Acta Biomaterialia 10 (2014) 1588–1600

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Review

# Heparin-functionalized polymeric biomaterials in tissue engineering and drug delivery applications ☆

Yingkai Liang [a], Kristi L. Kiick [a,b,c,*]

[a] Department of Materials Science and Engineering, 201 DuPont Hall, University of Delaware, Newark, DE 19716, USA
[b] Biomedical Engineering, University of Delaware, Newark, DE 19716, USA
[c] Delaware Biotechnology Institute, 15 Innovation Way, University of Delaware, Newark, DE 19711, USA

ARTICLE INFO

*Article history:*
Available online 2 August 2013

*Keywords:*
Heparin
Hydrogels
Nanoparticles
Tissue engineering
Drug delivery

ABSTRACT

Heparin plays an important role in many biological processes via its interaction with various proteins, and hydrogels and nanoparticles comprising heparin exhibit attractive properties, such as anticoagulant activity, growth factor binding, and antiangiogenic and apoptotic effects, making them great candidates for emerging applications. Accordingly, this review summarizes recent efforts in the preparation of heparin-based hydrogels and formation of nanoparticles, as well as the characterization of their properties and applications. The challenges and future perspectives for heparin-based materials are also discussed. Prospects are promising for heparin-containing polymeric biomaterials in diverse applications ranging from cell carriers for promoting cell differentiation to nanoparticle therapeutics for cancer treatment.


## 1. Introduction

Heparin is a linear polysaccharide which consists of $1 \rightarrow 4$ linked disaccharide repeating units of uronic acid and glucosamine residues (Fig. 1). Discovered nearly 100 years ago, heparin has been clinically employed as a blood anticoagulant since 1935; this activity is a result of its ability to bind to the serine protease inhibitor antithrombin, causing the inhibitor to inactivate thrombin [1,2]. Unfractionated heparin is usually isolated from natural tissues, such as porcine intestine or bovine lung, with an average molecular weight of about 15 kDa, and is highly heterogeneous in chemical structure and molecular weight.

The prevalence of sulfate and carboxylate groups endows heparin with a high negative charge (approximately −75), which mediates its electrostatic interactions with many proteins, such as growth factors, proteases and chemokines [3]. These interactions in many cases serve to stabilize proteins or increase their affinity for cell receptors [4]; such stabilization by heparin of the growth factors fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) has thus been employed in the design of materials as engineered scaffolds for tissue regeneration and controlled release platforms for growth factor delivery [5].

Given its function as an anticoagulant, heparin can exhibit undesirable side effects, such as hemorrhagic complications, heparin-induced thrombocytopenia and/or low bioavailability, when administered non-intravenously [6]. As a result, low-molecular-weight heparin (LMWH), which has a better defined chemical composition, has been developed to provide more predictable anticoagulant dose, longer half-lives and reduced side effects [7]. Many studies have indicated that LMWH is more effective in the inhibition of tumor growth via its regulation of the binding of many angiogenic growth factors (e.g. FGF and VEGF) when compared with unfractionated heparin. LMWH can also interfere with tumor metastasis by reducing the activity of heparanase and thus reducing tumor metastatic potential and growth, or by competing with P-selectin binding, thereby inhibiting the adhesion of tumor cells [8,9]. More recent research has suggested that heparin can also interact with transcription factors to induce apoptotic cell death [10]. Therefore, LMWH has been widely explored as a component in tumor-targeted delivery systems, and a few chemically modified LMWH derivatives, such as LMWH–deoxycholic acid, have been developed with reduced anticoagulant activity and high antiangiogenic efficacy [11,12]. Recent research has also suggested that LMWH may be potentially used as an antifibrotic agent in patients with chronic hepatitis B [13].

Given the pharmacokinetics difficulties posed by heparin's chemical heterogeneity and diversity of biological activities, heparin-mimetic compounds such as negatively charged polymers and sulfonate-modified peptides have been exploited [14–19]. It has been demonstrated that sulfonated polymers such as poly(styrene-sulfonate-co- poly(ethylene glycol) (PEG) methacrylate) and pep-

☆ Part of the Special Issue on Biological Materials, edited by Professors Thomas H. Barker and Sarah C. Heilshorn.

* Corresponding author at: Department of Materials Science and Engineering, 201 DuPont Hall, University of Delaware, Newark, DE 19716, USA. Tel.: +1 302 831 0201.
*E-mail address:* kiick@udel.edu (K.L. Kiick).

