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Review article

Biomedical applications of engineered heparin-based materials

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ABSTRACT

Heparin is a negatively charged polysaccharide with various chain lengths and a hydrophilic backbone. Due to its fascinating chemical and physical properties, nontoxicity, biocompatibility, and biodegradability, heparin has been extensively used in different fields of medicine, such as cardiovascular and hematology. This review highlights recent and future advancements in designing materials based on heparin for various biomedical applications. The physicochemical and mechanical properties, biocompatibility, toxicity, and biodegradability of heparin are discussed. In addition, the applications of heparin-based materials in various biomedical fields, such as drug/gene delivery, tissue engineering, cancer therapy, and biosensors, are reviewed. Finally, challenges, opportunities, and future perspectives in preparing heparin-based materials are summarized.

1. Introduction

Heparin is glycosaminoglycan (GAGs) composed of two disaccharides repeating; the 1,4-linked uronic acid (D-glucuronic (GlcA) or L-iduronic acid (IdoA)) and the D-glucosamine (GlcN). Heparin is a linear polysaccharide with high negative charges due to the carboxylic and sulfonate groups in its structure. Heparin biosynthesis inside the body by the endoplasmic reticulum and the Golgi apparatus of mast cells in connective tissues, including intestines, liver, and lungs, via activation of several enzymatic reactions [1–5].

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The quality and application of heparin are controlled mainly by the molecular weight of heparin. The higher molecular weight of heparin dissolves and degrades more slowly in water than the lower molecular weight of heparin, making it appropriate for sustained drug delivery or low-degradation applications. Unfractionated heparin (UFH), low molecular weight heparin (LMWH), and ultra-low molecular weight heparin (ULMWH) are three commercially available sub-types of heparins. UFH is the least processed form of heparin adopted from animal tissue after purification. LMWH is produced by controlled depolymerization of UFH, and ULMWH is a chemically synthesized form of heparin with the same pentasaccharide sequence as UFH and LWMH [6].

Due to its high negative charge, heparin (and its derivatives) can interact with different components, including growth factors, coagulating and fibrinolysin proteins, and immune active compounds (i.e., chemokines and cytokines). Heparin is well known as the most prevalent clinical anticoagulant agent, especially during surgery or following trauma, to inhibit the coagulation pathway via suppressing thrombin (FIIa) and/or factor X (FXa). This anticoagulant property of heparin results from the presence of pentasaccharide sequences that can strongly bind to the antithrombin. This binding will change the conformation of antithrombin, making them appropriate for attachment to the targeted coagulating proteins. This anti-coagulation property of heparin makes it a promising candidate to be employed in the structure of blood-contacting devices (i.e., venous catheters or extracorporeal blood filters) [7,8], resulting in heparin-coating technique as a gold standard for improving the hemocompatibility of materials [9].

In addition to anticoagulation activity, heparin can be an anti-inflammatory agent by interacting with inflammatory mediators, such as histamine [10–12]. The biocompatibility, biodegradability, angiogenesis inhibition capability, and high potency to load anti-cancer drugs are other characteristics of heparin that make it a fascinating candidate in various biomedical and drug delivery applications [13,14]. In addition, heparin has other therapeutic applications, such as antiviral activity, burn injury alleviation, wound healing capacity, bone, and tissue regeneration, sensing, and detection abilities [15]. These attractive properties and applications of heparin make it one of the best biomaterials in biomedical engineering and drug delivery applications.

With the recent advancements in nanotechnology and impressive achievements using nanotechnology in drug delivery and regenerative medicine, researchers employed more and more nanotechnology in their studies [16]. Thus, several nanomaterials with different sizes, morphology, and structural features have been introduced and used in therapeutic applications [17–21]. Utilizing these nanoplatforms could enhance treatment performance by increasing therapeutic agents' bioavailability in their targeted sites and reducing their side effects [22–24]. One of the exciting materials which could be incorporated into the structure of nanomaterials is heparin. For example, heparin could appear as a coating agent for nanocarriers and provide a platform for attaching anti-inflammatory agents to make them promising candidates for anti-inflammation applications. On the other hand, heparin can bind to different types of cytokines and chemokines and suppress the activation of the nuclear transcription factor-κB (NF-κB) pathway to enhance the anti-inflammatory responses of cytokines and chemokines [25]. Also, heparin derivatives can be used as an anti-cancer agent by restraining tumor metastasis and angiogenesis via selective binding to the pro-angiogenic growth factors and inhibiting heparinase [26].

Different types of heparin-based platforms, from polymeric composites to nanoparticles, hydrogels, and nanofibers, have been synthesized and employed for biomedical applications, showing that heparin has enormous potential applications in biomedical and tissue engineering [27–29]. This review summarizes some of the most recent advances in biomedical applications of heparin-based materials. We describe heparin's structure, chemistry, physicochemical properties, and bioactivity. Then we discuss the different biomedical applications of heparin, focusing on drug/gene delivery, cancer therapy, tissue engineering, and biosensors.

2. Structure and biochemistry

2.1. Chemical structure and synthesis

Heparin, a diverse polysaccharide, consists of various monomeric units (Fig. 1A and B). The biosynthesis process of heparin occurs naturally within the endoplasmic reticulum and the Golgi apparatus, following a common pathway involving 22 enzymes (Fig. 2A) [30].

This process begins with the attachment of a tetrasaccharide linker to the serine residue of the core protein. The linker comprises four monosaccharides: xylose, two galactose units, and glucuronic acid. The xylose unit couples with the serine residue first, facilitated by xylosyltransferase-1 or -2. This action is followed by the addition of two galactose units by galactosyltransferase-1 and -2, and a β-D-glucuronic acid by glucuronosyltransferase [31,32].

2.2. Maturation and modifications

The maturation of heparin involves modifications to the sugar units, primarily conducted by Golgi-associated sulfotransferases (STs) from different families. The steps are as follows:

(1) Glucosaminyl-*N*-acetylase/N-sulfotransferase (NDST) performs *N*-deacetylation and *N*-sulfation of GlcNAc. (2) 6O-sulfotransferases (HS6STs) attach a sulfate group to the C6 position of the previously modified glucosamine residues. (3) D-glucuronyl C5-epimerase (Glce) converts D-glucuronic acid to L-iduronic acid. (3) 2O-sulfotransferases (HS2STs) transfer a sulfate group to the 2-position of uranyl residues, primarily iduronic acid (IdoA). (4) 3O-sulfotransferases (HS3STs) perform the 3O-sulfation of previously *N*-sulfated glucosamine residues (Fig. 2B) [33].

2.3. Different derivatives of heparin

2.3.1. Unfractionated heparin (UFH)

Unfractionated Heparin (UFH) is a pharmaceutical-grade heparin derived from animals. Composed of a mixture of heparin molecules with different molecular weights, UFH production still relies on purification from animal tissues, a method that has been used for years [34]. For safety reasons, animals used in this process should be healthy and not given medication suitable for human consumption. Various types of analyses are performed on UFH to eliminate contamination risks [35].

The production of UFH, however, faces challenges. The high costs and low production yields limit the feasibility of synthesizing full-length heparin chains. In response, innovative methods have been developed, such as the use of catalysts like TiO_2 in the photo-depolymerization process, which produces high yields of UFH with reduced side effects [36,37].

2.3.2. Low molecular weight heparin (LMWH)

Low Molecular Weight Heparin (LMWH) is obtained through chemical synthesis or enzymatic depolymerization of UFH chains. Various types of LMWH can be produced, depending on the type of depolymerization reaction employed, resulting in different molecular weights, sulfation degrees, and content of AT-binding domains. However, the synthesis methods can be costly and potentially reduce efficiency. To combat these issues, new methods have been developed to obtain LMWH that are safe, easy, inexpensive, and highly efficient [38].

2.3.3. Ultra-low molecular weight heparins (ULMWHs)

Ultra-Low Molecular Weight Heparins (ULMWHs) are synthetic, bioavailable products typically seen in oligosaccharide forms. Despite their high price and limited clinical applications, ULMWHs have found a niche in the clinical setting, although they represent a small percentage of usage compared to LMWH and UFH [38].

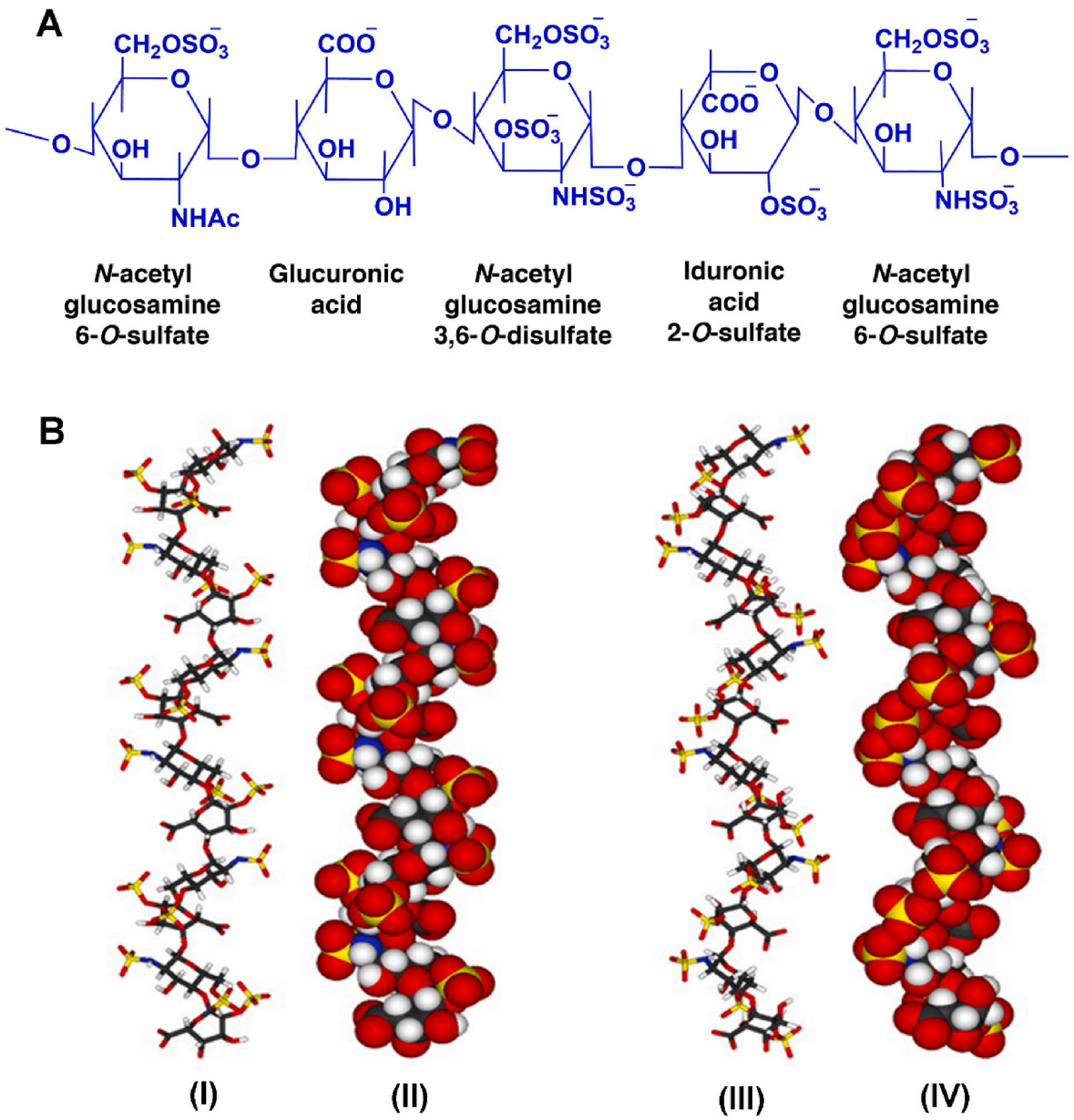

Fig. 1. (A) The chemical and (B) the three-dimensional structure of heparin. Three-dimensional heparin structures (Ball-and-stick models) are depicted using a combination of NMR spectroscopy and molecular modeling methods. In one model, all 2-*O*-sulfo-α-L-iduronic acids are in the $^{2}S_{0}$ conformation (**I** and **II**), while in the other one (**III** and **IV**), they are in the $^{1}C_{4}$ conformation.

3. Physicochemical properties of heparin

Heparin, a member of the glycosaminoglycans (GAGs) family, exhibits various intriguing physicochemical properties, albeit understanding their correlation with bioactivity proves challenging [39] This unique biopolymer has high acidity and shows significant molecular heterogeneity due to diverse patterns of sulfonation and the presence of hexuronic acid epimers [39–41]. The conformation of heparin varies based on its physical state and internal structural dynamics. In both solid and liquid states, its general conformation is rod-like. However, the conformational changes in iduronate residues lead to substantial internal movement, influencing its three-dimensional spatial configuration [41]. To reveal the fine details of heparin structure, specialized techniques are required, such as disaccharide compositional analyses, impurity analyses, and spectroscopic methods. These techniques can distinguish minor differences and have allowed scientists to develop hyphenated methods, which combine chromatographic and spectral methods [42]. Nuclear Magnetic Resonance (NMR) spectroscopy is a particularly powerful tool for characterizing heparin. It provides a detailed 'fingerprint' of heparin samples, revealing features like 3-*O*-sulfation of heparin, which enhances its pharmacological action and inhibits fibroblast growth factors (FGFs) [43]. Structural alterations in heparin molecules have been shown to modify their anticoagulant spectra [44].

Heparin exhibits a high degree of molecular heterogeneity, which is associated with the varying patterns of sulfonation and the presence of hexuronic acid epimers. This heterogeneity is reflected in its three main glycan structures: the highly sulfated domains with absolute uniformity, the precursor polysaccharide formed from an unsulfated disaccharide of β-D-glucuronic acid and *N*-acetylated α-D-glucosamine, and the minimum structure required for high-affinity antithrombin binding-the pentasaccharide [45]. The thermal properties of heparin also provide crucial information. Studies have shown that the thermal properties of heparin can be altered when complexed with other compounds, such as

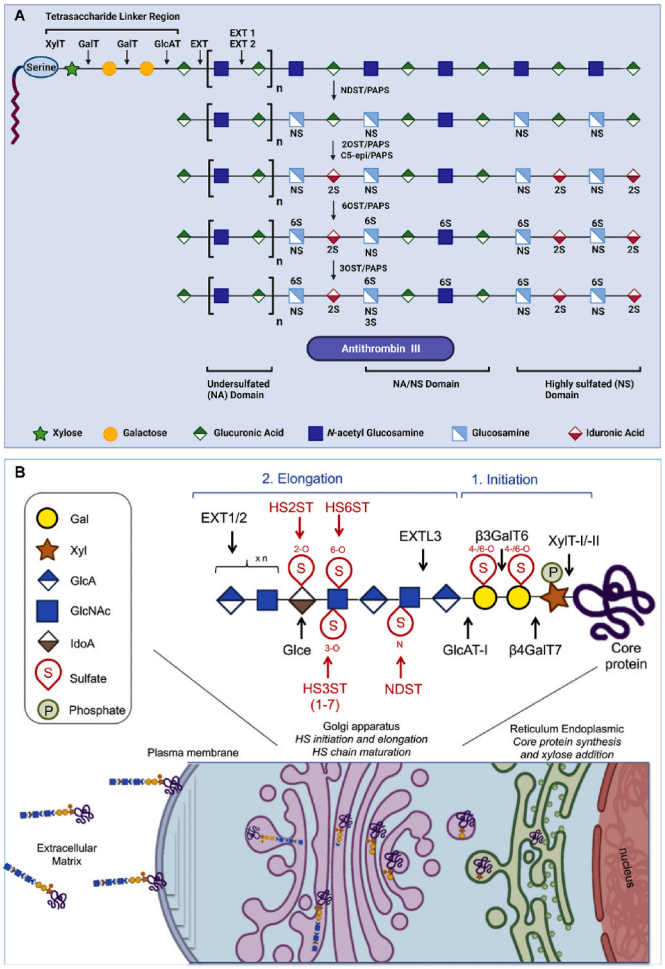

Fig. 2. Intracellular biosynthesis of heparin and heparan sulfate and maturation in the Golgi and the endoplasmic reticulum via several enzymatic reactions. (**A**) The core proteins are produced in the ER at first. Then a tetrasaccharide linker is attached step by step to the protein's serine residue, followed by the polymerization of polysaccharide chains. Xyl: xylose, Gal: galactose, Ser: Serine, NA: Undersulfated domain, NS: Highly sulfated domain. EXT: Exostosin; Exostosin-1 and 2 are proteins that did by the EXT1 and EXT2 genes in humans, respectively. Antithrombin III is a small glycoprotein anticoagulant that inactivates several enzymes of the coagulation system. (**B**) Heparan sulfate proteoglycan is transferred in the extracellular matrix or is attached to the plasma membrane to do its biological functions [33].

water-soluble chitosan. The thermal degradation of heparin and its complexes has been examined through Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) [46].

Heparin's absorption properties have been studied with UV–visible spectra, revealing that the absorbance at 538 nm can be used as a measure of heparin concentrations [47]. Despite these findings, the full range of heparin's spectral properties remains to be elucidated. Table 1 shows the summary of the physicochemical properties of heparin.

Table 1
Summary of physicochemical properties of Heparin.

Solubility	Heparin is soluble in dimethyl sulfoxide and water, while insoluble in inorganic solvents	[48]
Thermal stability	Based on TGA analysis, the degradation of heparin occurs suddenly at 210–310 °C	[49]
Morphology	Heparin is found in polysaccharide sulfated form, which has various sites like sulfonic, carboxyl, and hydroxy groups for reactions which further helps in the drug delivery systems	[50]

3.1. Biodegradability

Biodegradability is the ability of biological materials to decompose by interacting with suitable elements within appropriate conditions. Biodegradation usually occurs in the presence of enzymes or chemical deterioration properties of living organisms. Recently, interest in "biodegradable" biomaterials has increased in many medical applications due to their low toxic effects [51].

Heparin has already been acknowledged as a non-toxic and biodegradable polysaccharide that can bind to different materials. Heparin is degraded *in vivo* by the heparinase enzyme secreted by various cells, such as platelets [52]. Although this degradation does not pose a significant risk, it could affect the anticoagulation property of heparin. In other words, it could produce fragments with low anticoagulation properties [53]. This enzymatic activity could be used to fabricate a controlled-release formulation. For instance, heparinase/heparinase enzymes were used to maintain the release of growth factors (FGF-2) from the alginate microsphere containing heparin-FGF-2 in the presence of heparinase (Fig. 3) [54].

Heparin-based nanomaterials have been utilized as intelligent carriers for anticancer drugs and diagnostic instruments. In addition, some heparin delivery systems have been suggested to increase regional effects and control systemic toxicity. However, since heparin is an effective antithrombotic compound and current local delivery agents do not possess the capability to regulate the release kinetics accurately, the site-specific therapy is probable to bleed. For example, if heparin-functional biomaterials are exposed to blood products at the implantation site, they undergo an enzymatic degradation by the platelet-secreting heparinase. Because of this heparinase activity, the heparin-dependent released process has occurred.

In ECM, the presence of heparin in the environment is essential for the enzymatic biodegradation from biomaterial to mimic the growth factor-sequestering and to release the property of heparin [57]. In a study, heparin was modified with methacrylate groups, which allowed easy covalent incorporation into hydrogels. Thus, covalently bound heparin was degraded by heparinase, and growth factor signaling was increased [57].

Heparin has also been employed to modify polymeric characteristics. Studies on heparin-based hydrogels have explored their swelling, stiffness, and adhesion properties to circumvent the short *in vivo* half-life of bioactive factors. Owing to its binding affinity for these factors, heparin could protect them from proteolytic degradation, thus improving their lifespan and bioavailability [58]. However, the impact of such modifications on reducing acute and chronic inflammatory responses *in vivo* remains ambiguous [59–62].

Heparin was modified with different materials to create a more porous structure on the surface of the scaffold, and the porosity was increased by enhancing the degradation rate and permeability, which in turn could decrease the mechanical stiffness [63–65]. Using biodegradable biomaterials modified with heparin as a vascular graft provides a controlled degradation over time, reduces blood loss, and improves the efficiency of the healing process [63,66].

3.2. Mechanical properties

Mechanical properties of biomaterials are critical because they determine how these materials respond under different force conditions. For example, if the mechanical properties of biomaterials are weak, they cannot provide sufficient mechanical support for bone tissue regeneration. In addition, cells are always exposed to mechanical forces from the extracellular environment. In different tissues, they can sense the elasticity of a matrix and convert mechanical signals into different physiological responses [67,68]. Consequently, biomaterials with harmonized mechanical properties in the imperfect tissue will improved simplify tissue rebuilding. It has been proven that the transfer of mechanical information to the inner part of the cell, the nucleus, is done by

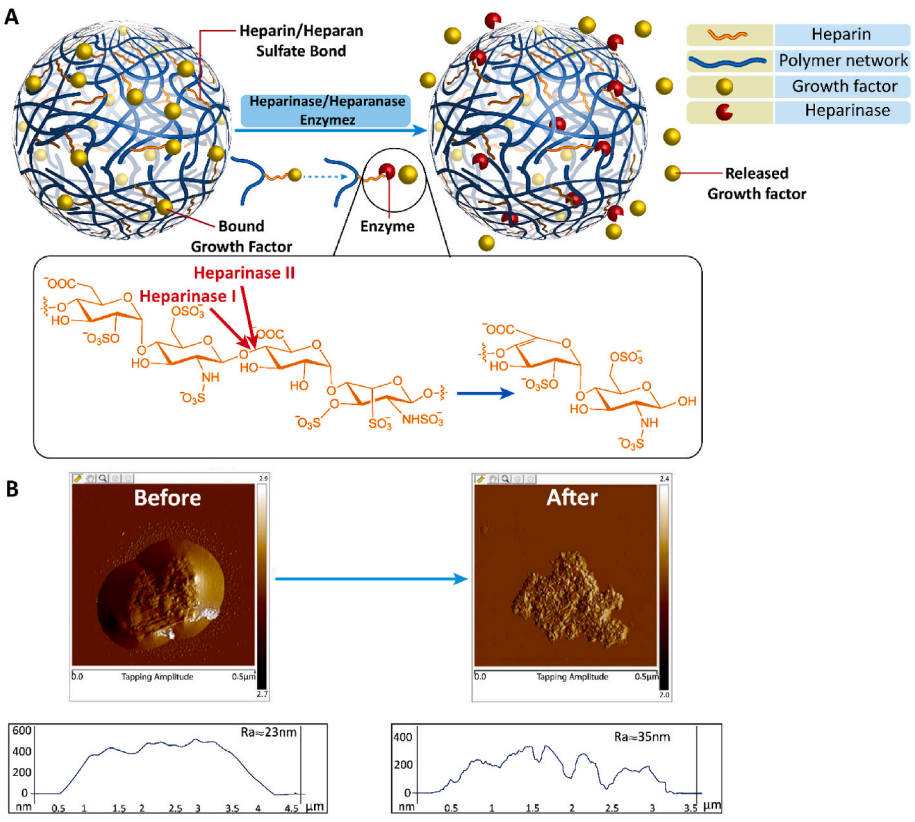

Fig. 3. Schematic representation of synthesizing the heparin/heparan sulfate bond and how heparinase enzymes are used to release growth hormones. (A) Active growth factor release mechanism occurs due to the disruption of heparin/heparan sulfate bonds in the presence of heparinase/heparinase enzymes. Reprinted with slight modifications from Ref. [55]. **(B)** Atomic force microscopy image of heparin containing nanocapsules before and after exposure to Heparanase. Reprinted with permission from Ref. [56].

dynamically regulating the integrity and elasticity of the cytoskeleton [69].

