Acta Biomaterialia 10 (2014) 1581–1587

Contents lists available at ScienceDirect

Acta Biomaterialia

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

Review

Incorporation of heparin into biomaterials ☆

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ARTICLE INFO

Article history:
Available online 8 September 2013

Keywords:
Drug delivery
Affinity delivery system
Self-assembling materials
Heparin-mimetics

ABSTRACT

This review provides an overview of the incorporation of heparin into biomaterials with a focus on drug delivery and the use of heparin-based biomaterials for self-assembly of polymer networks. Heparin conjugation to biomaterials was originally explored to reduce the thrombogenicity of materials in contact with blood. Many of the conjugation strategies that were developed for these applications are still popular today for other applications. More recently heparin has been conjugated to biomaterials for drug delivery applications. Many of the delivery approaches have taken advantage of the ability of heparin to bind to a wide variety of growth factors, protecting them from degradation and potentiating interactions with cell surface receptors. More recently, the use of heparin as a base polymer for scaffold fabrication has also been explored, often utilizing non-covalent binding of heparin with peptides or proteins to promote self-assembly of hydrogel networks. This review will highlight recent advances in each of these areas.

1. Introduction

Heparin and heparin sulfate are linear polysaccharides. Both are synthesized from a common precursor proteoglycan. Heparin is only produced in mast cells, where it is cleaved from the core protein (serglycin) at the end of synthesis [1]. Heparin sulfate (HS) is found in most tissues and remains attached to the core protein. Both are sulfated and also contain carboxylic acids, which contribute to an overall net negative charge [2]. Heparin/HS polymer chains are made up of repeating disaccharides, primarily uronic acid and glucosamine with varying degrees of sulfation and N-acetylation (Fig. 1). While their interactions with proteins are largely electrostatic, there are clearly contributions from hydrophobic effects and hydrogen bonding, as well as the promotion of secondary structure in the proteins binding to heparin, which imparts some selectivity and specificity [3]. In addition to binding to growth factors, heparin also binds to a number of enzymes (e.g. antithrombin III), plasma proteins (platelet factor 4, PF4), and extracellular matrix (ECM) proteins (e.g. fibronectin, laminin) [4,5]. In some cases specific heparin sulfation codes have been identified that facilitate binding with growth factors (e.g. bFGF) or enzymes (e.g. antithrombin III) [6,7].

2. Heparin modification of materials to reduce thrombogenecity

Heparin was discovered in 1916 and has been used clinically as an anticoagulant since 1935 [1]. Modification of biomaterials with heparin has been performed for over 50 years. Initially heparin was immobilized via ionic interactions to reduce the thrombogenecity of materials in the 1960s [8,9]. This approach took advantage of electrostatic interactions of the negatively charged sulfate groups on heparin with colloidal graphite and benzalkonium chloride (cation) in alternating layers [8]. Leininger et al. adapted this method for use on plastic surfaces by forming quaternary ammonium sites on the material surface to promote electrostatic interactions with heparin [10].

In the 1980s, methods for covalent conjugation were developed that used end-point immobilization, in which a primary amine on the material of interest was reacted with an aldehyde group generated by heparin chain depolymerization [11]. The literature on heparin immobilization is vast and has been reviewed extensively elsewhere [12–15]. This end-point immobilization has been use to conjugate heparin to vascular grafts and has been commercialized for expanded polytetrafluoroethylene (ePTFE) and Dacron grafts [16,17]. More recently work with heparin immobilization on vascular grafts has explored mechanisms other than antithrombotic effects that may be influenced by heparin, including elastin synthesis [18]. Additional studies explored coating vascular stents with heparin, however, more recent studies suggest that this may stimulate restenosis by sequestration of growth factors that promote smooth muscle cell proliferation [19].

3. Heparin modification of materials for drug delivery

Many types of drug delivery systems have been developed for the controlled release of small molecule and protein-based drugs for biomedical applications [20]. For delivery of protein-based drugs, such as growth factors, there are many advantages to the

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

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http://dx.doi.org/10.1016/j.actbio.2013.08.045

