
	Recombinant heparan sulfate PG	Electrospun in collagen fibrils to foster cellular growth	11
		Modify collagen fibrils to support cellular growth	46
		Support collagen cells in PLA scaffolds	47
	Recombinant dermatan sulfate peptidoglycosaminoglycan	Modulate collagen structure and tissue stiffness	53,62
		Aid cellular adhesion, prevent apoptosis	63
		Promote wound healing and minimize scaring	64
	Peptide-decorated chondroitin sulfate chains	Reduced proteolysis of neoPG	65
Nano-GAG composites	Chondroitin sulfate nanofibers	Enhance cartilage tissue formation in PVA scaffolds	66
	Chondroitin sulfate–collagen fibrils	Used as scaffolds that increased cellular proliferation	10
	CS–collagen–calcium phosphate nanocomposites	Used for bone and cartilage tissue engineering	67–69
	DS–alginate–chitosan microcomplexes	Support of cellular proliferation in tissue regeneration	70
	Heparin-coated nanotubes	Antiseptic, antibacterial properties for wound healing	73,74
	Heparin-coated gold and silver nanoparticles	Promote angiogenisis, exhibit anti-inflammatory properties	75,76
	Hyaluronan-coated silver nanoparticles	Antimicrobial activity	77
	Heparan sulfate chitosan nanoparticles	Support the growth of cartilage tissue	78
		Bound, released growth factors supporting cellular growth	79
Polymer–GAG complexes	Sulfated HA-collagen scaffolds	Used for osteogenic differentiation	80
	Heparin sulfate-coated PLGA microspheres	Support cell adhesion and cellular growth	81
	Heparin- and LMWH-bound star-poly (ethylene glycol) scaffolds	Cardiovascular tissue engineering	82
		Nervous system tissue regeneration	83
		Used to bind and release growth factors	84
	Chondroitin-bound poly(ethylene glycol) hydrogels	As aides to cell growth in wound healing	85–87
		Support stem cell differentiation in cartilage repair	55
	Chondroitin- and hyaluronan-bound alginate gels	Support the growth of chondrocytes in cartilage engineering	91
	Heparin-bound hyaluronan scaffolds	Bound, released growth factors in bone engineering	92,93
	Chondroitin-bound hyaluronan–gelatin scaffolds	Growth of tooth progenitors	94
		Used to support cartilage repair	95,96