Therefore, to describe the rheological properties of heparin, irrespective of the methodology, recent developments have provided a better understanding of the formulation, processing, characteristics, and quality of drug efficacy. Such attributes can further be characterized by rheological properties at analyze particle size, morphology, concentration, and molecular shape [70]. Using rheological properties, most blood and cardiovascular diseases can be characterized through blood viscosity [71] where heparin, as a potent antithrombotic agent, has been reported to reduce the blood viscosity, which is an underlying determinant of blood flow [72] to avoid coagulation [73]. The intrinsic mechanism works by forming complexes with antithrombin III, resulting in high affinity to bind to thrombin and inactivate factors IX, Xa, XI, and XII and the tissue factor–VIIa complex, which in turn prevents thrombin generation and avoids occlusive thrombosis in arteries [74,75].

In mechanical terms, the literature reveals that heparin can regulate numerous biological processes while binding with bFGF, VEGF, and BMP-2 proteins [76–78]. For instance, it has been reported that the mechanical strength of a collagen-based scaffold can significantly increase through the immobilization of heparin on the scaffold. This increase in mechanical and binding abilities is associated with more calcium deposition and higher alkaline phosphate activity, which enhances bone osteogenesis [79,80]. This process springs from the structural domains of heparin that exhibit affinity with various growth factors and form stable complexes (e.g., increasing osteoblastic stimulation with stable rhBMP-2, in this case). Such properties further increase the suitability of heparin in immobilization and controlled delivery of proteins with prolonged bioactivity [81,82].

The incorporation of heparin in the structure of a gelatin-based cryogel led to an improvement in the mechanical properties of the gel due to its cross-linking with gelatin components. The heparin not only affected the structural features of cryogel but also acted as an agent for controlling the release of growth factor from the gel so that by increasing the amounts of heparin, the primary burst release effect could be prevented [83].

4. Heparin-based biomaterials

Biomaterials containing heparin are becoming increasingly popular for use in biomedicine due to their beneficial effects in medicinal and biological applications [84]. By using different preparation methods, scientists can customize the structures and features of these materials, which can be extremely useful for specific applications. This section will focus on the integration of heparin into biomaterials, before delving into their bioactivity and various biomedical applications.

Heparin-based biomaterials exhibit diverse structures, including homogeneous blends, core-shell configurations, or encapsulation of heparin incorporated with nanomaterials, polymer, nanofiber, and nano gel [84–86]. The morphology of heparin-based biomaterials can range from the shape of nanoparticles to fibrous or porous materials structures, depending on the fabrication technique [84,87]. Blending heparin into biopolymers such as PLGA, chitosan, or PEG has become one of the most common preparation methods [87]. In situ, encapsulation is another commonly used technique where heparin is incorporated into the matrix of material during the fabrication process [84,86,88]. On the other hand, nanoprecipitation and emulsion/solvent evaporation techniques can be used to prepare heparin-based nanoparticles [84]. Moreover, other methods can also be performed, such as surface functionalization or chemical conjugation of heparin to the polymer backbone [84,89]. As a fabrication technique, electrospinning is widely used in various studies that have been reported to produce heparin-containing polymer materials [84,86,88]. Several materials and their heparinized method with their applications were also summarized in Table 2.

Combinations of nanomaterials, including polymeric, metal, and core/shell with biological materials as the new composites are attracting scientists as a new tool in biomedicine. Recently, the idea of gathering or linking a nanomaterial and heparin showed some improvements in its

Table 2
Several heparinized materials and their potential applications.

Heparin-based materials	Chemistry	Application	References
Doxorubicin loaded heparin–deoxycholic acid (DOCA) nanoparticles	Hydrophobic interaction with the DOCA moiety of the particles	Cytotoxicity in mouse models-anticancer effects	[90]
Nanomaterials			
Synthetic polymer			
Poly-ε-caprolactone	Electrostatic	Anticoagulant drug delivery	[91]
Poly(methyl methacrylate)	Self-assembled amphiphile	Drug delivery	[92]
Metal			
Gold Nanoparticles	Reduction of metal salts with heparin chains Amine on diaminopyridine moiety on heparin interacts with surface	Biocompatibility, drug carrier	[93]
Magnetic Iron oxide	Electrostatic layer-by-layer with poly (l-lysine)	Targeted drug delivery	[94]
Biopolymer			
Chitosan-heparin polyplexes	Polyelectrolyte complex formation between polycationic chitosan and polyanionic	Gene therapy, gene delivery	[95]
Chitosan–Heparin	Ionic gelation	Anti-Helicobacter pylori therapy, drug delivery	[93]
Core/shell			
Heparin-Loaded Core-Shell Nanofiber Sutures	Coaxial electrospinning,	Achilles Tendon Regeneration, drug release	[96]
Pluronic/Heparin	Heparin crosslinks activated poloxamer of [poly (ethylene oxide)–poly(propylene oxide)–poly (ethylene oxide)]	Drug carrier and imaging agent	[93]
Hydrogels			
NGF and bFGF loaded a Heparin hydrogel	Electrospinning	nerve regeneration	[88]
Nanofibers			
Poly(l-lactide-*co*-ε-caprolactone) nanofibers	Coaxially electrospun PLLACL nanofibrous mats with heparin incorporated	Blood vessel tissue engineering, *in vitro* proliferation test	[97]

known properties, and it opens the door to new composites in the therapeutic area. Heparin-loaded nanoparticles maintained their anticoagulant activity, increasing anti-cancer drug effectiveness caused destroying tumor cells. In their study, Jiao et al. [92] examined the heparin's oral bioavailability when conjugated to various polymers, including biodegradable poly-ε-caprolactone, nonbiodegradable polymethacrylates Eudragit RS and RL and poly(lactic-*co*-glycolic acid) (PLGA), in rabbits. The authors used a multiple emulsion process to entrap heparin within polymeric nanoparticles, which ranged in diameter from 260 to 300 nm. The anticoagulant activity of these nanoparticle composites was assessed *in vitro* and *in vivo*. Results indicated that heparin-loaded particles retained their anticoagulant activity, suggesting that the nanoparticles protected heparin from degradation and slowly released it in an unaltered state. Notably, orally administered free heparin was less effective than heparin-loaded particles.

In a study conducted by Park et al. [90], they were able to successfully trap the chemotherapy drug doxorubicin into nanoparticles made of amphiphilic heparin-deoxycholic acid (heparin-DOCA) through hydrophobic interaction with the deoxycholic acid (DOCA) component of the particles. The researchers tested the cytotoxicity of these nanoparticles in mouse models and found that they did not cause any side effects, unlike the free doxorubicin which caused considerable weight loss in the models. Additionally, the doxorubicin-loaded nanoparticles were found to significantly inhibit the growth of squamous cell carcinoma (SCC) and human umbilical vascular endothelial cell in comparison to free heparin. Furthermore, the researchers evaluated the nanoparticles' ability to suppress SCC tumor growth *in vivo* using mouse models and found that the entrapped doxorubicin in heparin-DOCA nanoparticles was the most effective in suppressing tumor growth compared to free doxorubicin and heparin-DOCA nanoparticles. This is because the nanoparticles have a prolonged circulation in the bloodstream, which exposes cancer sites to the drug for a longer period. The authors also noted that these particles have a dual action in inhibiting cancer by first destroying tumor cells with the doxorubicin drug and then inhibiting cellular proliferation with the heparin present in the nanoparticles. In their research, Passirani et al. [91] utilized poly (methyl methacrylate) (PMMA) nanoparticles that were coated with either heparin or dextran. Their objective was to evaluate the *in vivo* behavior of these particles by examining their ability to inhibit the complement system and their blood-circulation time. Their experimentation discovered that PMMA nanoparticles coated with heparin chains successfully prevented the complement cascade activation.

Li R. et al. [97] evaluated the efficacy of a heparin-poloxamer thermosensitive hydrogel loaded with basic bFGF and nerve growth factor (NGF) in enhancing peripheral nerve regeneration in diabetic rats. The researchers created a peripheral nerve injury model in diabetic rats and applied the hydrogel loaded with bFGF and NGF to the injured site. The nerve regeneration and functional recovery in comparison to control groups were analyzed. The results demonstrated that the hydrogel effectively delivered the growth factors, improving nerve regeneration and functional outcomes in diabetic rats. This study highlighted the potential of heparin-poloxamer hydrogels as a promising approach for promoting nerve regeneration in diabetic conditions.

As mentioned above, heparin-incorporated nanofiber materials have attracted much attention. Su Y et al. [88] investigated the encapsulation and controlled release of heparin from nanofibers composed of poly (l-lactide-*co*-ε-caprolactone) (P(LLA-CL)) using the electrospinning technique. The nanofibers were prepared to incorporate heparin using an electrospinning technique to evaluate the behavior of heparin release from the produced material over time. The results demonstrated that the electrospun PLLACL nanofibers successfully encapsulated heparin and exhibited controlled release characteristics, with the release rate influenced by the composition of the nanofibers. This further showed the potential of heparin/PLLACL nanofibrous mesh in blood vessel tissue engineering.

In the study conducted by Ye, Y.J. et al. [96], the effectiveness of heparin-loaded core-shell fiber sutures for Achilles tendon rupture repair was investigated. High-concentration heparin sutures (PPH3.0) outperformed low-concentration and commercial sutures (CSs). PPH3.0 sutures reduced inflammatory cell recruitment and exhibited good histocompatibility. Histological analysis showed decreased immune-inflammatory responses and comparable transforming growth factor-staining scores to control groups. Vascular endothelial growth factor (VEGF) study showed a significant increase in VEGF concentration with PPH3.0 sutures. The tensile strength of PPH3.0 sutures matched healthy tendons, highlighting their efficacy in promoting the healing and regeneration of Achilles tendon ruptures [96]. Moreover, in the context of genetic drug delivery, incorporating heparin into chitosan-based polyplexes enhances oligonucleotide release, reduces toxicity, and improves transfection efficiency. Chitosan-heparin polyplexes demonstrate significant improvements in gene release, transfection efficiency, and gene silencing *in vitro*, suggesting their potential for enhancing transfection efficiency with non-viral vectors [95].

5. Bioactivity

Heparin is a natural polyanionic polysaccharide with biological features such as biodegradability, biocompatibility, and nontoxicity against endogenous components and could play a significant role in different areas of biology [38,98]. Despite its excellent features, it has some drawbacks like low tissue permeability, short serum half-life, and poor stability, which limit its direct therapeutic applications [99]. One of the most effective methods to solve this problem is the functionalization of heparin with biomaterials to improve biocompatibility, decrease platelet adhesion and eliminate the loss of blood cells. This functionalization could also enhance their mechanical properties, chemical stability, pH resistance, thermal stability, and solidness, thus improving their widespread application in the biomedical field. The design and development of novel heparin-based biomedical systems with specific features, including anticoagulation ability, biodegradability, and biocompatibility, are described in more detail in the following parts.

5.1. Anticoagulation ability

Heparin is an anticoagulant that stops the growth of new blood clots and the spread of already-formed clots. To put it another way, under physiological circumstances, the ester and amide sulfate groups of heparins deprotonate and draw positive counterions to create a heparin salt [31,100]. The anticoagulation mechanism of heparin is based on the attachment between lysine residues of antithrombin to the heparin (at pentasaccharide sequence) that leads to the induction of a non-reversible conformational change in antithrombin. This conformational change, which occurs at the arginine-reactive site, leads to an increase in the binding affinity of antithrombin to the thrombin (Factor IIa, FIIa) and factor Xa (FXa) (for about twice and 100 fold, respectively), resulting in inactivating these two components. Inactivation of thrombin could inhibit fibrin formation and prevent platelets and factors V and VIII activations (Fig. 4) [101–103].

The anticoagulant ability of heparin (and its derivative) makes it appropriate to be applied as a therapeutic agent in the case of diseases like COVID-19, which are accompanied by high coagulation risk or could be used in the structure of other therapeutic materials which are described in details in the following parts of this manuscript [104,105].

5.2. Biocompatibility

Biocompatibility was defined as “the ability of a material to perform with an appropriate host response in a specific application,” which is related to the response of the living system to materials. An optimal biocompatible material must show no adverse effect on the body, such as blood clotting, inflammatory reaction, toxicity, or even no response by the host [106–108]. The broad concept of biocompatibility also includes

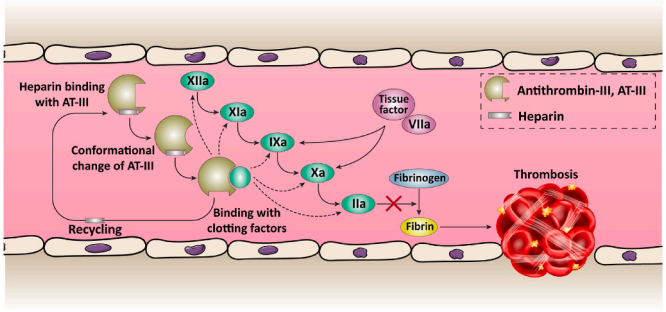

Fig. 4. Schematic of anticoagulation activity of heparin. Interaction between heparin and antithrombin (AT)-III leads to a conformational change in the structure of AT-III that appropriates it for attachment to the coagulation factors and inhibits the coagulation pathway.

bioactivity, which is even more advantageous for a biomaterial that could induce cell attachment and proliferation [109]. In this case, short-term and long-term analysis of material biocompatibility should be assessed closely so that the patients have no potential adverse effects. To understand the first initial response, cytotoxicity and hemocompatibility analysis must be performed to understand how the material interacts with living cells and blood [110].

Heparin could be employed as a coating agent to provide better biocompatibility and improve the cell attachment capability of biomaterials. Moreover, it could inhibit non-specific protein absorption and increase cell proliferation via integrating with growth factors [111]. Several studies have contributed to evaluating the improvement of biocompatibility using heparin. For instance, collagen-modified heparin was examined to enhance the biocompatibility of devices contacting blood [112]. Within this context, *in-situ*, *in vivo*, and *in vitro* biocompatibility of heparin, porous heparin-based hydrogels, and heparin-poloxamer were studied. For example, the heparin-based hydrogels displayed acceptable biocompatibility and, therefore, are appropriate for use in biomedical practices [113–115]. Moreover, the *in vitro* cytotoxicity analysis of heparin-based hydrogels on NIH/3T3 cells showed no toxicity after 24 h [114].

Heparin-loaded scaffolds were used to improve the rate of vascularization for porous orbital enucleation implant applications. More precisely, porous hydroxyapatite scaffolds were functionalized with collagen (COL)/heparin multilayers containing VEGF to accelerate the differentiation of mesenchymal stem cells (MSCs) to endothelial cells, which leads to an improving vascularization rate. *In vivo* analysis of hydrogels with and without heparin on days 7, 14, and 21 showed that the density of newly formed blood vessels was considerably high in heparin-contained hydrogels (Fig. 5) [116]. Heparin was also combined with bone marrow in a collagen scaffold, which showed vasculature formation with no toxicity [117]. Furthermore, no significant toxicity was observed in another *in vivo* analysis when quantum dots containing heparin deoxycholic acid conjugates were introduced orally [118].

Heparin has noble biocompatibility, cell adhesion, and proliferation properties that epochally led up to being used in bone formation and regeneration studies [118,120]. A study reported an enhanced cell proliferation and adhesion of osteoblast and adipose-derived stem cells (ADSCs) on heparin-based hydrogels compared to the poly(ethylene glycol) (PEG) and hyaluronic acid (HA) based hydrogels [121]. This finding explains the beneficial use of heparin to induce bone formation and regeneration.

Apart from the aforementioned beneficial features, it has been reported that heparin could be used in cancer treatment since it suppresses the growth of cancerous cells and prevents the metastatic spread of cancer cells [118,122,123]. Since the body absorbs heparin, it could also be used to detect cancer. For instance, heparin-coated gold nanoparticles were successfully detected cancer via computer-aided tomography. The effectiveness of heparin-nanoparticle interaction as chemotherapy agents was determined for various types of tumors; spleen, colon, lung, and kidney. Heparin-containing nanoparticles were also tested on HeLa cells and showed no significant toxicity [118]. Furthermore, an *in vitro* MTT assay showed high cell viability when heparin combined with heparin-combined PLGA nanoparticles were introduced to L929 cells [124].

Hemocompatibility is a crucial factor for a material to be considered biocompatible. It is possible to assess hemocompatibility by the interaction between the material blood and the ingredients of blood [125], such as blood plasma proteins, leukocytes, and erythrocytes. The absence of hemocompatibility leads to either rejection or loss of function, primarily via the activation of the blood coagulation cascade and immune responses. Although researchers have concentrated on preventing protein adhesion to the material surface, a few new approaches tried to curb adhesion to control the blood-contacting character. In other words, the protein and cellular adhesion on the surface of materials inside blood could lead to stenosis and thrombus formation, which could

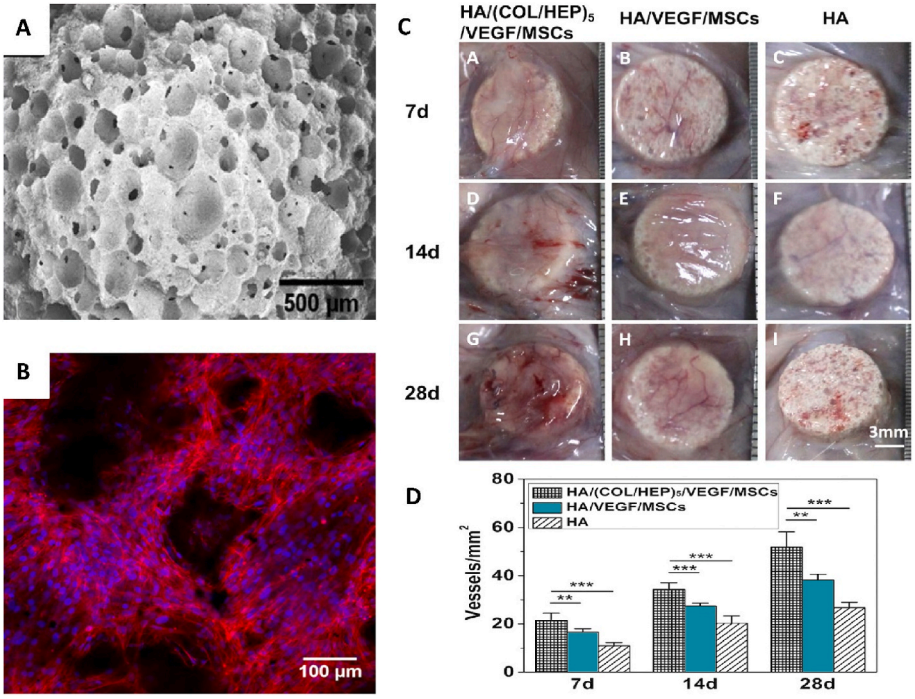

Fig. 5. Utilizing heparin in the structure of scaffolds to improve the vascularization rate. (**A**) SEM image of the porous HA/(COL/HEP)$_5$ scaffold. (**B**) Confocal microscopic image of mesenchymal stem cell distribution inside the scaffold. (**C**) Differences in the rate of vascularization around the implants are based on the presence and absence of heparin. (**D**) The number of new blood vessels formed after 7, 14, and 28 days of exposing tissue sections with different components (**p < 0.01; ***p < 0.001). Abbreviations; HA: hydroxyapatite, COL: collagen; HEP: heparin; VEGF: vascular endothelial growth factor; MSCs: mesenchymal stem cells. Reprinted with permission from Ref. [119].