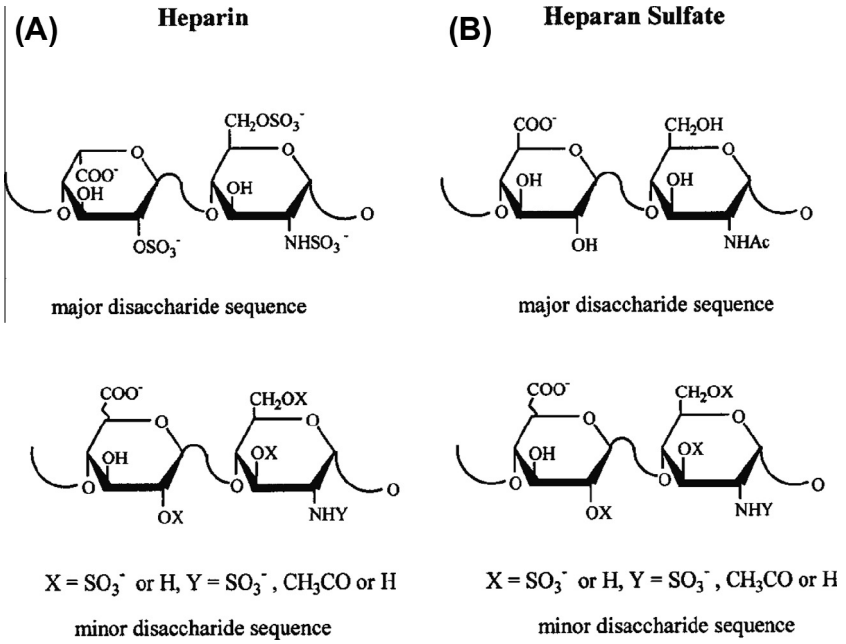

Fig. 1. (A) Structure of the major and minor disaccharide sequences of heparin. (B) Structure of the major and minor disaccharide sequences of heparan sulfate. Reprinted with permission from LeBrun and Linhardt [94].

use of affinity drug delivery systems, such as heparin-based delivery systems. These affinity delivery systems utilize specific non-covalent interactions to stabilize drugs and immobilize them within a biomaterial matrix, thus protecting their biological activity and slowing their diffusion from the matrix. The interactions with growth factors and affinity delivery systems can mimic those that naturally occur with native ECM proteoglycans.

Because a large number of growth factors bind to heparin with either moderate or high affinity ($\sim 10^{-6}$–10^{-9} M K_D) heparin-based delivery systems have proven useful for the delivery of a wide range of growth factors for different biomedical applications [21]. In the case of heparin-based systems, these interactions can also modulate the binding of a growth factor to the cell surface receptor. For some growth factors, such as basic fibroblast growth factor (bFGF), heparin facilitates binding of bFGF to its receptor and actually increases activity [22]. However, for other growth factors, such as bone morphogenetic protein 2 (BMP2), heparin can inhibit binding to the cell surface receptor [23]. The effects of heparin on BMP2 signaling are complex, as heparin has also been shown to block inhibition of the BMP2 pathway by noggin [24], thus demonstrating that heparin can have direct interactions with growth factors and their receptors, as well as indirect and sometimes opposing effects on signaling cascades.

Growth factors that bind to heparin include commonly studied heparin-binding growth factors, such as bFGF and vascular endothelial growth factor (VEGF), as well as members of the transforming growth factor (TGF) (e.g. BMPs), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and hepatocyte growth factor families [25–28]. Other morphogens, such as sonic hedgehog (Shh), and pathogens, such as *Bacillus pertussis*, herpes simplex virus (HSV) and *Plasmodium falciparum*, also bind to heparin and can be delivered or sequestered using a similar approach [29,30].

3.1. Covalent conjugation of heparin to biomaterials for delivery

Early work in demonstrating the utility of heparin-based delivery was performed by Edelman and Langer. Their initial system utilized heparin-conjugated Sepharose beads to bind bFGF within alginate microspheres. Their preliminary studies in vitro demonstrated that active bFGF could be released for at least 2 weeks, and heparin enhanced growth factor activity [31]. They went on to demonstrate the delivery of bFGF from heparin–Sepharose beads in alginate-stimulated angiogenesis and neointimal proliferation in a rat carotid artery model [32]. Later a Phase I clinical trial using these materials showed that bFGF improved revascularization after coronary artery bypass in a small number of patients [33]. Additionally, a Phase II trial demonstrated improved revascularization with bFGF treatment and a trend toward increased left ventricular ejection fraction [34]. These studies demonstrate that heparin-based delivery can be used to provide sustained release of growth factors in a clinical model.

Another approach to covalently immobilize heparin on biomaterials is to covalently link it to a protein, such as collagen or albumin, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) [35]. Heparin was conjugated to albumin using this method and emulsified to form microspheres that could then be covalently crosslinked with glutaraldehyde [36]. A similar approach was used crosslink heparin to collagen matrices for the delivery of bFGF, and bFGF delivery was found to enhance endothelial cell proliferation in vitro [37,38]. Later in vivo studies demonstrated that bFGF delivery from similar collagen matrices increased vascularization for 3 weeks in a rat subcutaneous implant model [39,40].

A similar EDC chemistry can be use to immobilize heparin onto poly(L-lactide-co-glycolide) (PLGA)-based materials. Jeon et al. developed heparin-conjugated PLGA nanospheres by first reacting PLGA with t-Boc-protected glycine and then deprotecting. Nanospheres of PLGA were formed using an oil/water emulsion and reacted with heparin in the presence of EDC/N-hydroxysuccinimide (NHS) [41]. Controlled release of bFGF and increased cell proliferation was observed over 28 days in vitro. They observed increased capillary density with bFGF and PLGA nanospheres versus controls in a mouse ischemic limb model. Similar heparin conjugation methods have also been used with salt-leached PLGA scaffolds to deliver BMP2, and BMP2 delivery increased bone formation in an ectopic bone formation model compared with PLGA scaffolds without heparin [42].