1742-7061/$ - see front matter © 2013 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.actbio.2013.07.031

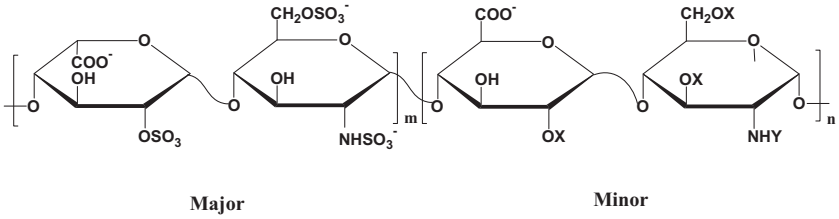


**Fig. 1.** Major (ca. 85%) and minor (ca. 15%) disaccharide sequences of heparin.

tide amphiphile nanofibers (composed of heparin-mimetic peptides decorated with bioactive chemical groups such as sulfonate, carboxylic acid, and hydroxyl groups) have better-defined structures and more specific bioactivity, and are capable of stabilizing growth factors and promoting angiogenesis. Additionally, some polysulfonated heparin mimics, such as poly(2-acrylamido-2-methyl-1-propane sulfonic acid), poly(anetholesulfonic acid), poly(4-styrenesulfonic acid) and polymethacrylic derivatives of 5-amino-2-naphthalenesulfonic acid, are found to be potent inhibitors of angiogenesis. They can potentially modulate angiogenic processes with low toxicity based on the interaction between the sulfonic acid groups and FGF, preventing FGF-induced endothelial cell migration and proliferation [20–22]. The use of these heparin mimics may reduce the risk associated with batch-to-batch variation and impurities of heparin.

Due to its advantageous biological activities, the incorporation of heparin in biomaterials has been highly attractive. Heparin is often physically encapsulated or covalently conjugated to hydrogels to provide sustained release of the anticoagulant [23]. Also, heparin-containing hydrogels have been widely used for the sequestration and controlled release of growth factors to promote angiogenesis and bone regeneration [24,25]. Heparin-functionalized, naturally derived hydrogels have been developed by taking advantage of their biocompatibility, low toxicity, relatively low cost and benign gelation conditions while heparin-synthetic polymer biohybrid hydrogels have been studied to provide increased control over their mechanical and chemical properties [26]. Additionally, surfaces and scaffolds modified with heparin have been explored to suppress non-specific protein absorption and localize growth factors to promote cell attachment and proliferation [27–29].

In clinical application, most of the scaffolds and hydrogels either need to be implanted through a surgical intervention or require injection into the pathological site. The former mode of administration is inconvenient and poses risks to patients, while materials administered via the latter route can be limited in scope of use because many pathological sites are not easily accessible via injection. To address this concern, nanoparticle-based approaches, which render all administration routes possible, have been developed. Nanoparticles pass through capillary vessels with prolonged circulation half-lives in the blood stream, and can also penetrate cells and tissue barriers to reach target organs [30]. Heparin-functionalized nanocarriers can both stabilize the growth factors against denaturation or proteolysis and provide controlled release behaviors, but also promote therapeutic efficacy by increasing the cellular uptake of the delivered molecules [31–34].

Nanoparticulate drug delivery systems have demonstrated great promise in cancer nanotechnology due to their ability to accumulate and reside in tumor tissues via the enhanced permeation and retention (EPR) effect. Their surface versatility permits conjugation of a broad range of biomolecules to enhance targeting and therapeutic efficacy [35,36]. Given the increasing appreciation of the antiangiogenic and apoptotic effects of heparin, heparin-based nanoparticles have been widely explored for cancer therapy as well.

In this review, recent progress in the development of heparin-based hydrogels and nanoparticles is systematically summarized, with an emphasis on those aiming at tissue engineering and drug delivery applications. Types of polymeric networks (crosslinking strategies and stimuli-responsiveness) and the formation of the nanoparticles (physical and chemical synthesis methods) are introduced and some of the important applications for these types of materials are highlighted. Perspectives on the challenges and promise of heparin-based materials are also discussed.

## 2. Heparin-based hydrogels

Heparin-based hydrogels have been increasingly employed as antithrombogenic materials, growth factor carriers and scaffolds for tissue regeneration via covalent or non-covalent strategies. In addition, several chemically labile crosslinks have been explored to temporally regulate hydrogel degradation in response to environmental stimuli, to encourage the development of tissue-like structures and achieve on-demand or targeted delivery.

### 2.1. Physically crosslinked heparin hydrogels

Physical crosslinking occurs as a result of entanglements between dynamic macromolecular species or due to specific non-covalent interactions between polymer chains. Typically, the motifs employed in the formation of physically crosslinked hydrogels include hydrogen bonding, multivalent ionic, metal-ligand and host-guest interactions, and stereocomplexation [37]. Physical hydrogels avoid the addition of toxic crosslinking reagents, which might potentially affect the integrity and bioactivity of encapsulated therapeutic agents. In addition, physically crosslinked hydrogels permit injection due to their shear-thinning capability and the facile re-establishment of the noncovalent bonds [38].

Among the various physical crosslinking mechanisms, biomimetic interactions, including the heparin–protein interaction, have been used widely in the formation of physically crosslinked heparin hydrogels. Several heparin-binding peptides (HBPs), such as those derived from antithrombin III, heparin interacting protein (HIP) and human platelet factor 4, have been exploited for the non-covalent assembly of hydrogel networks. Based on the fact that growth factors, including basic fibroblast growth factor (bFGF), VEGF and hepatocyte growth factor, can bind to short sequences in heparin, multivalent species that incorporate the heparin-binding domains of growth factors could also be potentially employed as multifunctional crosslinkers in the formation of physical networks [39].

In the earliest studies of physically crosslinked, heparin-containing hydrogels, Panitch and co-workers employed the