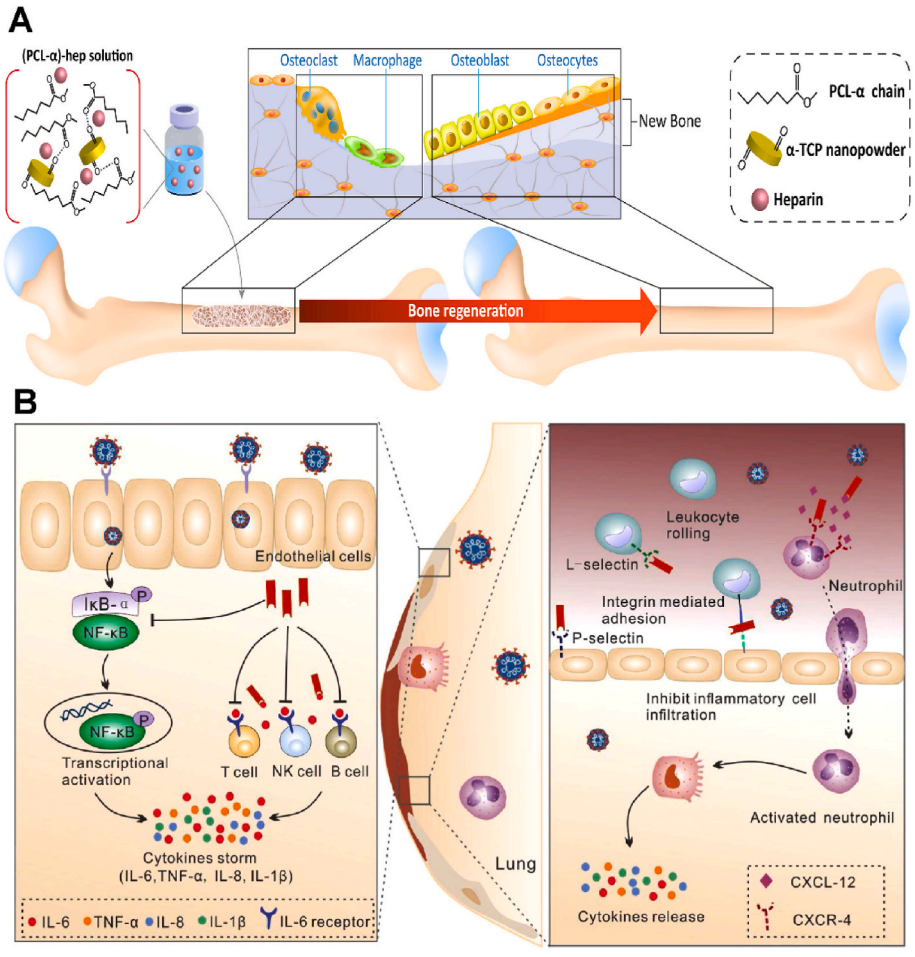

Fig. 6. Synthesis of heparin-loaded PCL-α fibrous membranes and their anticoagulant and hemocompatibility studies. (**A**) Schematic drawing of heparin-loaded PCL-α-TCP fibrous membranes for bone defects. (**B**) **The anti-inflammatory mechanisms of heparin against COVID-19 inflammation in lungs.** Heparin could act as an anti-inflammatory agent against COVID-19 by preventing IL-6 function and inhibiting the infiltration of inflammatory cells. Abbreviations; PCL: poly(ε-caprolactone); α-TCP: α-tricalcium phosphate; hep: heparin. Part (**A**) reprinted with permission from Ref. [133], and part (**B**) was reprinted with permission from Ref. [132].

be prevented via surface modification [112].

Heparin is a gold standard for surface coating. It is not only used as an anticoagulant for drug delivery systems, but also it can be combined with other materials, e.g., polymers, to enhance their biocompatibility, antimicrobial activity [126], and blood compatibility. The *in vitro* analysis confirmed that heparin/silk/poly(l-lactide-*co*-caprolactone) nanocomposite showed excellent blood compatibility over 15 days [127]. Therefore, heparin is an appropriate candidate for surface coating applications so that the surface of polymers can be modified to provide better hemocompatibility [128] due to its antiadhesive property [129,130].

The blood compatibility of heparin makes it favorable for utilization in the structure of membranes for bone regeneration application. For instance, it was loaded in the form of a nanocomposite of poly(ε-caprolactone) (PCL)- α-tricalcium phosphate (α-TCP) fibrous membranes in three different concentrations; 0.1, 1, and 2 wt%. Kinetic clotting and hemolytic analyses showed that increasing the amount (wt%) of heparin improved the anticoagulant and hemocompatibility characteristics of the membrane. It could also improve cellular interactions due to physical and chemical cues (Fig. 6A) [131].

The biocompatibility of heparin and its anti-inflammatory and anticoagulation activities make it an appropriate candidate against SARS-CoV-2. In other words, heparin could eliminate the inflammatory responses in the lungs by preventing the chemotactic property of neutrophils and, thus, migration of leukocytes and inhibition of the chemokines responsible for neutrophils trafficking into the inflammatory region. Moreover, it could eliminate the adhesion of leukocytes to the vessel walls and endothelium via interaction with P/L-selectin and integrin, respectively. It could also prevent the activation of inhibiting NF-κB and reduce the release of interleukin-6 (IL-6), which has a high expression level in COVID-19's inflammatory responses. Besides, it shows an affinity for binding to the IL-6 and has competition with the IL-6 receptor and SARS- IL-6 receptor (Fig. 6B) [132].

6. Biomedical applications

6.1. Tissue regeneration

The increasing incidences of tissue/organ damage and failure have motivated the research community to devise adequate strategies to repair and restore them in native-like structural and functional forms [134,135]. In this regard, the domain of tissue engineering (TE) has shown promising prospects [136,137]. TE aims to develop biomimetic tissue using a combination of cells, biomaterials, and soluble factors, which can be used for tissue regeneration, diagnostic, and disease modeling applications [138–140].

Regarding material perspective, heparin has witnessed widespread applicability in TE, particularly due to its anticoagulant activity and ability to interact and stabilize various proteins, including growth factors [141]. However, from the tissue engineering perspective, heparin-based engineered tissue constructs are often prepared using through physical mixing, layer-by-layer conjugation, direct conjugation, or indirect conjugation via a spacer molecule (Fig. 7) [120]. In this section, we will discuss current advances in heparin-based engineered constructs for regenerating diverse tissue targets (e.g., skin, blood vessels, pancreas, liver, kidney, muscles, cartilage, and bone) (Table 3).

Physical blending: This is a simple and cost-effective strategy that involves loading biopolymeric formulations with heparin/heparin-based particles [143]. This strategy relies on interaction, such as van der Waals or electrostatic interactions, between heparin and the bulk matrix.

Exploiting this strategy, poly(ethylene arginyl aspartate diglyceride) (PEAD)-heparin coacervate laden-functional hydrogels, composing of thiolated gelatin/poly(ethylene glycol) diacrylate (PEGDA), were used to deliver ADSCs and insulin-like growth factor-1 (IGF-1) to cartilage defects [144]. Owing to heparin's ability to bind IGF-1, coacervate-laden-hydrogels exhibited slower yet sustained IGF-1 release profiles than non-coacervate hydrogel systems over 30 days. Moreover, IGF-1 loaded coacervate particles promoted healing of cartilage defects in the rabbit models. Similarly, FGF-2-loaded PEAD-heparin coacervates promoted the recovery of full-thickness excision wounds in mice models through controlled FGF-2 release [145]. Cell proliferation, induction of VEGF, collagen deposition, and granulation tissue formation were all enhanced in coacervate-treated groups compared to control groups. Heparin methacrylamide microparticles-loaded alginate hydrogel was used to deliver bone morphogenic protein-2 (BMP-2) and VEGF for bone regeneration [146]. Two different drug delivery systems were developed. First, microparticles individually loaded with VEGF and BMP-2

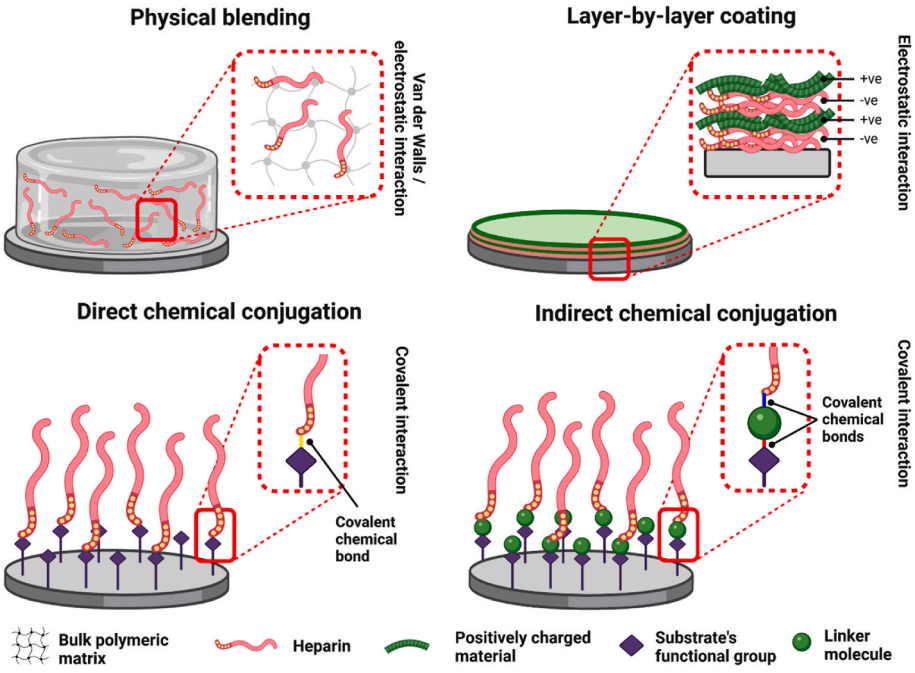

Fig. 7. Different heparinization strategies–physical blending, layer-by-layer coating, direct/indirect chemical conjugation. In physical blending, heparin is blended into the bulk matrix and interacts via van der Waals or electrostatic interactions (top-left). Layer-by-layer coating mainly involves electrostatic interactions to form a polyelectrolyte complex with other positively charged materials (top-right). In chemical conjugation strategies, heparin is covalently attached to the bulk matrix directly (bottom-left) or *via* a linker molecule (bottom-right). Adapted with permission from Ref. [142].

Table 3
Representative recent studies highlighting the use of heparin for tissue engineering applications.

Target tissue	Construct type	Base material	Method of heparin incorporation	Cargos	Cells	*In vivo* studies	Ref.
Cartilage	Hydrogel	HA-glutamine and HA-lysine	Heparin-glutamine	TGF-β1	hCC	–	[160]
	Hydrogel	–	PEGMA-gelatin-heparin	TGF-β3	Bone marrow MSCs	Subcutaneous implantation	[161]
	Hydrogel	–	StarPEG-heparin (RGD peptide and collagen-binding peptide-conjugated, MMP-sensitive)	–	Bone marrow MSCs and articular chondrocytes	Subcutaneous implantation	[162]
	Hydrogel	–	Heparin-conjugated alginate and iron oxide nanoparticles	TGF-β1	ATDC5	–	[163]
	Scaffold	Collagen-chitosan-silk fibroin	Encapsulated: PLL-heparin nanoparticles	TGF-β1	Bone marrow MSCs	Rabbit articular cartilage defect	[164]
	Scaffold	PLGA	Encapsulated: heparin and Tetronic 1107 nano complex	BMP-7 and TGF-β3	ADSCs	–	[165]
	Scaffold	–	Heparin conjugated, fibroblast cell-derived ECM-coated PLGA-PLLA microfibers	TGF-β1	Umbilical cord blood-derived MSCs	Rabbit articular cartilage defect	[166]
Osteochondral	Hydrogel	Thiolated gelatin and PEGDA	PEAD-heparin coacervate fabrication	IGF-1	ADSCs	Rabbit femoral trochlear osteochondral defect	[167]
	Hydrogel	–	StarPEG-heparin (MMP-sensitive)	–	Bone marrow MSCs and articular chondrocytes	Subcutaneous implantation	[168]
Intervertebral disc	Formulation	–	PEAD-heparin coacervate fabrication	GDF-5	ASCs	Coccygeal vertebral defect	[169]
Bone	Double cryogel scaffold	Outer: Gelatin-chitosan	Inner: Gelatin-heparin	VEGF and BMP-2	ADSCs	Mouse cranial defect	[170]
	Hydrogel	Fibrin-glue hydrogel	Casein-heparin co-functionalized $CaCO_3$ microspheres	BMP-2	Bone marrow MSCs	Rabbit tibia bone defect	[171]
	Scaffold	β-tricalcium phosphate scaffold	Filler: Collagen-heparin gel	BMP-2	Dental pulp MSCs	Subcutaneous implantation	[172]
	Scaffold	PCL scaffold	Filler: Fibrin gel encapsulating heparin conjugated-decellularized bone particles	PDGF-BB	ACSs	Mouse calvarial defect	[173]
	Membrane	Poly(vinylidene) fluoride or Poly(vinylidene) fluoride- cobalt ferrite oxide composite	Collagen-heparin layer-by-layer coating	–	Bone marrow MSCs	–	[174]
	Hydrogel	4-arm StarPEG (RGD and MMP-conjugated)	Heparin-MAL	BMP-2	–	Medication-related osteonecrosis of the jaw	[175]
Tendon/ ligament-to-bone	Scaffold	–	Heparin-conjugated silk fibroin	TGF-β2 and GDF-5	ASCs	–	[77]
Muscle-bone	Hydrogel	Alginate	Encapsulated: Heparin methacrylamide microparticles	VEGF and BMP-2	–	Femoral segmental bone defect and volumetric muscle loss	[176]
Muscles	Hydrogel	–	Aldehyde-modified HA, glycol chitosan, PEDOT: Hep	–	C2C12 cells	–	[150]
	Hydrogel	–	Gelatin and heparin conjugated alginate, porcine skeletal muscle-derived extracellular matrix	–	Skeletal muscle progenitor cells	–	[177]
Blood vessles	Hydrogel	–	StarPEG-heparin (RGD peptide-conjugated, MMP-sensitive; different degree of GAGs sulfation)	VEGF	HUVECs	–	[178]
	Hydrogel	–	StarPEG-heparin (RGD peptide-conjugated, MMP-sensitive)	FGF-2, VEGF, SDF-1	HUVECs	Subcutaneous implantation	[179]
	Multi-layer electrospun tubular scaffold	Outer: PCL mat	Inner: PLCL-heparin mat	–	–	Sheep carotid artery defect	[180]
	Multi-layer tubular scaffold	Outer: PU75 electrospun mats	Inner: Gelatin/chitosan-heparin	–	HUVECs	Rabbit carotid artery defect	[181]
	Decellularized matrix scaffold	Human great saphenous vein scaffold	Inner surface functionalization: HA-heparin	–	–	Rat aorta or inferior vena cava defect	[182]
	Electrospun tubular scaffold	PU75	Surface functionalization: heparin	–	HUVECs	Rat abdominal artery defect	[159]
	Electrospun tubular scaffold	–	PCL-silk fibroin-heparin		Endothelial cells	Rabbit carotid artery model	[183]

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Table 3 (*continued*)

Target tissue	Construct type	Base material	Method of heparin incorporation	Cargos	Cells	*In vivo* studies	Ref.
	Electrospun scaffold	PCL	Gelatin-heparin layer-by-layer coating	VEGF	HUVECs	–	[184]
	Electrospun scaffold	PCL	Collagen-PLL-heparin layer-by-layer coating	–	HUVECs	–	[151]
	Scaffold	Polyurethane-coated decellularized scaffold	Chitosan-heparin layer-by-layer coating	–	Endothelial progenitor cells	Porcine carotid artery model	[185]
Skin	Formulation	–	PEAD-heparin coacervate fabrication	FGF-2	–	Mouse skin wound defect	[145]
	Scaffold	Chitosan	Surface functionalization: heparin	SDF-1	HUVECs	Diabetic rat skin wound defect	[186]
Pancreas	Cryogel	–	StarPEG-heparin (RGD peptide-conjugated)	–	Islet cells and bone marrow MSCs	Subcutaneous implantation	[187]
	Scaffold	Esterified collagen	Surface functionalization: heparin	–	Islet cells and ADSCs	Diabetic mouse	[188]
	Capsular scaffold	PLCL	Surface functionalization: heparin	VEGF	Islet cells	Diabetic mouse	[189]
Liver	Hydrogel microparticles	PEGDA-Acry-PEG-biotin	Surface functionalization: biotin-conjugated heparin	–	Bipotential mouse embryonic liver 9A1 cells	–	[190]
Kidney	Hydrogel	–	StarPEG-heparin (MMP-sensitive)	–	HK-2 and human proximal tubule epithelial cells	–	[191]
Mammary gland	Hydrogel	–	StarPEG-heparin (MMP-sensitive)	–	MCF10A	–	[192]
NS	Hydrogel	–	StarPEG-heparin (RGD peptide-conjugated, MMP-sensitive; different degree of GAGs sulfation)	PDGF-BB	Bone marrow MSCs	–	[193]

*Abbreviation - NS: not specified; SDF-1: stromal cell-derived factor-1; PDGF-BB: platelet-derived growth factor-BB; PLCL: Poly(lactide-*co*-caprolactone).

were encapsulated in alginate hydrogel, while BMP-2-loaded microparticles were entrapped in VEGF-loaded alginate gels in the second system. The first system released both growth factors simultaneously at the same rate, while in the dual system; VEGF was released faster than BMP-2. However, both approaches showed equivalent regenerating effectiveness in rat models with unilateral composite bone and muscle lesions. In another study, composite electroconductive hydrogels, consisting of aldehyde-modified HA, glycol chitosan, and PEDOT: heparin, were reported for cardiac tissue regeneration (Fig. 8) [147]. The developed hydrogels exhibited excellent adhesive properties, self-healing behavior, electrical conductivity, and supported viability and maturation in C2C12 myoblasts.

However. it is important to note the limitation of this strategy, such as poor long-term biostability, low shear resistance, and relatively lower efficiencies, which can affect the performance of the blended formulations for certain applications [120].

Layer-by-layer strategy: It exploits intrinsic electrostatic charges of the polymers to form a polyelectrolyte multilayer (PEM) on the substrates. By combining negatively charged polymers, such as heparin, with positively charged materials like chitosan and poly-L lysine (PLL), alternate layers are deposited on the substrate, offering several advantages [120,143].

One of the major advantages of this method is the fine controllability over the physical and chemical architecture of the coated layers, allowing precise tailoring of the coating properties to meet specific requirements. Furthermore, retention of heparin's bioactivity and structural stability ensure that its therapeutic benefits are maintained in the coated structure.

Recently, polyurethane/decellularized matrix-based patches with chitosan/heparin PEM were reported for vascular healing [148]. The study highlighted that the coated sets significantly increased blood coagulation and plasma calcification time, and maintained carotid artery blood flow five months after animal implantation. On the other hand, those treated with uncoated patches had blocked arteries. In another investigation, aminolyzed poly–caprolactone (PCL) electrospun scaffolds were coated gradually with gelatin, PLL, and heparin to deposit 20 PEM layers and tested for vascular tissue engineering (Fig. 9) [149]. The modified scaffolds exhibited anti-thrombogenic properties due to the release of heparin, which was greatly dependent on scaffold cross-linking or matrix metalloproteinase (MMP) exposure. Moreover, the modified scaffolds had greater HUVEC adhesion and proliferation than bare PCL or those treated with only gelatin and PLL. Similarly, heparin/collagen layer-by-layer coated poly(vinylidene) fluoride membranes (containing magnetostrictive cobalt ferrite oxide nanoparticles) were reported to promote MSC proliferation, providing a smart solution to recapitulate bone piezoelectric microenvironment REF.

However, it is worth noting that this method involves multistep coating-washing-drying procedure, which can be quite tiresome and complicated. This aspect poses a challenge to its practical implementation and scalability [120].

Direct and indirect chemical conjugation strategies: It involves covalent attachment of heparin molecule to matrices. In the direct method, heparin is covalently linked to the matrix without the need of a linker molecule, while in the indirect strategy, a linker molecule is used to assist the attachment. Covalently immobilized heparin molecules are more stable and resistant to shear stress than ionically linked heparin molecules [120]. However, due to limited molecular flexibility and availability, the bioactivity of covalently immobilized heparin molecules is often compromised [120]. Using a linker-based conjugation strategy is more likely to improve bioactivity by increasing the availability of binding site mobility [152].

Recently, heparin-grafted poly(lactide-*co*-caprolactone) capsular scaffolds were reported for pancreatic tissue engineering [153]. These scaffolds exhibited an improved VEGF binding ability, thereby promoting cell infiltration and vascularization post-implantation into the rat omentum. Further injection of islet cells in pre-vascularized scaffolds prolonged their survival and function up to 50 days. Similarly, heparin-functionalized biphasic silk fibroin scaffolds with anisotropic, isotropic, and transition zones were fabricated for tendon/ligament-to-bone tissue regeneration [77]. Heparin functionalization promoted retention and regulated delivery of TGF-β2 and GDF5 growth factors, which are crucial for this composite tissue regeneration. Dual growth factor-loaded scaffolds differentiated MSCs better than single ones, although their lineage depended on zonal isotropy. In

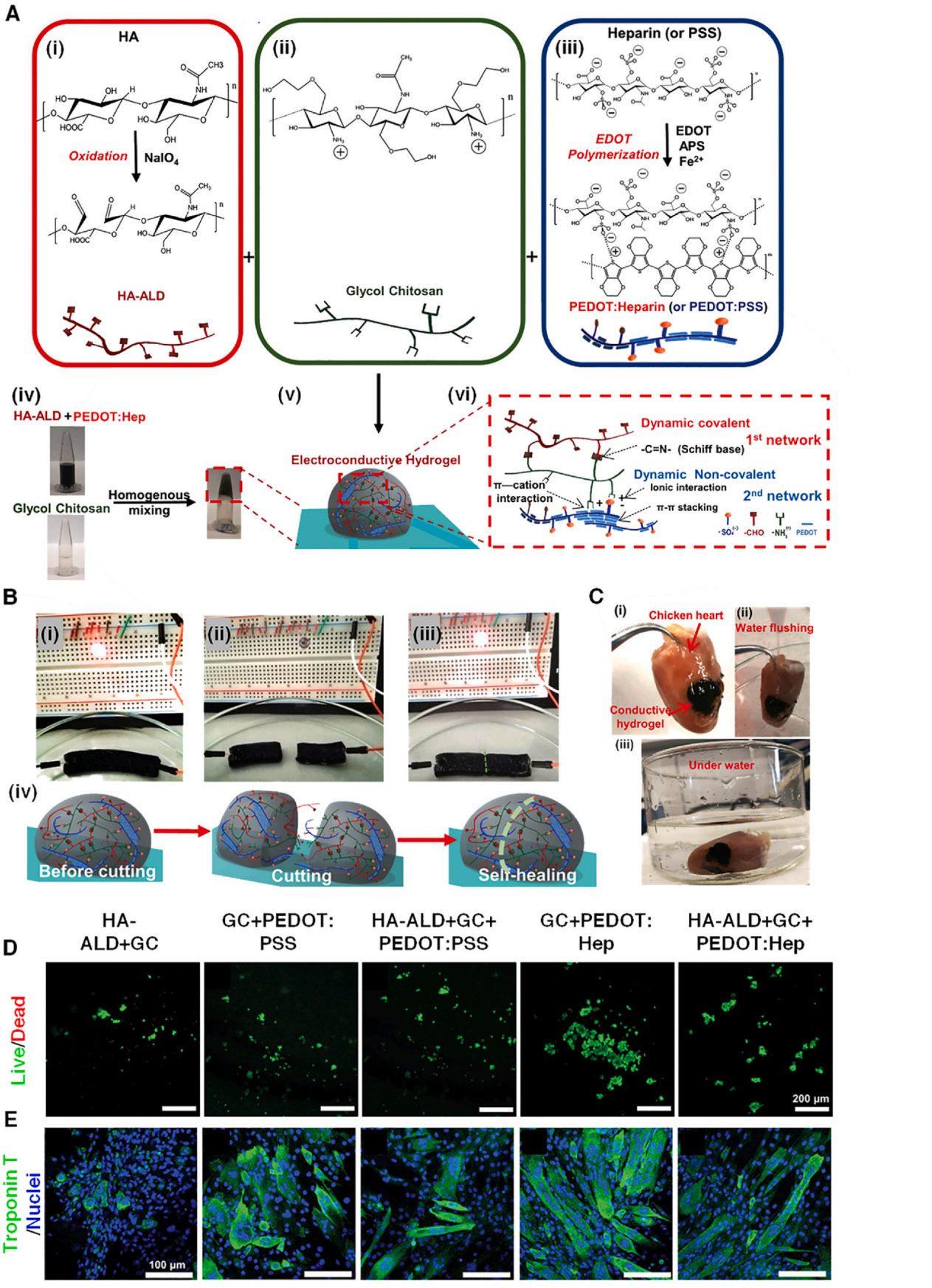

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Fig. 8. Synthesis of aldehyde-modified HA, glycol chitosan, and PEDOT composite electroconductive hydrogels for cardiac tissue regeneration. (A) Preparation of electroconductive composite HA-ALD/GC/PEDOT: Heparin hydrogels. (i) HA-ALD Structure generated post oxidation of HA with $NaIO_4$. (ii) Structure of GC. (iii) PEDOT: Heparin synthesis. EDOT was polymerized to form PEDOT with Heparin as a dopant. In the case of PEDOT: PSS, PSS was used as a dopant (not illustrated). (iv) Hydrogels were formed by mixing HA-ALD/PEDOT: heparin with GC homogenously in microtubes. (v) Graphical representation of dual cross-linked hydrogel network in 3D. (vi) Scheme of the molecular interactions involved in the developed hydrogels. **(B)** Self-healing property of the hydrogels depicted using an electrical circuit. (i) Before cutting, the circuit was closed, which lit up the LED. (ii) Upon cutting the hydrogel, the circuit was opened. (iii) Self-healed hydrogels formed a closed circuit again. (iv) Schematics demonstrating the event of self-healing in 3D hydrogels. **(C)** Adhesive properties of electroconductive hydrogels. (i) Hydrogels adhered effectively with chick heart. Hydrogels remained adhered even after (ii) flushing with water or (iii) immersion in water. **(D)** Representative images post-live/dead staining of C2C12 cells after 7-day culture in hydrogels (scale: 200 μm). **(E)** Representative images of C2C12 cells immunostained with Troponin T (green), suggesting induction of myogenesis (scale: 100 μm). The nucleus was stained with DAPI.
*Abbreviations; HA: hyaluronic acid; $NaIO_4$: Sodium periodate; HA-ALD: aldehyde-modified hyaluronic acid; GC: glycol chitosan; PEDOT: poly(3,4-ethylene dioxythiophene). Reproduced with permission from Ref. [150].

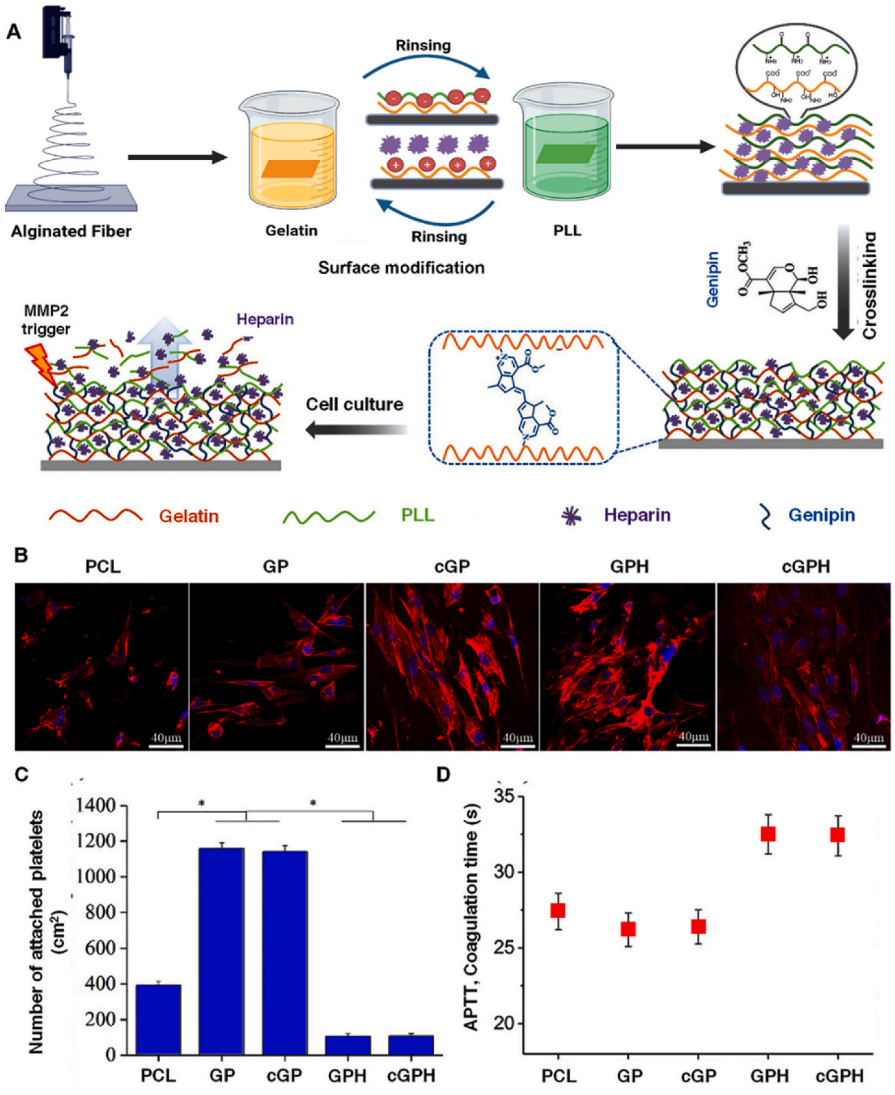

Fig. 9. Aminolyzed poly-ε-caprolactone electrospun scaffolds coated with gelatin, PLL, and heparin, for vascular tissue engineering. (A) Schematic illustration of the preparation of electrospun PCL scaffolds coated layer-by-layer with gelatin, PLL, and heparin to form nano-coating. The scaffolds were then crosslinked with Genepin and further used for cell culture application, where heparin was released in an MMP2-dependent manner. **(B)** Representative fluorescent micrographs of HUVECs at day 5 (scale: 40 μm). Actin cytoskeleton was stained with fluorescent red phalloidin, while the nucleus was stained with DAPI. **(C)** Quantification of platelet adhesion on different scaffolds. **(D)** APTT coagulation time of platelet-poor plasma with different scaffolds. *Abbreviations; PCL: untreated PCL scaffold; GP: uncrosslinked PCL-coated with gelatin/PLL; cGP: crosslinked PCL-coated with gelatin/PLL; GPH: uncrosslinked PCL-coated with gelatin/PLL/heparin; cGPH: crosslinked PCL-coated with gelatin/PLL/heparin; MMP2: matrix metalloproteinase 2; APTT: activated partial thromboplastin time. Reproduced with permission from Ref. [151].

particular, cells in isotropic zones expressed cartilage markers while transition zones expressed enthesis markers, emphasizing the impact of microenvironmental cues on cellular fate. In another study, heparin-grafted poly(ester-urethane)urea/gelatin electrospun tubular scaffolds were tested for vascular tissue engineering (Fig. 10) [154]. The scaffolds were biocompatible, exhibited decreased platelet adhesion, and promoted smooth muscle cell and endothelial cell migration in rat abdominal arteries after implantation.

Another noteworthy work used heparin-conjugated HA injectable hydrogel to sustain TGF-β1 administration and stimulate cartilage regeneration (Fig. 11) [155]. The human chondroprogenitor cells, entrapped in these hydrogels, exhibited higher viability, proliferation, and underwent better chondrogenesis as compared to non-heparinized hyaluronan hydrogels. Recently, Star-PEG hydrogels were designed to research renal tubulogenesis [156]. The biochemical and degradability features of these hydrogels could easily be tuned by incorporating heparin and MMP-sensitive peptides. In this study, three different hydrogels, namely degradable PEG-MMP-PEG, degradable PEG-MMP-heparin, and non-degradable PEG-heparin, were tested for tubulogenesis in human renal proximal tubule epithelial cells. The study found that only degradable PEG-MMP-heparin hydrogels formed polarized renal tubules with clear lumen, while others were insufficient

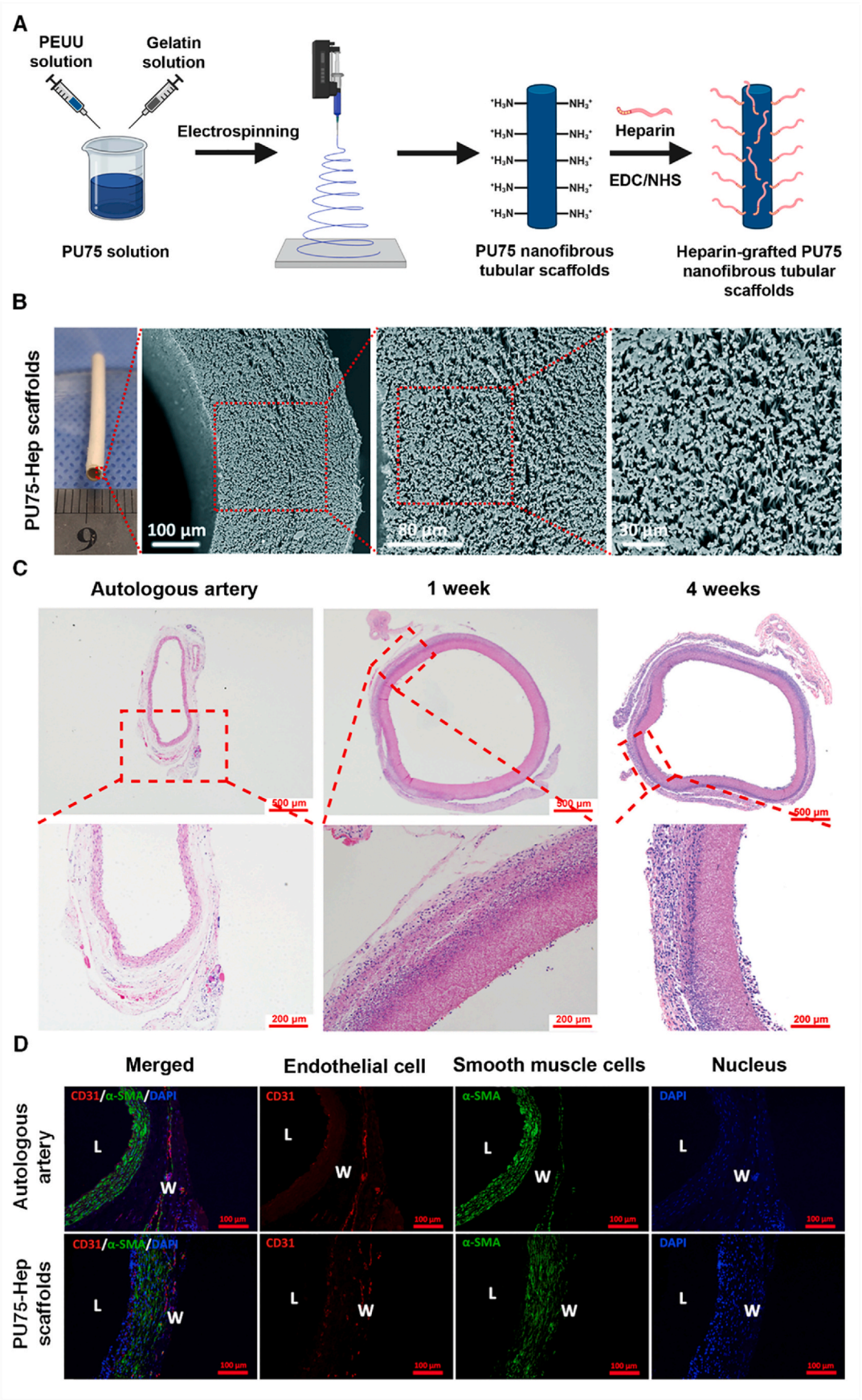

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Fig. 10. Synthesis of heparin-grafted poly(ester-urethane)urea/gelatin electrospun tubular scaffold for vascular tissue engineering. (A) Schematic representation of the fabrication of PU75-Hep nanofibrous tubular scaffold. **(B)** Digital image and scanning electron micrographs of PU75-Hep scaffolds. **(C)** Representative micrographs of H&E stained autologous artery and PU75-Hep scaffold (1 and 4 weeks post-implantation) (Scale bar: 500 μm; zoomed images: 200 μm). **(D)** Representative images of the autologous artery and PU75-Hep scaffold (4 weeks post-implantation), immunostained with CD31 (endothelial cells) and α-SMA (smooth muscle cells) (Scale bar: 100 μm). The nucleus was stained with DAPI. *Abbreviations; PU75-Hep: heparin-grafted poly(ester-urethane)urea/gelatin; CD31: cluster of differentiation 31; α-SMA: smooth muscle actin alpha; H&E: hematoxylin and eosin; DAPI: 4′,6-diamidino-2-phenylindole. Parts B to D reproduced with permission from Ref. [159].

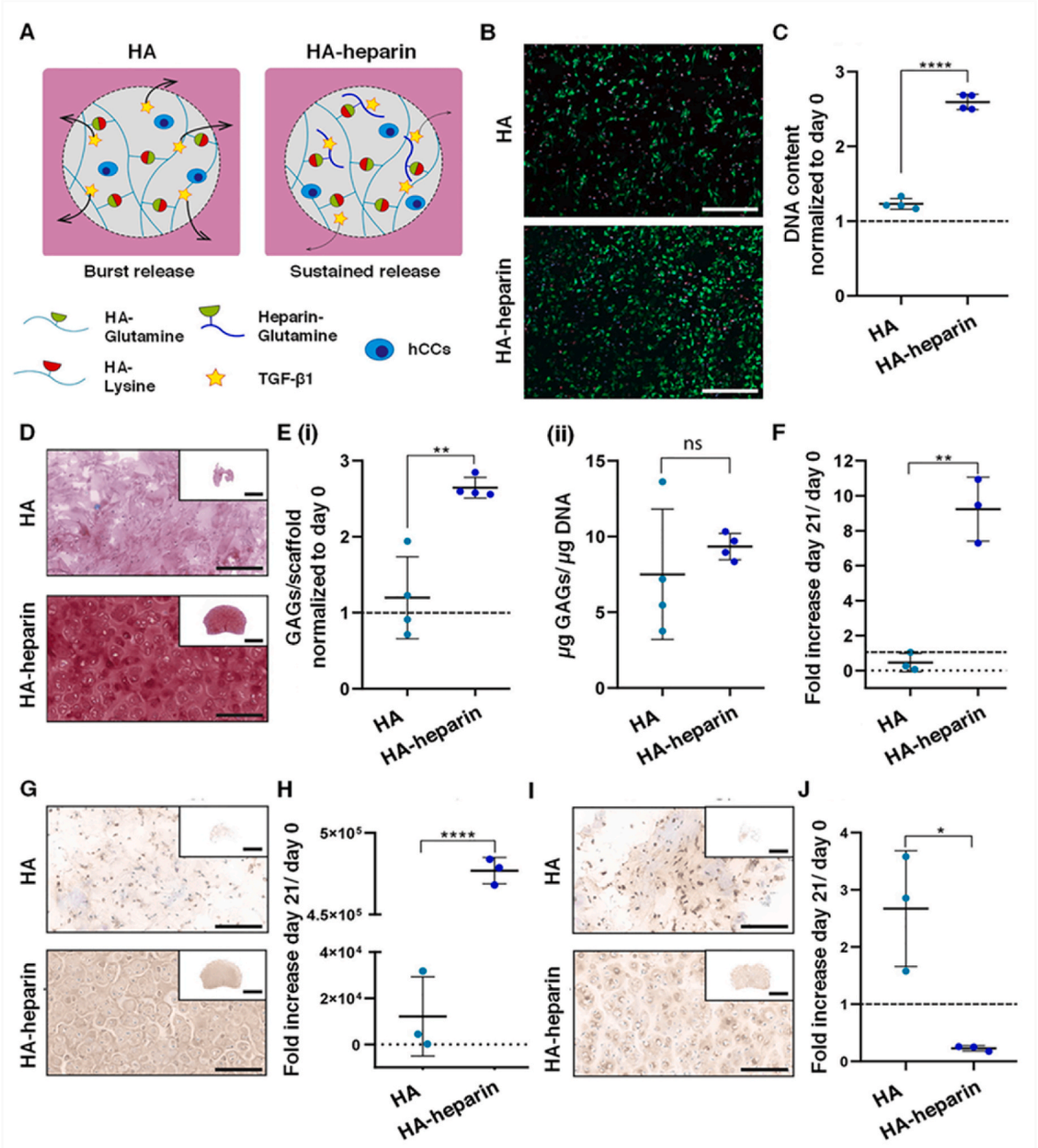

Fig. 11. (A) Illustration of TGF-β1-loaded HA and HA-heparin hydrogels, enzymatically crosslinked with transglutaminase factor XIII. Heparin-containing hydrogels exhibited TGF-β1 sustained release profiles. **(B)** Representative images of hCCs in hydrogels post-live/dead staining (scale bar: 200 μm). **(C)** Quantification of cell proliferation in hydrogels. **(D)** Representative images of Safranin O-stained hydrogels (scale: 200 μm). **(E)** Quantification of GAGs deposited, normalized to (i) day 0 or (ii) DNA content. **(F)** Quantification of Aggrecan gene expression at day 21 concerning day 0 in both the hydrogels. Representative images of immunostained hydrogel sections against **(G)** collagen type II and (I) collagen type I (scale: 200 μm). Quantification of gene expression of **(H)** collagen type II and **(J)** collagen type I at day 21 concerning day 0 in both the hydrogels. Insets represent micrographs of the full hydrogel section (scale: 2 mm). *Abbreviations; HA: hyaluronic acid; hCC: human chondroprogenitor cells; GAGs: glycosaminoglycans. Reproduced with permission from Ref. [160].

for the purpose. A comparable chemically defined hydrogel platform was employed to explore biophysical and biochemical cues involved in mammary epithelial tissue [157]. In addition, heparin sulfation can also affect growth factor sequestering and release. Star-PEG/heparin (with varied sulfation degrees) were utilized to investigate angiogenesis. In particular, heparin desulfated at *6O* position enhanced angiogenic network formation in the presence of VEGF165 at 1 μg/mL [158].

In conclusion, heparin presents superior prospects for tissue engineering applications and various strategies have been opted to introduce it into the engineered matrix. Each of these strategies has their advantages and considerations, and often the choice depends on the specific application. Further research is needed to optimize these strategies and overcome the challenges for wider applicability.

6.2. Gene delivery

Gene mutations are responsible for the development of various diseases including cancer, neurological disorders, diabetes, and cardiovascular diseases, among others. The purpose of gene therapy is to change the expression level of a certain gene or alter the biological characteristics of living cells with a therapeutic aim [194]. Gene therapy has provided promising results in the treatment of blood cancers and rare diseases, and its application in the treatment of solid tumors is being currently performed [195,196]. Gene therapy is powerful in affecting "undruggable targets" and due to advances in various fields of biology and medicine, several gene therapy approaches, including RNA interference and exogenous nucleic acids such as DNA, mRNA, and antisense oligonucleotides (ASOs) have been employed in disease treatment [197]. However, the efficacy of gene therapy is limited *in vivo* due to the degradation of nucleic acid drugs in blood circulation by RNase enzymes, low accumulation at tissues, and off-targeting features [198]. Hence, targeted delivery of nucleic acid drugs by nanostructures appears to be a promising strategy for the protection of genes against degradation, increasing their potential in gene silencing and preventing off-targeting [199].

The nature-derived polysaccharides including pectin, hyaluronic acid, cellulose, chitosan, and alginate, are ideal candidates for the purpose of gene therapy due to their high biocompatibility, safety profile, and biodegradability. Since genes possess a negative charge, the biomaterials employed for gene therapy should have a positive charge to generate a stable complex. For instance, chitosan as a linear polysaccharide has a positive charge and can be used for gene delivery [200]. Noteworthy, there have been efforts in the functionalization of polysaccharides for generating positively charged biomaterials from negative ones. Hence, polysaccharide-based nanostructures can be employed as non-viral vectors for delivery and concentrating large genes/plasmids [201].

Heparin is a highly negatively charged polysaccharide capable of condensing nucleic acid drugs and has the potential to be employed in the preparation of copolymers. Heparin-based copolymers with effective performances produced by poly(ethylene glycol) methyl ether methacrylate (PPEGMEMA), poly(dimethylaminoethyl methacrylate) (PDMAEMA), or their block copolymer (PPEGMEMA-*b*-PDMAEMA). The electrostatic interaction mediated conjugation of cationic PDMAEMA and negatively charged heparin backbone. Due to the negative charge of heparin that provides competition with negatively charged pDNA, heparin-based copolymers have lower condensation capacity compared to PDMAEMA (Fig. 12). However, enhancing weight ratios could prevent the migration of pDNA from heparin-based nanostructures. It is worth mentioning that increasing the length of PPEGMEMA blocks diminishes the condensation capacity of heparin-based nanoplatforms. These nanostructures had particle sizes less than 250 nm and could be internalized in cells via endocytosis. It was also shown that heparin introduction to nanostructures promotes transfection efficiency and it also promotes biocompatibility [202].

Another study prepared polyethyleneimine (PEI)-R8-heparin nanogels for pDNA delivery. In order to promote the selectivity of PEI-heparin nanogels towards colon cancer cells (HCT-116 cells), surface modification of nanostructures with R8 peptide was performed. Such surface modification with R8 increased cellular uptake of PEI-heparin nanogels in cancer cells and mediates endo-lysosomal escape in improving transfection efficiency. The PEI-R8-heparin nanogels had a particle size of 125 nm and a zeta potential of 28 mV, showing high stability for gene delivery. These pDNA-loaded PEI-R8-heparin nanogels induce apoptosis (*in vitro*) and reduce tumor growth (*in vivo*) [203].

Although systemic gene delivery has demonstrated high potential in the treatment of various diseases, targeting specific cells *in vivo* with limited impact on healthy cells is still a challenge [204]. For this

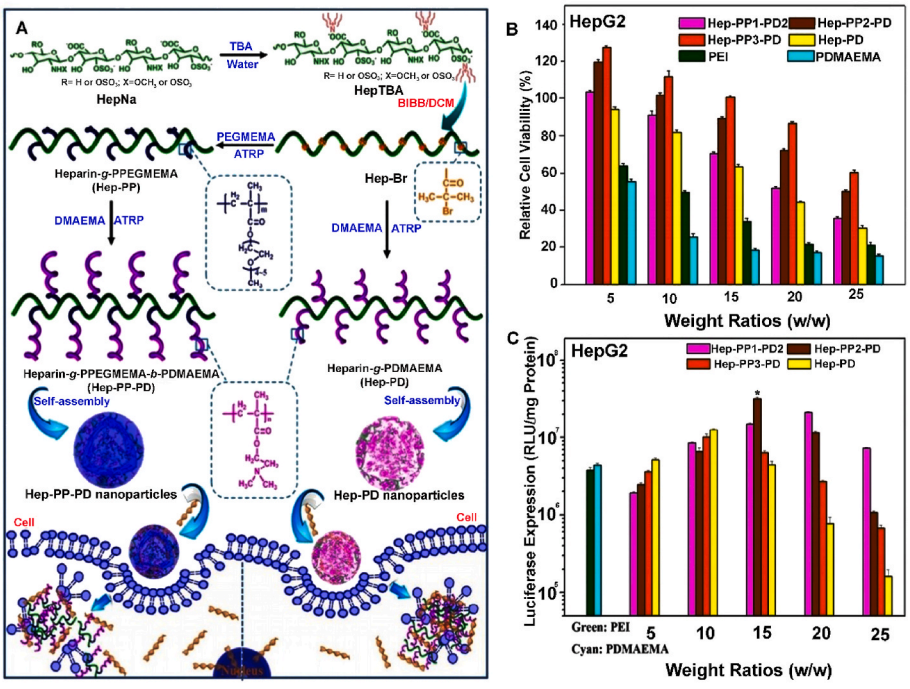

Fig. 12. (**A**) Graft modification of heparin via Atom transfer radical polymerization (ATRP) and the resultant gene delivery process of the heparin-based nanoparticles. (**B**) MTT assay of PPEGMEMA-b-PDMAEMA(Hep-PP-PD), Heparin-g-PDMAEMA(Hep-PD), polyethylenimine(PEI) and poly(dimethylaminoethyl methacrylate) (PDMAEMA), in HepG2 cell lines at various weight ratios (mean ± SD, n = 6) (**C**) *in vitro* gene transfection efficiencies in HepG2 cell lines mediated by Hep-PP-PD and Hep-PD at various weight ratios, in comparison with the control PEI (at its optimal N/P ratio of 10) and PDMAEMA (at its optimal N/P ratio of 15) (mean ± SD, n = 3, *p < 0.05). Reprinted with permission from Ref. [202].

purpose, an experiment using nanocarriers for DNA delivery and their surface modification with heparin has been performed. The heparin modification of DNA-loaded nanostructures is in favor of preventing passive gene transfer in target and off-target tissues. In order to provide spatial control towards gene release at targeted tissue, ultrasound-targeted microbubble destruction (UTMD) has been employed that activates heparin-inhibited gene transfer at the target site. The liver is the major site of liposome accumulation. The surface modification of DNA-loaded liposomal nanocarriers with heparin prevented off-target gene expression in the liver and decreased gene expression more than 700-fold compared to non-modified PEGylated liposomes and then, UTMD provided activation of gene transfer [205]. Such spatial control of gene delivery by heparin-modified nanoparticles appears to be interesting in the treatment of diseases.

The endothelial progenitor cells (EPCs) transplantation has been employed for the generation of new vessels and treatment of various ischemic diseases [206,207]. However, there are some problems associated with using EPCs for angiogenesis induction, and one of them is difficulties in the differentiation of these cells [208]. Therefore, gene therapy has been used to mediate the differentiation of EPCs and to induce angiogenesis. For this purpose, an experiment has developed heparin-modified supramolecular pluronic nanogels for the delivery of bFGF and VEGF165 genes. The modification of nanogels with PEI enables complexation with pDNA and heparin produces high-affinity complexes with bFGF to improve its stability. These gene-loaded heparin-modified nanogels internalized into EPCs and stimulated vascular formation. Furthermore, *in vivo* experiments revealed enhanced neovascularization in an animal model of limb ischemia [209].

As it was mentioned in previous sections, the use of biomaterials derived from nature is essential in the preparation of nanostructures. Chitosan is extensively utilized in gene delivery and the treatment of various diseases [210]. However, a number of changes should be introduced to chitosan-based nanoparticles to improve their capacity for gene their capacity for gene delivery. The efficiency of chitosan in gene delivery is limited by its binding strength to DNA and RNA. In order to improve the binding strength of chitosan with cargo (gene), heparin modification of nanostructures has been proposed. In research work, chitosan-heparin nanostructures with a size of 145 nm have been developed by a one-step process. The mass ratio of polycation to polyanion determined the particle size of polyplexes. The nanostructures released genes in response to pH due to polyplex swelling and collapse of the polysaccharide network. Based on this study, the introduction of heparin to chitosan nanostructures led to a) increased siRNA release from chitosan nanostructures, b) improved biocompatibility and c) enhanced transfection efficiency [211].

Stimuli-responsive nanomaterials have been used for cargo delivery. Developing physically, chemically, or biologically stimuli-sensitive heparin-based nanoplatforms may improve the targetability of these carriers for gene therapy. Preparing photo-sensitive poly-*l*-lysine-heparin structures improved the efficacy of the interpolyelectrolyte complexes for oligonucleotide delivery applications. The linker, 4-bromomethyl-3-nitrobenzoic acid, has been used to achieve the photosensitive properties of the polyplex. To provide the appropriate gene release kinetics, the poly-L-lysine to heparin ratios needs to be optimized. Using heparin displaces the genetic construction from the polyplex [212]. Overall, experiments are in line with the fact that heparin can improve the efficacy of nanostructures in gene delivery by stabilizing genes, promoting transfection efficiency, and improving the viability of nanocarriers. The only drawback of heparin is its negative charge, which is similar to genetic materials. Before the introduction of heparin, genes were complexed with positively charged materials such as polyethylene imine and chitosan. The interesting point is the development of stimuli-responsive heparin-based nanoplatforms such as light- and pH-responsive for targeted gene delivery and improving selectivity. However, there is still a long way to revealing the true potential of heparin-based nanoplatforms in gene delivery, and other kinds of genes, such as microRNAs, CRISPR/Cas system, and shRNA, among others, should be delivered by these nanocarriers in disease treatment.

6.3. Drug delivery

In addition to gene therapy, drugs are extensively applied in the treatment of various diseases. Similar to genes, therapeutic drugs also have their drawbacks [213,214]. Overall, both synthetic and naturally occurring compounds are employed in disease therapy. However, the bioavailability of these compounds appears to be low, which restricts their therapeutic index. Besides, pH and other factors, such as enzymes, have the capacity to modify drug structure and affect its activity. Therefore, carriers should be developed for their delivery. Experiments have revealed that nanostructures can prolong the blood circulation time of drugs to improve their therapeutic index and, by encapsulating them, protect them against harmful changes. Furthermore, by enhancing the accumulation of drugs in tissues and cells, a significant increase occurs in their therapeutic index.

The drug-loaded heparin-based nanocarriers have been developed for the treatment of various diseases. A recent study has developed heparin-based nanoparticles for curcumin delivery with the purpose of wound healing. In the first step, curcumin/PLGA nanofiber membranes were developed and their surface modification with heparin was performed. These nanofibers provide a high release of curcumin to promote cell migration rate and diminish oxidative stress. The *in vivo* experiment also revealed re-epithelization, angiogenesis, and improving collagen deposition. Due to the modification of nanocarriers with heparin, the attraction of growth factors occurs, resulting in wound healing acceleration [215].

Thiolated heparin also exhibited effective wound healing potential with a high-speed recovery in wound closure [216]. The pH and temperature-sensitive heparin-based structures were designed through the copolymer of poly(urethane sulfamethazine) and poly(ethylene glycol), coupled with thiolated heparin via Michael-addition reaction. Sample solutions were injected into each rat to see its formation and stability [217]. Following the injection of the solution, the aqueous solutions were immediately transformed into hydrogels by changes in pH and temperature. The shape of the hydrogel inside the skin was continuously maintained. Any inflammation problems were not observed during the measurement period of four weeks. The results indicate that the presence of heparin in the structure of this type of hydrogel improves its ability to control the pH-responsive sustained release of therapeutic components (like vascular endothelial growth factor (VEGF)) (Fig. 13). Besides, according to the results, this type of hydrogel could be used for subcutaneous injection both *in vivo* and *in vitro* without toxicity effect.

Another application of heparin-based nanostructures can be in the treatment of anti-inflammatory diseases such as asthma. Anti-inflammatory agents such as montelukast can ameliorate asthma. An experiment developed large porous particles via the double-emulsion-solvent-evaporation method and then, montelukast was loaded in polymeric nanostructures using PEI as a porogen. Then, the resulting nanocarriers were coated with LMWH and showed uptake by rat alveolar macrophages. These biomaterials reduced infiltration of inflammatory cells by 74% and *in vivo* experiments revealed reduced airway wall thickness [218]. In addition to asthma, inflammation participates in the development of inflammatory bowel disease (IBD). A recent experiment has prepared heparin-coated serum albumin nanostructures in ameliorating inflammation for colitis therapy. The nanostructures had a particle size of 120 nm, 180 nm, and 250 nm with a zeta potential of −38, −43, and −44 mV. The budesonide (BUD) and vancomycin (Vanco) were loaded on heparin-coated serum albumin nanoparticles, and encapsulation efficiency was 46% and 24%, respectively. These nanoparticles significantly enhanced the intestinal permeability of both drugs and promoted their efficacy in inflammation alleviation. The levels of TNF-α and nitric oxide significantly decreased by drug-loaded

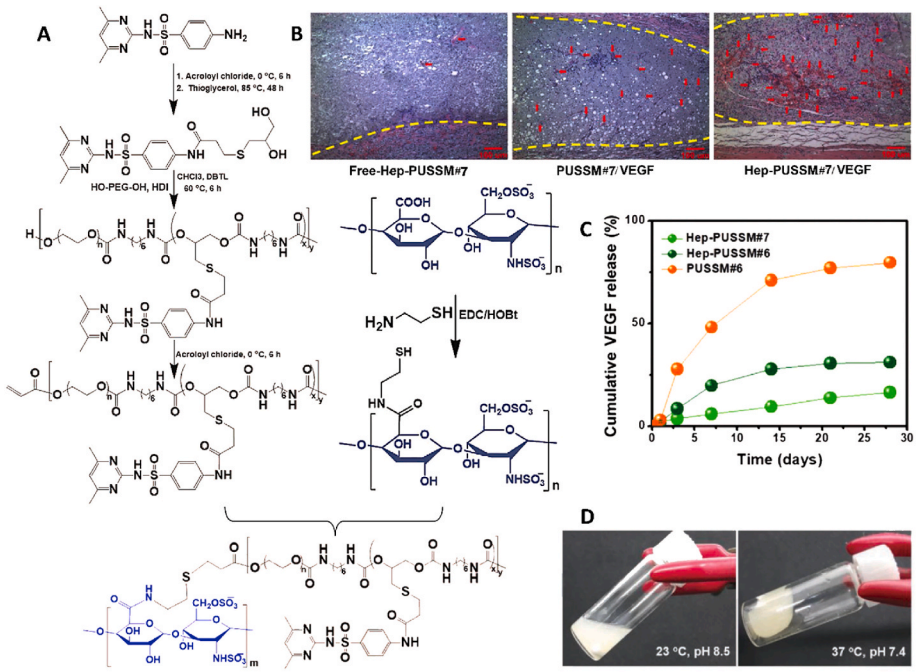

Fig. 13. Schematic representation of pH and temperature-sensitive heparin hydrogels. (**A**) Synthesis of pH and temperature-sensitive heparin contained hydrogel. (**B**) H & E staining images of blood vessel formation in the presence of hydrogel contained VEGF (Scale bar = 100 μm). (**C**) *In vitro* release pattern of VEGF from hydrogel in the presence and absence of heparin. (**D**) *In vitro* pH-responsive gel formation. Abbreviations; Hep: Heparin; PUSSM: Poly (urethane sulfamethazine); VEGF: vascular endothelial growth factor. Reprinted with permission from Ref. [114].

heparin-modified nanostructures and *in vivo* experiments revealed the potential of nanoparticles for binding to the inflamed intestine [219].

The ability of heparin to interact with cellular receptors makes them appropriate for use in the structure of antibacterial drug delivery systems [220]. For instance, the nano-complex of berberine, heparin, and chitosan was used to localize the berberine as an anti-bacterial agent. Heparin could interact with the C-terminal domain of vacuolating cytotoxin A of *Helicobacter pylori*, to release drug components. Utilizing the composite hydrogels of heparin with polypeptide is a promising tool for wound dressing applications. Their low hemolytic activity, good cell compatibility, and perfect antibacterial activity make them ideal materials for *in vivo* applications [221]. These composite hydrogels could also contain growth factors to accelerate the healing process [222]. Moreover, the heparin's interaction with growth factors could lead to the

Table 4
Summary of heparin-based nanomaterials, fabrication techniques and their applications.

Fabrication technique	Components of delivery system	Delivered Cargo/cell	Application	References
Self-assembled heparin-based nanomaterials	Heparin nanoparticles	Pheophorbide a	Photothermal cancer therapy	[226]
	Low molecular weight heparin/Stearyl amine conjugate nanoparticle	Docetaxel	Cancer therapy	[227]
	Heparin based comb nanoparticles	pDNA	Gene delivery	[228]
	Heparin/Deoxycholic acid conjugates	–	–	[229]
	Heparin-alpha-tocopherol succinate nanoparticles	Paclitaxel	Intracellular delivery	[230]
	Solid lipid nanoparticle	Heparin coated iron oxide nanoparticles	Theranostic	[231]
	Folate-PEG-heparin/PBLA bifunctional nanoparticles	Doxorubicin	Cancer Therapy	[232]
Core shell-based heparin nanomaterials	Heparin modified superparamagnetic iron oxide nanoparticle	Doxorubicin	Anticancer drug delivery	[233]
	Heparin modified poly(l-lactide) grafted polyethylenimine cationic nanoparticles	Doxorubicin and curcumin	Breast Cancer	[234]
	Heparin immobilized cellulose nanofibers	Iron oxide nanoparticles	Anticoagulants/Biomedical application	[235]
	Heparin conjugated PVA core shell magnetic nanoparticle	Heparin	Anticoagulants	[236]
	Heparin coated G4-PAMAM dendrimers nanoplatforms	Cytosine-phosphate-guanine oligonucleotides and Doxorubicin	Immune activation and multiple anti-metastatic effects	[237]
	Heparin-grafted poly-l-lactic acid-chitosan core-shell nanofibers	–	Vascular gasket	[238]
Another polyelectrolyte-based heparin nanomaterials	Electrospinning polyelectrolyte complex nanoparticles	Fibroblast growth factor 2	Growth factor delivery	[239]
	Polyelectrolyte multilayer nanoparticle	Heparin, Naproxen	Anti-inflammatory activity	[240]
	Chitosan/heparin nanoparticulate complex	Bovine serum albumin	Enhanced entrapment efficiency	[241]
	Chitosan/heparin polyelectrolyte complex	Heparin	Solubility enhancement	[242]
	Polyhedral gold nanoparticle nanoarchitecture	Gold nanoparticles	Heparin detection	[243]
	Chitosan/heparin multilayer implants	Silver nanoparticles	Cell proliferation and antimicrobial properties	[244]
	Chitosan/heparin polyelectrolyte composites	Silver nanoparticles	Metallic nanoparticle stabilization and its release	[245]

raised adjustable release of growth factors [223]. Table 4 summarizes numerous applications of heparin-based nanomaterials based on commonly used fabrication techniques. It should be noted that the table is focused on studies focusing on drug delivery, cancer, gene delivery and related applications.

For preventing the chance of vascular bypass graft failure, re-endothelialization and neointima hyperplasia suppression are essential. Therefore, the application of agents with angiogenesis induction activity and re-endothelialization effect is vital. An experiment has prepared type I collagen hydrogel for the delivery of Pleiotrophin (PTN) as an inducer of angiogenesis. The modification of hydrogels with heparin has been performed to protect and release growth factors. Furthermore, heparin modification of hydrogels is correlated with the sustained release of PTN. The heparin can also promote the hemocompatibility of hydrogels. Such a combination enhances the viability of endothelial cells and reduces vascular graft failure [224]. Another study also confirms the role of heparin-modified hydrogels for the prolonged release of osteoprotegerin in triggering vascularization, inflammation alleviation, and improving bone disorders [225]. Overall, these studies highlight the fact that modification of platforms with heparin significantly improves their biocompatibility and results in sustained release of drugs, improving therapeutic index.

Various internal and external stimuli-responsive materials have been used to trigger drug delivery systems. Light, temperature, pH, and ultrasound are some examples of these stimuli [246]. Recently, protamine-responsive micro platforms have been used to release therapeutics. In this approach, amine-modified dextran (DEXAM) synthesized by the reductive amination reaction, has been combined with heparin. The prepared DEXAM/HP microspheres were triggered by protamine, a high-affinity agent for heparin. The controlled release property of these microspheres was related to the heparin amount, amine content in DEXAM, and protamine concentration. The system avoiding chemical modification delivered the cargo efficiently [247].

Hydrogels prepared from synthetic or natural polymers are extensively applied for drug delivery purposes [248]. The polymeric hydrogels can be functionalized with different agents to possess targetability or remote controllability [249]. Recently, hydrolytically degradable heparin-based hydrogels (heparin/PEG-diacrylate) have been used to sustain the release of crystal violet as an anti-inflammatory drug. The negatively charged heparin, as a highly sulfated GAG, binding the positively-charged small molecule made the hydrogels an efficient drug delivery system. Heparin sulfation and the degradation of the hydrogel significantly affected the release kinetics and rates of crystal violet over time. The prepared hydrogels exhibited near-zero order release kinetics over 5–15 days. The *N*-desulfated heparin contained hydrogels exhibiting ~90% crystal violet loading efficiency to maintain the bioactivity of the small molecule to treat inflammatory diseases (Fig. 14A) [250].

Neuroinflammation mediates several neurological disorders, including multiple sclerosis, Alzheimer's disease, Parkinson's disease, epilepsy, and stroke. Interleukin-13 (IL-13) can play crucial anti-inflammatory roles, but its brain delivery is challenging. Recently, IL-13-loaded heparin-based macroporous cryogels have been used for immunomodulation and overexpression of ARG1 (anti-inflammatory phenotype marker) both *in vitro* and *in vivo* (Fig. 14B). 1 mg of the intrastriatal injectable microscale carriers containing heparin could be loaded with at least 500 ng of IL-13 as a building block and the affinity center was able to control the release of IL-13 (13% over a range of three weeks) [251].

6.4. Cancer therapy

Heparin-based materials have revealed great promise in the therapy

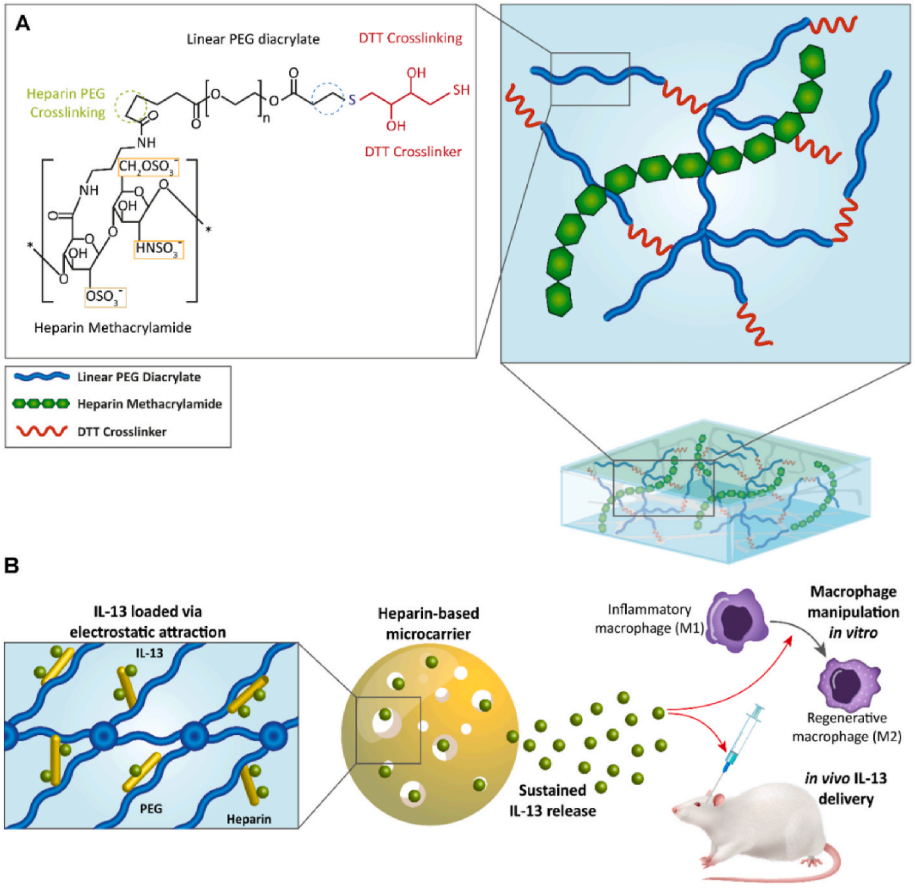

Fig. 14. Heparin-based hydrogels (heparin/PEG-diacrylate) for sustained release of crystal violet as an anti-inflammatory drug. (**A**) Hydrogel fabrication with heparin methacrylamide, linear PEG-diacrylate, and DTT crosslinker. (**B**) Heparin-based microcarriers were used to modify the phenotypic of macrophages *in vitro* and create a pro-regenerative phenotype in macrophages/microglia in the animal brain Abbreviations; PEG: Polyethylene glycol. Part (**A**) is reprinted with permission from Ref. [251].

of several cancers in over 50 clinical trials [252]. Angiogenesis, cell adhesion, proliferation, migration, immune system regulation, and cancer cell invasion are all influenced by these materials. The mechanisms and pathways used by these molecules to inhibit the proliferation of cancer cells mainly include angiogenesis, P/L selectin, interference with the C-X-C Motif Chemokine Ligand 12/C-X-C chemokine receptor type 4 (CXCL12/CXCR4) axis, and inhibition of heparanase [253]. Recent experiments on the anti-cancer effects of heparins have confirmed their role in inhibiting cancer progression and introduced them as promising anti-cancer agents to be utilized in potential treatments [254]. Reducing the metastasis rates and enhancing the survival of heparin-treated patients suggest the heparin's role in the suppression of cloak's formation by hindering the interactions between platelet's adhesion molecule P-selectin and its ligands on cancer cells. This heparin's antimetastatic effect has been measured by the adhesion between platelets and human non-small cell lung cancer cells (A549) by developing single-cell force spectroscopy. The results of this study showed an additional reduction of maximum adhesion force and detachment work of about 9.52% and 7.12%, respectively, after heparin addition [255].

Lymphatic vessel suppression and angiogenesis inhibition effects of heparins have been indicated in several studies to specify novel therapeutic mechanisms by which heparins suppress metastasis [256,257]. Recently, a four bis-deoxycholates conjugated LMWH, LHbisD4, has been found to inhibit lymphatic angiogenesis and reduce lymph node metastasis by blocking vascular endothelial growth factor C (VEGF-C) signaling pathway by suppressing the phosphorylation of VEGF receptor 3 (VEGFR-3) which is induced by VEGF-C [258]. In addition to inhibition of angiogenesis as a critical process required for a tumor's survival, growth, and metastasis, heparins can also protect the endothelial barrier. For example, the permeability of the VEGF-induced endothelial barrier has been attenuated by using LMWH tinzaparin regardless of its anticoagulant activity [259].

The number of small soluble cytokine proteins involved in oncogenesis increases with the development of cancer in multiple stages. Regarding this, the possible impact of heparins has been verified on cancer progression. For instance, an orally active LMWH-based polyanionic component named LHTD4 has been developed for targeting advanced metastatic human breast cancer. This LMWH-taurocholate compound consists of tetrameric deoxycholic acid physically complexed with a synthetic bile acid enhancer (DCK) to increase the lipophilicity and oral bioavailability of LHTD4. To confirm the binding of LHTD4 with TGF- β1 and CXCL12 as the two responsible factors in enhancing metastatic activity during breast cancer's initiation and progression stages, surface plasmon resonance (SPR) analysis, and computer simulation studies were performed. The *in vitro* treatment of MDA-MB-231 cells with LHTD4 in TGF-b1R1 and CXCR4 phosphorylation assays exhibited an effective inhibition of receptor phosphorylation and prevention in the expressions of epithelial to mesenchymal transition marker proteins (e.g., vimentin). The migration of MDA-MB-231 cancer cells was inhibited explicitly by LHTD4, which blocks the signaling pathway of transforming growth factor-beta 1 (TGF-β1) and the CXCL12-CXCR4 axis followed by a subsequent ligand-receptor response. *In vivo* therapeutic efficacy of LHTD4 was studied on a transplant tumor mice model of 4T1 breast cancer, which was treated daily with 5 mg/kg of LHTD4 for 8 weeks. A significant reduction in the metastases formation was observed that proving the effectiveness of LHTD4 in preclinical studies with no noticeable toxicity [260].

Heparin can also prevent cancer relapse through various mechanisms of modulation and inhibition of cancer stem cells (CSCs) [261,262]. For instance, G2.2, a sulfated non-saccharide GAG mimetic of heparin hexasaccharide, was selectively inhibited *in vitro*, *in vivo*, and *ex vivo* colonic CSCs self-renewal which was mediated by the p38 mitogen-activated protein (MAP) kinase activation [263].

The anti-metastasis effect of heparin and heparan sulfate was also confirmed via heparanase, which led to the reduction of endothelial cell adhesion by up to 40% and the elevation of filopodia formation. Meanwhile, the migration of endothelial cells affected the cancer cell migration and invasion as necessary steps for both angiogenesis and metastasis by inhibiting the synthesis of ECM proteins and plasmin (a PLG gene-encoded proteolytic enzyme) [264].

Heparin and its derivatives could also diminish cell proliferation and metastasis by regulating the expression of major ECM macromolecules, which might also be helpful in therapeutic targeting [265]. Heparin, mainly LMWH, has revealed significantly improved survival benefits, primarily in limited-stage small-cell lung carcinoma, when administered as prophylaxis for thrombosis to lung cancer patients without any other anticoagulant indications [266].

Heparins have also been applied alone or in combination with other chemotherapeutic drugs in chemotherapy applications. They could play a significant protective role in decreasing chemotherapy-induced coagulopathies, causing an eventually increased survival in patients [267].

Several studies have verified the role of heparanase as a viable target for cancer therapy and the only enzyme that lyses heparan sulfate proteoglycan protein (HSPG) in tumor angiogenesis, invasion, and metastasis. Thus, several heparin mimetics have been developed for cancer treatment [268,269]. Although a common side effect of heparin mimetics is anticoagulant activity, PG545, as promising heparin mimetic, is recently found to reveal only a mild anticoagulant activity with an anti-lymphoma solid effect. The results of the pro-apoptotic molecular mechanism of PG545 indicated that it stimulates apoptosis by activating the NF-κB pathway, inducing endoplasmic reticulum (ER) stress and autophagy [270].

A study used the integration of intelligent, responsive drug-loaded polymeric micelles and heparin to promote antitumor efficiency. This novel, innovative, and biocompatible pH-sensitive heparin-based polymeric micelles nanocarrier was designed based on heparin–α-tocopherol conjugate, which shows promising results for enhanced intracellular delivery of docetaxel in breast cancer treatment [271].

In another study, heparin-chlorambucil polymeric prodrug nanoparticles have been applied to selectively kill tumor cells due to the high redox potential in HeLa tumor cells while being biocompatible with normal cells (HaCaT). Co-delivery of heparin and chlorambucil via a redox-responsive prodrug approach could enhance the anti-tumor activities of chlorambucil (with 61.33% chlorambucil grafting efficiency). This designed prodrug delivery vehicle could be a better alternative for the controlled release of chlorambucil in cancer cells [272].

In another study, LMWH-modified liposomes have been developed to deliver the antitumor drug doxorubicin for bone targeting in orthotopic osteosarcoma and breast cancer bone metastatic tumors. The LMWH-coated liposomes were functionalized by the alendronate, which was used as a bone targeting and anti-osteoporosis agent. The poor permeability and the less hemoperfusion of bone tissue could restrict the efficiency of chemotherapeutic treatments in the bone mentioned above metastatic tumors. As the hydrophilic layer of the liposomes, LMWH enhanced anti-metastasis efficiency by improving the blood circulation time of liposomes (3.55 ± 0.41 h) compared to doxorubicin alone (0.95 ± 0.18 h). Both cancer models demonstrated satisfying *in vivo* efficiency, which proved the remarkable efficacy and synergistic effects of this LMWH-based nanosystem in tumor growth suppression and metastasis inhibition [273].

A significant challenge in glioma systemic chemotherapy using nanoparticle-based drug delivery systems is the inadequate delivery efficacy due to deficient tumor penetration. To significantly enhance anti-glioma efficiency and achieve effective drug delivery, doxorubicin/heparin-based nanoparticles (DNPs) were loaded onto the surface of natural grapefruit extracellular vesicles. The patching approach provides an exceptional 4-fold drug loading capacity compared to traditional extracellular vesicle encapsulation. This system could significantly extend circulation time and promote cellular internalization and antiproliferation capability. The high-abundance accumulation of extracellular vesicle-DNPs found in glioma tissues enabled the maximum brain tumor uptake of extracellular vesicle-DNPs and

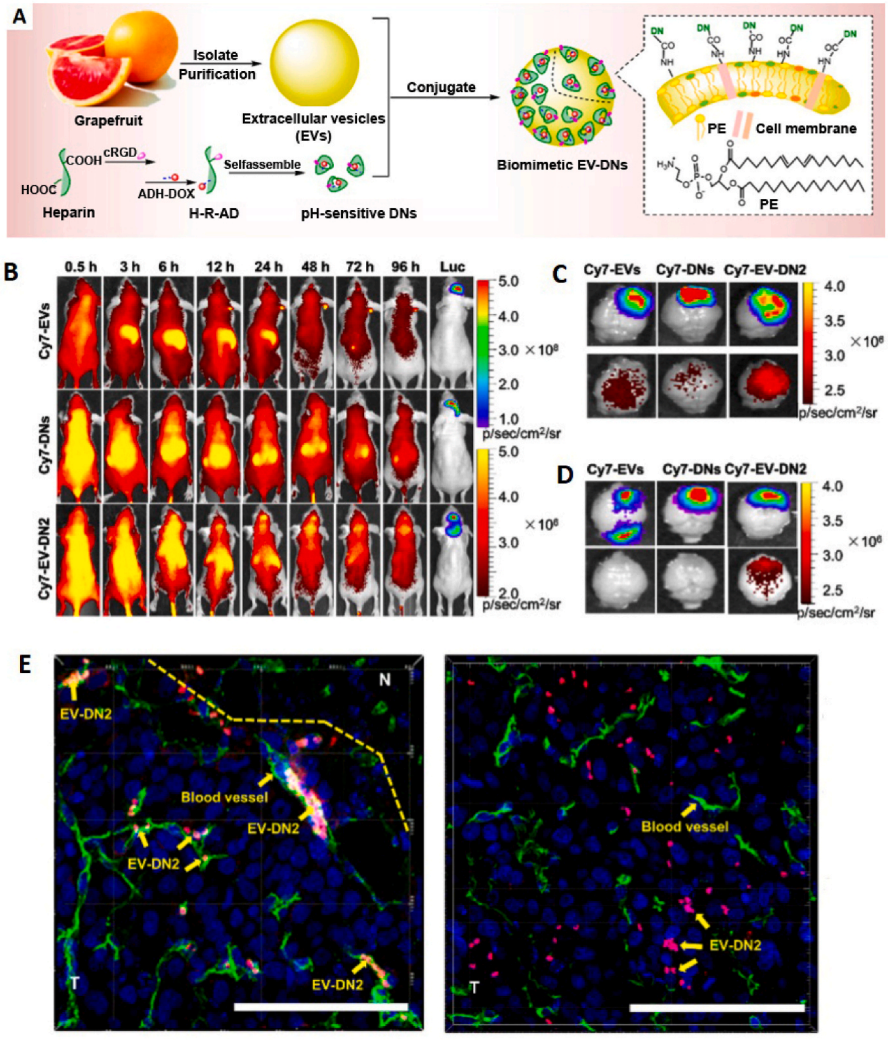

Fig. 15. Doxorubicin/heparin-based nanoparticles are fabricated onto the surface of natural grapefruit extracellular vesicles. (A) Preparation of biomimetic EV-DNPs delivery system. **(B)** *In vivo* fluorescence images of bearing intracranial LN229-luc glioma mice at different time points after Cy7-EVs, Cy7-DNPs, and Cy7-EV-DN2 administration. Brains with tumors removed from LN229-luc glioma-bearing mice were examined for *ex vivo* Cy7 and Luc signals, 96 h after Cy7-EV, Cy7-DNP, and Cy7-EV-DN2 injection. **(C)** Without and **(D)** with perfusion. **(E)** The EV-DN2 intravenous injection-induced 3D confocal pictures of the glioma-bearing brain tissues (left image, glioma edge; right image, glioma core). Endothelial cells were extravasated from tumor blood arteries (green), nuclei stained with Hoechst 33342, and EV-DN2 (red) and accumulated within tumor tissues. (Scale bar: 100 m; T = tumor tissues; N = for normal tissues.) extracellular vesicles, or EV. Reprinted with permission from Ref. [274].

excellent *in vivo* anti-glioma efficiency (Fig. 15) [274].

Heparin-based hydrogels have also been widely used in drug/protein-loading cancer therapy systems due to their crosslinking structures and satisfying biocompatibility. Some key factors should be considered while designing heparin-based hydrogels in cancer drug delivery systems, such as the hydrogel's loading capacity, size, shape, surface, and mechanical properties. In addition, mesh size construction is as important as the stimuli-responsive interactions between hydrogels and drugs to achieve an efficient and successful controlled release of drugs. Furthermore, the hydrogels should be biocompatible enough to protect the drugs from degradation [275–277].

In a study, hemo- and cytocompatible graphene oxide hybridized heparin-analog hydrogels have been reported as potential implantable biomaterials for anti-cancer drug/protein delivery. With a porous morphology that decreased with increasing the graphene oxide concentration, these hydrogels could successfully improve the mechanical properties and optimize the drug/protein loading and release performances. Due to their high drug-loading capacity and persistent releasing ability, efficient anticancer cell activity was also observed for these doxorubicin-loaded graphene oxide/heparin-analog hydrogels [278].

A novel delivery system based on LMWH modified liposomes has been recently developed to decrease the tumor metastasis risk and reach improved phototherapeutic efficiency. Here, photosensitizer indocyanine green (ICG) was loaded into LMWH-modified liposomes (LMWH-ICG-Lip) to recognize the synergistic effects between drug vehicles and photosensitizer. This system could enhance the accumulating efficacy of the photosensitizer to tumor tissue and extend its circulation time. The anti-metastatic effect of LMWH could inhibit the adhesion of platelets into tumor cells and decrease the invasion and migration of tumor cells. Moreover, orthotopic 4T1 breast cancer mice models were evaluated for *in vivo* efficacy of LMWH-ICG-Lip. The results showed alleviated metastasis potential of residual tumor cells after irradiation and improved phototherapeutic antitumor and anti-metastasis efficacy (Fig. 16) [279].

As summarized above, different types of heparin derivatives could improve the chemosensitivity of cancer cells by inhibiting their proliferation, migration, and invasion. Heparin derivatives could also regulate hematogenous metastasis by reducing the interactions between cancer cells and platelets and controlling lymphatic metastasis through lymphangiogenesis inhibition. Additionally, these derivatives could successfully block signal pathways of integrins, TGF-β1, CXCL12-CXCR4, and VEGFC/VEGFR-3 axis by binding to a range of heparin-binding proteins. Furthermore, several heparin mimetics have been verified as anti-cancer agents, such as G2.2, in the *in vivo* selective inhibition of colonic cancer stem cells. Accordingly, heparin and its derivatives, with high biocompatibility and anti-metastatic ability, could be applied as novel promising adjuvant anti-cancer drugs that can function through various pathways to impact multiple phases of tumor progression.

6.5. Biosensors

A biosensor is a diagnostic tool that combines a bioreceptor and an

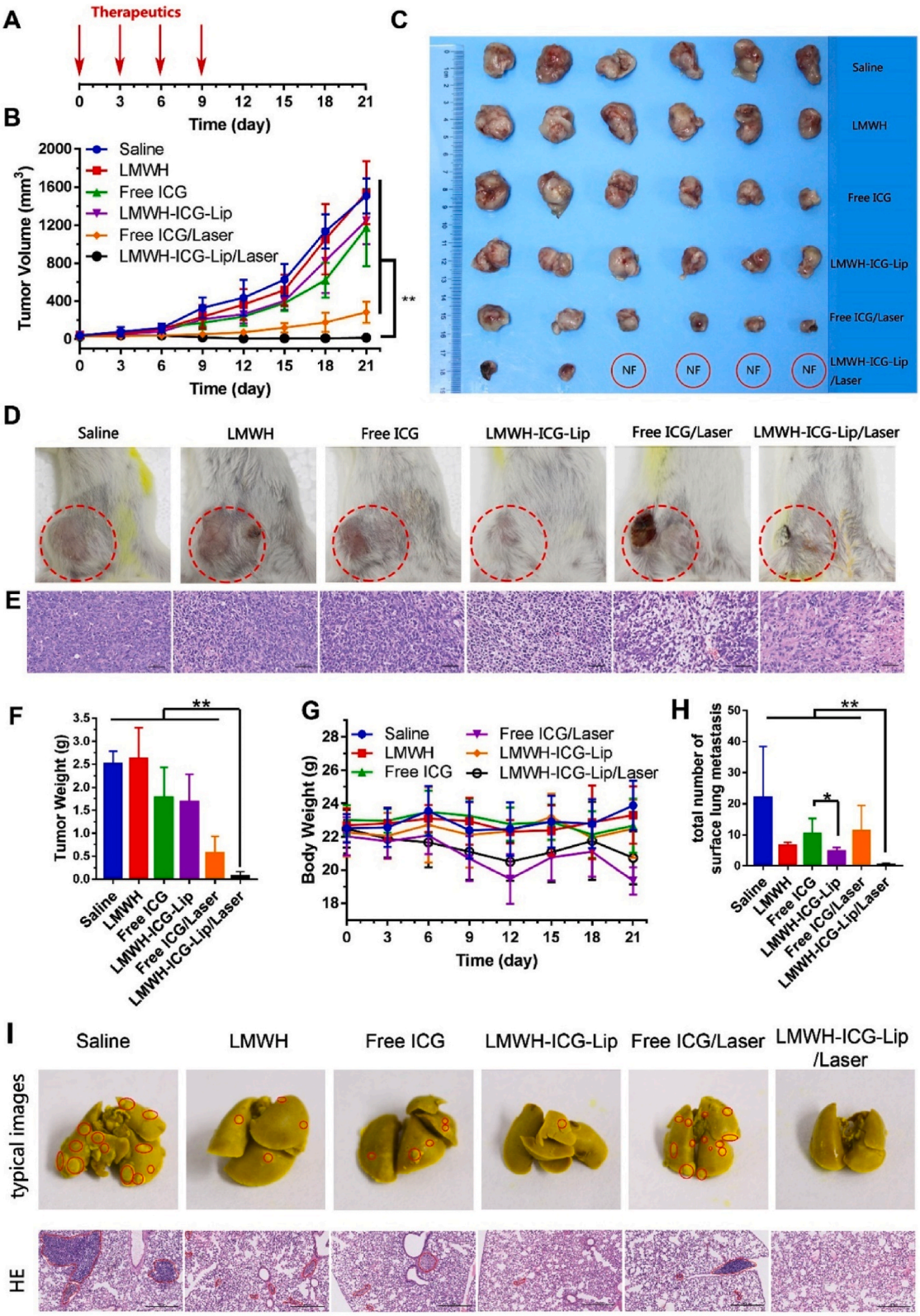

Fig. 16. ***In vivo*** **therapeutic efficacy of mice treated with saline, LMWH, free ICG, LMWH-ICG-Lip, free ICG/Laser, and LMWH-ICG-Lip/Laser (808 nm, 1.5 W/cm2, 5 min), n = 6.** (A) Schematic of the timeline of treatment; (**B**) tumor volumes; (**C**) tumors of all groups on Day 21; (**D**) 4T1 tumor-bearing mice with various treatments on Day 21. (**E**) Hematoxylin-eosin (HE) histopathology images of tumor tissues (Scale bar, 400 μm). (**F**) Tumor weights; (**G**) body weight changes. (H) A total number of nodules on lung tissues; (I) The metastatic tumor locations in mouse lungs treated with various formulations and HE stained lung tissue sections are shown with circles (Scale bar, 400 μm). Abbreviations; NF = tumor was not found. NS = no significant difference. *$p < 0.05$, **$p < 0.01$. Reprinted with permission from Ref. [279].

electronic interface to turn an analyte-specific binding event into quantifiable data [280,281]. These devices offer a precise, consistent low-cost analytical method with fast detection capabilities [282]. In general, a biosensor is composed of three main parts; a) Bio-receptor or biorecognition element, which determine the specificity of the biosensor and could be natural or synthetic agents like different types of enzyme, antibodies, nucleic acids, and aptamers; b) Transducers that convert the signal resulted from the interaction between the analyte and the natural element to a measurable signal and is included different types such as optical, physicochemical, electrochemical, piezoelectric, etc., and c) a signal processor that is responsible for visualizing the results in a user-friendly form [283,284].

According to some literature, heparin could act as a biorecognition element in the structure of biosensors to detect specific target agents. Some particular features of heparin, such as its anticoagulant activity, biocompatibility, and stability during storage in adverse conditions, are appropriate for biosensor application, especially for blood samples [6]. In addition, heparin-based biosensors give the advantage of compatibility with clinical examples. For instance, in studies performed on the detection of blood components, centrifuging the serum is mainly used for separating different parts that require complex laboratory equipment and take a long time to prepare samples, which may produce contaminations. Besides, the results detected from a serum sample cannot present the actual situation in the whole blood [285,286]. Hence, it is of significant implication to detect the expected analyte directly in the whole blood. For example, a Lipase-(ε-polylysine-heparin)-glassy carbon electrode was designed to detect the triglycerides level in whole blood directly. The combination of polylysine and heparin resulted in a biosensor with anticoagulation capability and antithrombosis ability that could be now used in whole blood and provided new analytical systems for clinical illness diagnosis. The fabricated biosensor showed high electrocatalytic activity for the detection of triglycerides with a detection limit of about 5.18 mg dL^{-1} in whole blood and sensitivity of about 0.40 μA mg^{-1} dL cm^{-2}. Moreover, it had a long shelf-life and excellent anti-interference capability. All these features, along with the presence of hemocompatible heparin in the structure of this biosensor, introduced it as a good candidate for utilization in clinical assessments (Fig. 17) [287].

Due to the large concentration of sulfate groups in heparin's structure, it possesses the greatest negative charge density of any known biological [288]. For instance, heparin is used in a highly sensitive, antibody-free, carbon chemiresistor, nanotube-based biosensor to detect four serotypes of the dengue virus. [289−291]. The biosensor consists of a single-walled carbon nanotubes (SWNT) network chemiresistor blocked by Tween 20 and attached with heparin in such a way that the carboxyl groups of heparin were cross-linked to primary amine groups on the pyrene-linker, 1- pyrenemethylamine, that earlier applied onto the SWNT. Dengue virus envelope protein has positively charged while the net surface charge is negative [292,293]. Therefore, the attachment of heparin and dengue virus would be possible, and the detection process would be achieved. This biosensor showed an excellent potential to perform as a bioreceptor for the dengue virus during biosensing. The ultrasensitive biosensor showed rapid detection of the virus in a deficient volume of sample (10 μL) with a detection limit of about 8.4×10^2 $TCID_{50}$/mL (median tissue culture infectious dose/mL). Good selectivity of the biosensor was also confirmed via utilizing the Influenza H1N1 virus along with the dengue virus, during which the biosensor reacts in the presence of the dengue virus [294,295].

Heparin was also used in the structure of an on/off nanosensor. This biosensor was composed of CdS quantum dots modified by heparin and mercaptopropionic acid (MPA) (Hep-MPA-CdS quantum dots) and was used to detect protamine and hemin. In this type of label-free fluorescent sensor, the negatively charged heparin-coated quantum dots could attract positively charged protamine, which enhanced the fluorescence intensity. The addition of hemin to the solution of this complex led to the detachment of protamine from heparin and their attachment to hemin, which is accompanied by reducing the fluorescent intensity. This is a low-cost fluorescent biosensor with good performance and high

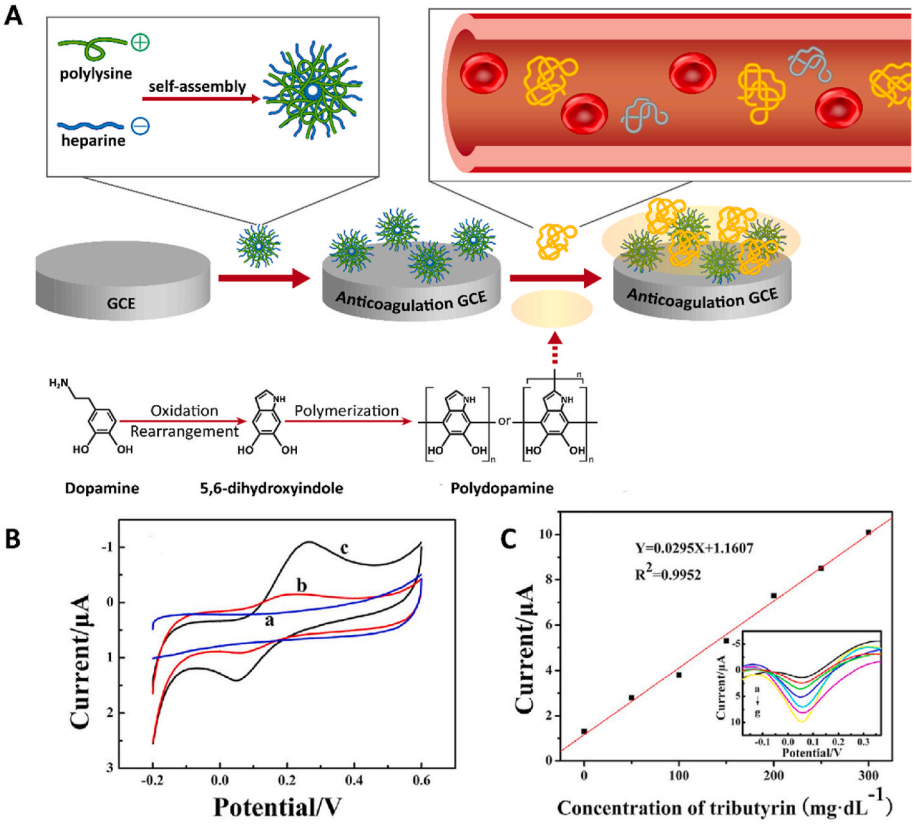

Fig. 17. Preparation of Lipase-(ε-polylysine-heparin)-glassy carbon electrode for electrocatalytic activities. (A) Schematic of fabrication of Lipase-(ε-polylysineheparin)-GCE biosensor. **(B)** Cyclic voltammograms of a) (ε-PL-Hep)-GCE, b) Lipase-Hep-GCE, and c) Lipase-(ε-PL-Hep)-GCE in PBS. **(C)** The calibration curve and the inset: differential pulse voltammetry (DPV) of tributyrin in the presence of lipase-(ε-polylysineheparin)-GCE biosensor. GSE: glassy carbon electrode. Reprinted with permission from [296].

reusability with a detection limit of 48.6 nmol L^{-1} and 5.2 nmol L^{-1} for hemin and protamine, respectively [297].

Heparin was also used in the structure of a dual-responsive electrochemical/fluorescent biosensor for detecting blood lead. The role of heparin in this biosensor was to induce the biofouling resistance property to this biosensor for its application in the blood environment. The application of this dual-responsive biosensor for the detection of lead ions in blood and water via fluorescent and electrochemical methods with a linear relationship of 0.5 pM–4.8 $\times 10^3$ pM and 1.5 pM- 4.8 $\times 10^3$ pM, relatively. The application of dual detection mode is appropriate for this biosensor for utilization in a blood sample with an excellent detection limit (1.0 pM and 4.4 pM for fluorescent and electrochemical detection, respectively) in comparison to other biosensors which are used in water aqueous conditions [298].

As mentioned before, heparin contains functional groups of sulfates ($-OSO^{3-}$, $-NHSO^{3-}$) and carboxylate ($-COO^{-}$) [299]. These properties make this material suitable for detecting some specific enzymes. For instance, it is indicated that heparin binds with anion-binding exposits-II of thrombin [300]. Correspondingly, electrochemical synthesis of a chromium-loaded CdS nanoprobe (CdS–Cr) is reported for use in ultrasensitive electrochemiluminescence (ECL) detection of thrombin. A glassy carbon electrode was covered with an electro-polymerized polyaniline nanofibers film (PANINF), a CdS–Cr nanoprobe was synthesized in-situ, heparin, and bovine serum albumin (BSA) applied to prevent unspecific adsorption. Previously, to detect thrombin, aptamer, a stranded DNA or RNA, has been used [301−304]. However, in comparison with aptamer, heparin is cheaper, easily available, and has a comparable bonding capability. Additionally, the aptamer needs further chemical modification. Hence, as an alternative material instead of aptamer, detecting thrombin using heparin in bioanalytical chemistry is greatly impacted. Compared to another similar biosensor, the fabricated CdS–Cr nanoprobes and heparin-based biosensor was a susceptible system with a good linear range (0.01 pM–100 pM) and excellent detection limit (0.0068) [305].

Another example of using a heparin-based biosensor is to detect thrombin, an important enzyme that plays a fundamental role in the blood clotting pathways. The majority of the methods to detect thrombin rely on fluorescence labeling assays or aptamers/antibodies as specific recognition biomolecules, which need complex procedures that result in a very expensive outcome. In a study by Mudlier et al., a simple, selective, and label-free fluorescence biosensor is designed to detect thrombin. This biosensor is based on the communication between thrombin and a fluorescent complex of heparin and Thioflavin-T [306]. This amperometric glucose biosensor was fabricated using polyurethane-heparin nanoparticles (PU-Hep NPs). This biosensor showed the ability to utilize whole blood directly, owing to the antibiofouling property of PU-Hep NPs. The detection system develops based on the interaction between cationic thrombin and anionic heparin to regulate the monomer-aggregate balance of the Thioflavin-T–Heparin scheme. Significantly, this scheme recommends a ratiometric reaction capable of robust quantifying thrombin concentration in different situations, even in complex mediums. This detection system's contribution of all available components is a key advantage. Additionally, this detection system demonstrates consistent response in diluted serum matrix. The biocompatibility and anticoagulant properties of heparin appropriate it to use in the structure of a biosensor applied for the quantitative detection of α-Fetoprotein (AFP). For this purpose, an immunosensor was fabricated *by* modifying the surface of a glassy carbon electrode (GCE) with heparin- γ-polyglutamic-polypyrrole (Hep-PGA–PPy) nanoparticles. This was a stable immunosensor with high selectivity and reproducibility that could detect AFP in whole blood samples with the detection limit of 0.099 ng mL^{-1} in a linear range from 0.1 to 100 ng mL^{-1} [307].

To reach the necessity of more medical treatment, biosensors with innovative surface modification approaches are still the focus of the biomedical investigation. Although commonly used coating such as calcium phosphate and nanotubes are capable of detecting and evaluating the release of drugs from the material surface, owing to the lack of pores and high surface area, these coating materials are not able to realize the high amount of medications in a long-term release (more than 7–10 days). For instance, long-term detection of antimicrobial drugs cannot be promised using the above coating methods. The exceptionally outstanding characteristics of heparin-based materials helped to solve many fundamental limitations of biosensing, such as instability during storage in adverse conditions and hemocompatibility issues. Accordingly, the advantages produced by the heparin-based biosensors, especially their compatibility with clinical samples, would help reach the final goal of the biosensors, which is the detection of toxic and harmful molecules with high sensitivity and selectivity.

7. Conclusion and outlook

In this review, the excellent features and applications of heparin and its derivatives have been studied in detail as one of the FDA-approved anticoagulant components, and owing to their exclusive features such as high anticoagulant activity, good biocompatibility, and biodegradability, heparin and its derivatives, whether synthetic or natural ones have been used in several biomedical fields. These fields include but are not limited to cancer treatment, tissue regeneration, wound healing, and biosensors. It has been demonstrated that some specific features of heparin have emerged only in particular types of heparins, which makes them appropriate for a particular application; for instance, the antimetastatic, anti-proliferative, anti-angiogenic, and liver first pass escape capability of LMWH index them in delivery applications. Moreover, the presence of different functional groups in the structure of this GAG component makes it appropriate for other physical and chemical interactions with various features to induce new properties of heparin and thus expand its current applications.

Heparin could also be easily integrated into other materials to fabricate new agents with predefined properties for biomedical applications. It could act as an anticoagulation coating and functional agent on the surface of materials used for implantable stents or blood vessels. Apart from laboratory-scale research, several clinical studies are also being conducted on heparin in biomedical fields. The combination use of heparin and some specific glycoprotein were also applied for the fabrication of a nanoscale extracellular matrix used for producing embryoid bodies and sophisticated patient-specific multicellular from induced pluripotent stem cells without affecting their particular properties like pluripotency, proliferation, and adhesion capability during its aggregation [308].

The anticoagulation ability of heparin makes it appropriate in blood-contacting fields such as drug delivery vehicles and diagnostic agents leading to cost-effective fabrication methods. The affinity of this component toward P-selectin, which is overexpressed in pathological states related to inflammation and thrombosis, makes it a targeting agent for the future design of targeted drug carriers for thrombolytic theranostic. Moreover, this polymer's affinity to different growth factors makes it an appropriate candidate for tissue regeneration applications in other parts of the body (like spinal cord injury) as hydrogel and 3D printed scaffolds [309]. Indeed, it could improve the controlled release pattern of growth factors and thus enhance the performance of regeneration. It is an excellent candidate to be employed in the structure of vascular grafts of small-diameter vessels due to its highly negative charges that prevent both protein precipitations and thrombosis and promote cell attachment, differentiation, and proliferation. The anticoagulation property and its capability to interfere with inflammatory cascade make it a good target for ulcerative colitis treatment. These, along with several other samples, confirm the potential of this polymer and its derivatives for clinical applications; however, long-term studies are needed to optimize their biological features and make them commercialized.

While recognizing the extensive biomedical applications of heparin

and its derivatives, it is crucial to underscore the need for comprehensive safety evaluations of engineered heparin-based materials. In-depth studies need to focus on their biocompatibility, immunogenicity, and cytotoxicity, especially in the context of long-term exposure and interactions with biological systems. Additional focus should be on the environmental and public health implications of these nanomaterials' degradation products. All these evaluations should take into account the varying fabrication methods and nanostructures that could alter these materials' safety profile. An interdisciplinary approach involving scientists, clinicians, and regulatory bodies will be crucial to ensuring the safe and effective use of these materials. In addition, although several studies have been conducted in the biomedical sector, there are still some limitations to their commercialization, like overcoming thrombogenicity in humans which needs several future studies to clarify. The low permeability and stability of heparin oligosaccharides along with their short half-life limit could limit their direct application as drug/gene delivery compound or tissue regeneration application. Besides, the probable overdose during the surgery could limit the usage of these compound for clinical applications [310]. The incorporation of this compound on the surface of biomaterials like stent couldn't prevent the activation of other pro-coagulant systems which are response for the platelet activation, and so other components are need in these cases to overcome this limitation [311]. Another limitation of the heparin derivatives (UFH and LMWHs) is their oral administration, which is resulted from their high molecular weight and high surface negative charge. Moreover, their short half-life and rapid removing by the cells of immune system limit their application as drug delivery system [312]. To overcome these drawbacks, some other anticoagulant agents have been introduced with similar capabilities as heparin without its limited features, which may limit the market of heparin as its substitute. Given that RNA and DNA vaccines became widely used after the outbreak of the Corona pandemic. Thus, the utilization of heparin properties for producing nanoplatforms for releasing nucleic acid-based vaccines should be investigated. In addition, heparin, along with other biological and synthetic substances, may be recommended to be used for gene editing purposes as well.

Drug delivery devices, e.g., microneedles, based on different natural materials such as hyaluronic acid and gelatin derivatives, were deployed. In light of this, the preparation of microneedle patches based on heparin could also be one of the prospective research interests. Although the widespread study in injectable hydrogel platforms based on heparin, the actual applications of heparin-based platforms could be one of the prospective instructions. Research on industrial and clinical applications of heparin-based platforms is insufficient. Another subject that could be addressed in the future is the synthesis of heparin-based copolymers or blends with inherently conductive polymers, e.g., polyaniline and its derivatives, polypyrrole, and polythiophene for the preparation of electroconductive hydrogels for the treatment and healing of wounds by photothermal and application in nerve regeneration. On the other side, there is little research on the use of heparin-based nanoplatforms in bioimaging and antimicrobial activity, so they are helpful areas that can be further worked on in the future to produce beneficial and commercial materials.

Notes

The authors declare no competing financial interest.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations and Acronyms

GAGs	glycosaminoglycans
GlcA	D-glucuronic
IdoA	L-iduronic acid
GlcN	D-glucosamine
UFH	Unfractionated heparin
LMWH	low molecular weight heparin
ULMWH	ultra-low molecular weight heparin
NF-κB	nuclear transcription factor-κB
Xyl	xylose
Gal	galactose
PU	polyurethane
PPEGMEMA	poly (poly-(ethylene glycol) methyl ether methacrylate)
PDMAEMA	poly(dimethylamino methyl) methacrylate
PEG	polyethylene glycol
Hep	Heparin
PUSSM	Poly(urethane sulfamethazine)
VEGF	Vascular endothelial growth factor
NMR	Nuclear magnetic resonance
FGF	fibroblast growth factor
TGA	Thermogravimetric analysis
ECM	Extracellular matrix
HA	Hydroxyapatite
COL	Collagen
ADSCs	Adipose-derived stem cells
PCL	Poly(ε-caprolactone)
α-TCP	α-tricalcium phosphate
TERM	Tissue engineering and regenerative medicines
PEAD	Poly(ethylene arginyl aspartate diglyceride)
PEGDA	Poly(ethylene glycol) diacrylate
IGF-1	Insulin-like growth factor-1
GC	Glycol chitosan
PEDOT	Poly(3,4-ethylene dioxythiophene)
PEM	Polyelectrolyte multilayer
PLL	Poly-L lysine
MMP	Matrix metalloproteinase
HUVEC	Human umbilical vein-derived endothelial cells
APTT	activated partial thromboplastin time
MMP2	Matrix metalloproteinase 2
cGP	Crosslinked PCL-coated with gelatin/PLL
GPH	Uncrosslinked PCL-coated with gelatin/PLL/heparin
cGPH	Crosslinked PCL-coated with gelatin/PLL/heparin
PU75-Hep	Heparin-grafted poly(ester-urethane)urea/gelatin
CD31	Cluster of differentiation 31
α-SMA	Smooth muscle actin alpha
H&E	Hematoxylin and eosin
DAPI	4′,6-diamidino-2-phenylindole
hCC	Human chondroprogenitor cells
SDF-1	Stromal cell-derived factor-1
PDGF-BB	Platelet-derived growth factor-BB
PPEGMEMA	Poly(ethylene glycol) methyl ether methacrylate
PDMAEMA	Poly(dimethylaminoethyl methacrylate)
ATRP	Atom transfer radical polymerization

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide
HepG2	HepatomaG2
TBA	Tetrabutylammonium
BIBB	2-bromoisobutyryl bromide
siRNA	Small interfering RNA
DEXAM	Amine-modified dextran
CSCs	Cancer stem cells
MAP	Mitogen-activated protein
HSPG	Heparan sulfate proteoglycan protein
ER	Endoplasmic reticulum
ICG	Indocyanine green
DENV	Dengue virus
SWNT	Single-walled carbon nanotubes
MPA	mercaptopropionic acid
PU-Hep NPs	Polyurethane-heparin nanoparticles
AFP	α-Fetoprotein
GCE	Glassy carbon electrode
CdS–Cr	Chromium-loaded CdS nanoprobe
ECL	Electrochemiluminescence
PANINF	Polyaniline nanofibers film
BSA	Bovine serum albumin
PU-Hep NPs	Polyurethane-heparin nanoparticles
Hep-PGA–PPy	Heparin- γ-polyglutamic-polypyrrole

References

[1] A. Onishi, K. St Ange, J.S. Dordick, R.J. Linhardt, Heparin and anticoagulation, Front. Biosci. Landmark 21 (2016) 1372–1392, https://doi.org/10.2741/4462.

[2] C. Cheng, S. Sun, C. Zhao, Progress in heparin and heparin-like/mimicking polymer-functionalized biomedical membranes, J. Mater. Chem. B 2 (2014) 7649–7672, https://doi.org/10.1039/c4tb01390e.

[3] C. Hao, H. Xu, L. Yu, L. Zhang, Heparin: an essential drug for modern medicine, Prog. Mol. Biol. Transl. Sci. 163 (2019) 1–19, https://doi.org/10.1016/bs.pmbts.2019.02.002.

[4] N. Goldberg, Y. Shmidov, O. Kryukov, D. Aranovich, S. Cohen, R. Bitton, Effect of heparin and peptide conjugation on structure and functional properties of alginate in solutions and hydrogels, Mater. Adv. 2 (2021) 440–447, https://doi.org/10.1039/d0ma00669f.

[5] N. Zandi, E. Mostafavi, M.A. Shokrgozar, E. Tamjid, T.J. Webster, N. Annabi, A. Simchi, Biomimetic proteoglycan nanoparticles for growth factor immobilization and delivery, Biomater. Sci. 8 (2020), https://doi.org/10.1039/c9bm00668k.

[6] E.I. Oduah, R.J. Linhardt, S.T. Sharfstein, Heparin: past, present, and future, Pharmaceuticals 9 (2016) 1–12, https://doi.org/10.3390/ph9030038.

[7] L. Hou, W.M.R.N. Udangawa, A. Pochiraju, W. Dong, Y. Zheng, R.J. Linhardt, T. J. Simmons, Synthesis of heparin-immobilized, magnetically addressable cellulose nanofibers for biomedical applications, ACS Biomater. Sci. Eng. 2 (2016) 1905–1913, https://doi.org/10.1021/acsbiomaterials.6b00273.

[8] S. Hu, S. Shao, H. Chen, J. Sun, J. Zhai, H. Zheng, M. Wan, Y. Liu, C. Mao, J. Zhao, Preparation and properties of Janus heparin-loaded ammoniated-hollow mesoporous silica nanomotors, J. Phys. Chem. C 122 (2018) 9680–9687, https://doi.org/10.1021/acs.jpcc.8b02079.

[9] Y. Lee, P. Le Thi, G.M. Seon, S.B. Ryu, C.M. Brophy, Y.T. Kim, J.C. Park, K. D. Park, J. Cheung-Flynn, H.J. Sung, Heparin-functionalized polymer graft surface eluting MK2 inhibitory peptide to improve hemocompatibility and anti-neointimal activity, J. Contr. Release 266 (2017) 321–330, https://doi.org/10.1016/j.jconrel.2017.10.002.

[10] S. Mousavi, M. Moradi, T. Khorshidahmad, M. Motamedi, Anti-inflammatory effects of heparin and its derivatives: a systematic review, Adv. Pharmacol. Sci. 2015 (2015), https://doi.org/10.1155/2015/507151.

[11] C.R. Aláez-Versón, E. Lantero, X. Fernàndez-Busquets, Heparin: new life for an old drug, Nanomedicine 12 (2017) 1727–1744, https://doi.org/10.2217/nnm-2017-0127.

[12] C. He, H. Ji, Y. Qian, Q. Wang, X. Liu, W. Zhao, C. Zhao, Heparin-based and heparin-inspired hydrogels: size-effect, gelation and biomedical applications, J. Mater. Chem. B 7 (2019) 1186–1208, https://doi.org/10.1039/c8tb02671h.

[13] Y. Wu, F. Li, X. Zhang, Z. Li, Q. Zhang, W. Wang, D. Pan, X. Zheng, Z. Gu, H. Zhang, Q. Gong, K. Luo, Tumor microenvironment-responsive PEGylated heparin-pyropheophorbide-a nanoconjugates for photodynamic therapy, Carbohydr. Polym. 255 (2021), 117490, https://doi.org/10.1016/j.carbpol.2020.117490.

[14] F. Seidi, R. Jenjob, T. Phakkeeree, D. Crespy, Saccharides, oligosaccharides, and polysaccharides nanoparticles for biomedical applications, J. Contr. Release 284 (2018) 188–212, https://doi.org/10.1016/j.jconrel.2018.06.026.

[15] M.D.P. Rodriguez-Torres, L.S. Acosta-Torres, L.A. Diaz-Torres, Heparin-based nanoparticles: an overview of their applications, J. Nanomater. 2018 (2018), https://doi.org/10.1155/2018/9780489.

[16] E. Mostafavi, P. Soltantabar, T.J. Webster, Nanotechnology and Picotechnology: A New Arena for Translational Medicine, 2018, https://doi.org/10.1016/B978-0-12-813477-1.00009-8.

[17] D.M. Cruz, E. Mostafavi, A. Vernet-Crua, H. Barabadi, V. Shah, J.L. Cholula-Díaz, G. Guisbiers, T.J. Webster, Green nanotechnology-based zinc oxide (ZnO) nanomaterials for biomedical applications: a review, J. Phys. Mater. 3 (2020), https://doi.org/10.1088/2515-7639/ab8186.

[18] E. Mostafavi, D. Medina-Cruz, A. Vernet-Crua, J. Chen, J.L. Cholula-Díaz, G. Guisbiers, T.J. Webster, Green nanomedicine: the path to the next generation of nanomaterials for diagnosing brain tumors and therapeutics? Expet Opin. Drug Deliv. 18 (2021) https://doi.org/10.1080/17425247.2021.1865306.

[19] M. Saravanan, H. Vahidi, D. Medina Cruz, A. Vernet-Crua, E. Mostafavi, R. Stelmach, T.J. Webster, M. Ali Mahjoub, M. Rashedi, H. Barabadi, Emerging antineoplastic biogenic gold nanomaterials for breast cancer therapeutics: a systematic review, Int. J. Nanomed. 15 (2020), https://doi.org/10.2147/IJN.S240293.

[20] L.B. Truong, D. Medina-Cruz, E. Mostafavi, N. Rabiee, Selenium nanomaterials to combat antimicrobial resistance, Molecules 26 (2021), https://doi.org/10.3390/molecules26123611.

[21] J. Cheon, W. Chan, I. Zuhorn, The Future of nanotechnology: cross-disciplined progress to improve health and medicine, Acc. Chem. Res. 52 (2019) 2405.

[22] M. del P. Rodriguez-Torres, L.S. Acosta-Torres, L.A. Diaz-Torres, Heparin-based nanoparticles: an overview of their applications, J. Nanomater. (2018) 2018.

[23] S. Kargozar, M. Mozafari, Nanotechnology and Nanomedicine: start small, think big, Mater. Today Proc. 5 (2018) 15492–15500.

[24] H. Al-Khoury, E. Espinosa-Cano, M.R. Aguilar, J.S. Román, F. Syrowatka, G. Schmidt, T. Groth, Anti-inflammatory surface coatings based on polyelectrolyte multilayers of heparin and polycationic nanoparticles of naproxen-bearing polymeric drugs, Biomacromolecules 20 (2019) 4015–4025, https://doi.org/10.1021/acs.biomac.9b01098.

[25] H. Groult, N. Poupard, F. Herranz, E. Conforto, N. Bridiau, F. Sannier, S. Bordenave, J.-M. Piot, J. Ruiz-Cabello, I. Fruitier-Arnaudin, Family of bioactive heparin-coated iron oxide nanoparticles with positive contrast in magnetic resonance imaging for specific biomedical applications, Biomacromolecules 18 (2017) 3156–3167.

[26] F. Yergoz, N. Hastar, C.E. Cimenci, A.D. Ozkan, M.O. Guler, A.B. Tekinay, T. Tekinay, M.O. Guler, Heparin mimetic peptide nanofiber gel promotes regeneration of full thickness burn injury, Biomaterials 134 (2017) 117–127, https://doi.org/10.1016/j.biomaterials.2017.04.040.

[27] J.J. Roberts, P. Naudiyal, L. Jugé, L.E. Bilston, A.M. Granville, P.J. Martens, Tailoring stimuli responsiveness using dynamic covalent cross-linking of poly (vinyl alcohol)-heparin hydrogels for controlled cell and growth factor delivery, ACS Biomater. Sci. Eng. 1 (2015) 1267–1277, https://doi.org/10.1021/acsbiomaterials.5b00321.

[28] F. Wu, T. Xu, G. Zhao, S. Meng, M. Wan, B. Chi, C. Mao, J. Shen, Mesoporous silica nanoparticles-encapsulated agarose and heparin as anticoagulant and resisting bacterial adhesion coating for biomedical silicone, Langmuir 33 (2017) 5245–5252, https://doi.org/10.1021/acs.langmuir.7b00567.

[29] S.N. Baytas, R.J. Linhardt, Advances in the preparation and synthesis of heparin and related products, Drug Discov. Today 25 (2020) 2095–2109, https://doi.org/10.1016/j.drudis.2020.09.011.

[30] S.N. Baytas, R.J. Linhardt, Advances in the preparation and synthesis of heparin and related products, Drug Discov. Today 25 (2020) 2095–2109, https://doi.org/10.1016/j.drudis.2020.09.011.

[31] L. Fu, M. Suflita, R.J. Linhardt, Bioengineered heparins and heparan sulfates, Adv. Drug Deliv. Rev. 97 (2016) 237–249, https://doi.org/10.1016/j.addr.2015.11.002.

[32] S. Gulberti, X. Mao, C. Bui, S. Fournel-gigleux, Seminars in Cancer Biology the role of heparan sulfate maturation in cancer : a focus on the 3O-sulfation and the enigmatic 3O-sulfotransferases (HS3STs), Semin. Cancer Biol. 62 (2020) 68–85, https://doi.org/10.1016/j.semcancer.2019.10.009.

[33] C. Hao, H. Xu, L. Yu, L. Zhang, Heparin: an essential drug for modern medicine, Prog. Mol. Biol. Transl. Sci. 163 (2019) 1–19.

[34] W. Jeske, A. Kouta, A. Farooqui, F. Siddiqui, V. Rangnekar, M. Niverthi, R. Laddu, D. Hoppensteadt, O. Iqbal, J. Walenga, J. Fareed, Bovine mucosal heparins are comparable to porcine mucosal heparin at USP potency adjusted levels, Front. Med. 6 (2019) 1–9, https://doi.org/10.3389/fmed.2018.00360.

[35] H. Sun, D. Cao, Y. Liu, H. Wang, X. Ke, T. Ci, Low molecular weight heparin-based reduction-sensitive nanoparticles for antitumor and anti-metastasis of orthotopic breast cancer, Biomater. Sci. 6 (2018) 2172–2188, https://doi.org/10.1039/c8bm00486b.

[36] J.Y. van der Meer, E. Kellenbach, L.J. van den Bos, From farm to pharma: an overview of industrial heparin manufacturing methods, Molecules 22 (2017), https://doi.org/10.3390/molecules22061025.

[37] C.R. Aláez-Versón, E. Lantero, X. Fernàndez-Busquets, Heparin: new life for an old drug, Nanomedicine 12 (2017) 1727–1744.

[38] H. Sun, D. Cao, Y. Liu, H. Wang, X. Ke, T. Ci, Low molecular weight heparin-based reduction-sensitive nanoparticles for antitumor and anti-metastasis of orthotopic breast cancer, Biomater. Sci. 6 (2018) 2172–2188, https://doi.org/10.1039/c8bm00486b.

[39] G. Liu, K. Li, H. Wang, Polymeric micelles based on PEGylated chitosan-g-lipoic acid as carrier for efficient intracellular drug delivery, J. Biomater. Appl. 31 (2017) 1039–1048, https://doi.org/10.1177/0885328216685755.

[40] F.P. Seib, M. Tsurkan, U. Freudenberg, D.L. Kaplan, C. Werner, Heparin-modified polyethylene glycol microparticle aggregates for focal cancer chemotherapy, ACS

Biomater. Sci. Eng. 2 (2016) 2287–2293, https://doi.org/10.1021/acsbiomaterials.6b00495.
[41] H. Jin, Q. Chen, Y.Y. Zhang, K.F. Hao, G.Q. Zhang, W. Zhao, Preactivation-based, iterative one-pot synthesis of anticoagulant pentasaccharide fondaparinux sodium, Org. Chem. Front. 6 (2019) 3116–3120, https://doi.org/10.1039/c9qo00480g.
[42] M. Mende, C. Bednarek, M. Wawryszyn, P. Sauter, M.B. Biskup, U. Schepers, S. Bräse, Chemical synthesis of glycosaminoglycans, Chem. Rev. 116 (2016) 8193–8255, https://doi.org/10.1021/acs.chemrev.6b00010.
[43] A.K. Korir, C.K. Larive, Advances in the separation, sensitive detection, and characterization of heparin and heparan sulfate, Anal. Bioanal. Chem. 393 (2009) 155–169, https://doi.org/10.1007/s00216-008-2412-2.
[44] S. Alban, Adverse Effects of Heparin, 2012, https://doi.org/10.1007/978-3-642-23056-1_10.
[45] C.J. Jones, S. Beni, J.F.K. Limtiaco, D.J. Langeslay, C.K. Larive, Heparin characterization: challenges and solutions, Annu. Rev. Anal. Chem. 4 (2011) 439–465, https://doi.org/10.1146/annurev-anchem-061010-113911.
[46] R. Gupta, M.P. Ponnusamy, Analysis of sulfates on low molecular weight heparin using mass spectrometry: structural characterization of enoxaparin, Expert Rev. Proteomics 15 (2018) 503–513, https://doi.org/10.1080/14789450.2018.1480110.
[47] D.R. Junqueira, L.M. Zorzela, E. Perini, Unfractionated heparin versus low molecular weight heparins for avoiding heparin-induced thrombocytopenia in postoperative patients, Cochrane Database Syst. Rev. (2017), https://doi.org/10.1002/14651858.CD007557.pub3, 2017.
[48] B.E. Thacker, D. Xu, R. Lawrence, J.D. Esko, Heparan sulfate 3-O-sulfation: a rare modification in search of a function, Matrix Biol. 35 (2014) 60–72.
[49] G.C. Jayson, J.T. Gallagher, Heparin oligosaccharides: inhibitors of the biological activity of bFGF on Caco-2 cells, Br. J. Cancer 75 (1997) 9–16.
[50] E.I. Oduah, R.J. Linhardt, S.T. Sharfstein, Heparin: past, present, and future, Pharmaceuticals 9 (2016) 1–12, https://doi.org/10.3390/ph9030038.
[51] R. Lever, B. Mulloy, Heparin - A Century of Progress, Springer Berlin Heidelberg, Berlin, Heidelberg, 2012, https://doi.org/10.1007/978-3-642-23056-1.
[52] D. Kweon, S. Lim, Preparation and characteristics of a water-soluble chitosan–heparin complex, J. Appl. Polym. Sci. 87 (2003) 1784–1789.
[53] P.V.A. Bueno, P.R. Souza, H.D.M. Follmann, A.G.B. Pereira, A.F. Martins, A. F. Rubira, E.C. Muniz, N,N-Dimethyl chitosan/heparin polyelectrolyte complex vehicle for efficient heparin delivery, Int. J. Biol. Macromol. 75 (2015) 186–191, https://doi.org/10.1016/j.ijbiomac.2015.01.030.
[54] S. Rong, Q. Chen, G. Xu, F. Wei, J. Yang, D. Ren, X. Cheng, X. Xia, J. Li, M. Gao, Novel and facile synthesis of heparin sulfur quantum dots via oxygen acceleration for ratiometric sensing of uric acid in human serum, Sensor. Actuator. B Chem. 353 (2022), 131146.
[55] N. Bogdan, E.M. Rodríguez, F. Sanz-Rodríguez, M.C.I. de la Cruz, Á. Juarranz, D. Jaque, J.G. Solé, J.A. Capobianco, Bio-functionalization of ligand-free upconverting lanthanide doped nanoparticles for bio-imaging and cell targeting, Nanoscale 4 (2012) 3647–3650.
[56] Z. Wang, C. Shi, X. Wu, Y. Chen, Efficient access to the non-reducing end of low molecular weight heparin for fluorescent labeling, Chem. Commun. 50 (2014) 7004–7006.
[57] M. Ismail, Y. Du, L. Ling, X. Li, Artesunate-heparin conjugate based nanocapsules with improved pharmacokinetics to combat malaria, Int. J. Pharm. 562 (2019) 162–171.
[58] Y. Zhao, Y. Jiang, Q. Wang, Y. Sun, K. Huang, Z. Yao, Rapid and sensitive detection of dextran sulfate sodium based on supramolecular self-assembly of a perylene diimide derivative in aqueous solution, Spectrochim. Acta Mol. Biomol. Spectrosc. 270 (2022), 120760.
[59] S. Rong, Q. Chen, G. Xu, F. Wei, J. Yang, D. Ren, X. Cheng, X. Xia, J. Li, M. Gao, Novel and facile synthesis of heparin sulfur quantum dots via oxygen acceleration for ratiometric sensing of uric acid in human serum, Sensor. Actuator. B Chem. 353 (2022), 131146.
[60] J.L. Tian, Y.Z. Zhao, Z. Jin, C.T. Lu, Q.Q. Tang, Q. Xiang, C.Z. Sun, L. Zhang, Y. Y. Xu, H.S. Gao, Z.C. Zhou, X.K. Li, Y. Zhang, Synthesis and characterization of Poloxamer 188-grafted heparin copolymer, Drug Dev. Ind. Pharm. 36 (2010) 832–838, https://doi.org/10.3109/03639040903520983.
[61] P.V.A. Bueno, P.R. Souza, H.D.M. Follmann, A.G.B. Pereira, A.F. Martins, A. F. Rubira, E.C. Muniz, N,N-Dimethyl chitosan/heparin polyelectrolyte complex vehicle for efficient heparin delivery, Int. J. Biol. Macromol. 75 (2015) 186–191, https://doi.org/10.1016/j.ijbiomac.2015.01.030.
[62] Q. Li, L. Ye, A. Zhang, Z. Feng, The preparation and morphology control of heparin-based pH sensitive polyion complexes and their application as drug carriers, Carbohydr. Polym. 211 (2019) 370–379, https://doi.org/10.1016/j.carbpol.2019.01.089.
[63] R. Song, M. Murphy, C. Li, K. Ting, C. Soo, Z. Zheng, Current development of biodegradable polymeric materials for biomedical applications, Drug Des. Dev. Ther. 12 (2018) 3117–3145, https://doi.org/10.2147/DDDT.S165440.
[64] B.L. Farrugia, M.S. Lord, J. Melrose, J.M. Whitelock, The role of heparan sulfate in inflammation, and the development of biomimetics as anti-inflammatory strategies, J. Histochem. Cytochem. 66 (2018) 321–336.
[65] A. Nilasaroya, A.M. Kop, D.A. Morrison, Heparin-functionalized hydrogels as growth factor-signaling substrates, J. Biomed. Mater. Res. 109 (2021) 374–384.
[66] A. Amirsadeghi, A. Jafari, L.J. Eggermont, S.S. Hashemi, S.A. Bencherif, M. Khorram, Vascularization strategies for skin tissue engineering, Biomater. Sci. 8 (2020) 4052–4073, https://doi.org/10.1039/d0bm00266f.
[67] A. Amirsadeghi, A. Jafari, L.J. Eggermont, S.S. Hashemi, S.A. Bencherif, M. Khorram, Vascularization strategies for skin tissue engineering, Biomater. Sci. 8 (2020) 4052–4073, https://doi.org/10.1039/d0bm00266f.
[68] L. Sun, X. Xiong, Q. Zou, P. Ouyang, R. Krastev, Controlled heparinase-catalyzed degradation of polyelectrolyte multilayer capsules with heparin as responsive layer, J. Appl. Polym. Sci. 134 (2017), https://doi.org/10.1002/app.44916.
[69] A. Nilasaroya, A.M. Kop, D.A. Morrison, Heparin-functionalized hydrogels as growth factor-signaling substrates, J. Biomed. Mater. Res. 109 (2021) 374–384.
[70] C. He, H. Ji, Y. Qian, Q. Wang, X. Liu, W. Zhao, C. Zhao, Heparin-based and heparin-inspired hydrogels: size-effect, gelation and biomedical applications, J. Mater. Chem. B 7 (2019) 1186–1208, https://doi.org/10.1039/c8tb02671h.
[71] V. Kumar, P. Gopinath, V.K. Lakshmanan, K. Navaneetha Pandiyaraj, Surface analysis technique for assessing hemocompatibility of biomaterials, in: Hemocompatibility of Biomaterials for Clinical Applications: Blood-Biomaterials Interactions, Elsevier Inc., 2018, pp. 119–161, https://doi.org/10.1016/B978-0-08-100497-5.00006-9.
[72] J.J. Nie, W. Zhao, H. Hu, B. Yu, F.J. Xu, Controllable heparin-based comb copolymers and their self-assembled nanoparticles for gene delivery, ACS Appl. Mater. Interfaces 8 (2016) 8376–8385, https://doi.org/10.1021/acsami.6b00649.
[73] S.H. Kim, T. Thambi, J.S. Lym, V.H. Giang Phan, D.S. Lee, Tunable engineering of heparinized injectable hydrogels for affinity-based sustained delivery of bioactive factors, Macromol. Mater. Eng. 304 (2019), https://doi.org/10.1002/mame.201900279.
[74] K. Amoako, R. Gbyli, Improving the hemocompatibility of biomedical polymers, in: Hemocompatibility of Biomaterials for Clinical Applications: Blood-Biomaterials Interactions, Elsevier Inc., 2018, pp. 223–252, https://doi.org/10.1016/B978-0-08-100497-5.00016-1.
[75] K. Amoako, R. Gbyli, 9 - improving the hemocompatibility of biomedical polymers, in: C.A. Siedlecki (Ed.), Hemocompatibility of Biomaterials for Clinical Applications, Woodhead Publishing, 2018, pp. 223–252, https://doi.org/10.1016/B978-0-08-100497-5.00016-1.
[76] V. Kumar, P. Gopinath, V.-K. Lakshmanan, K. Navaneetha Pandiyaraj, 6 - surface analysis technique for assessing hemocompatibility of biomaterials, in: C. A. Siedlecki (Ed.), Hemocompatibility of Biomaterials for Clinical Applications, Woodhead Publishing, 2018, pp. 119–161, https://doi.org/10.1016/B978-0-08-100497-5.00006-9.
[77] J.-J. Nie, W. Zhao, H. Hu, B. Yu, F.-J. Xu, Controllable heparin-based comb copolymers and their self-assembled nanoparticles for gene delivery, ACS Appl. Mater. Interfaces 8 (2016) 8376–8385, https://doi.org/10.1021/acsami.6b00649.
[78] L. Daneshmandi, M. Barajaa, A. Tahmasbi Rad, S.A. Sydlik, C.T. Laurencin, L. Daneshmandi, M. Barajaa, C.T. Laurencin Raymond, A. Tahmasbi Rad, C. T. Laurencin, Graphene-Based Biomaterials for Bone Regenerative Engineering: A Comprehensive Review of the Field and Considerations Regarding Biocompatibility and Biodegradation, 2020, https://doi.org/10.1002/adhm.202001414.
[79] J. D, Mastropietro, rheology in pharmaceutical formulations-A perspective, J. Dev. Drugs 2 (2013) 2–7, https://doi.org/10.4172/2329-6631.1000108.
[80] R. Khnouf, D. Karasneh, E. Abdulhay, A. Abdelhay, W. Sheng, Z.H. Fan, Microfluidics-based device for the measurement of blood viscosity and its modeling based on shear rate, temperature, and heparin concentration, Biomed. Microdevices 21 (2019), https://doi.org/10.1007/s10544-019-0426-5.
[81] M. Hitosugi, M. Niwa, A. Takatsu, Changes in blood viscosity by heparin and argatroban, Thromb. Res. 104 (2001) 371–374, https://doi.org/10.1016/S0049-3848(01)00359-0.
[82] R. Khnouf, D. Karasneh, E. Abdulhay, A. Abdelhay, W. Sheng, Z.H. Fan, Microfluidics-based device for the measurement of blood viscosity and its modeling based on shear rate, temperature, and heparin concentration, Biomed. Microdevices 21 (2019), https://doi.org/10.1007/s10544-019-0426-5.
[83] C. Loira-Pastoriza, A. Sapin-Minet, R. Diab, J.L. Grossiord, P. Maincent, Low molecular weight heparin gels, based on nanoparticles, for topical delivery, Int. J. Pharm. 426 (2012) 256–262, https://doi.org/10.1016/j.ijpharm.2012.01.044.
[84] M. Hitosugi, M. Niwa, A. Takatsu, Changes in blood viscosity by heparin and argatroban, Thromb. Res. 104 (2001) 371–374, https://doi.org/10.1016/S0049-3848(01)00359-0.
[85] S.T.M. Nillesen, P.J. Geutjes, R. Wismans, J. Schalkwijk, W.F. Daamen, T.H. Van Kuppevelt, Increased angiogenesis and blood vessel maturation in acellular collagen – heparin scaffolds containing both FGF2 and VEGF, Biomater. 28 (2007) 1123–1131, https://doi.org/10.1016/j.biomaterials.2006.10.029.
[86] S. Font Tellado, S. Chiera, W. Bonani, P.S.P. Poh, C. Migliaresi, A. Motta, E. R. Balmayor, M. van Griensven, Heparin functionalization increases retention of TGF-β2 and GDF5 on biphasic silk fibroin scaffolds for tendon/ligament-to-bone tissue engineering, Acta Biomater. 72 (2018) 150–166, https://doi.org/10.1016/j.actbio.2018.03.017.
[87] L. Liao, B. Shi, H. Chang, X. Su, L. Zhang, C. Bi, Y. Shuai, in: T h e r a n o s t i c s Heparin improves BMSC cell therapy : Anticoagulant treatment by heparin improves the safety and therapeutic effect of bone marrow-derived mesenchymal stem cell cytotherapy, 2017, https://doi.org/10.7150/thno.16911, 7.
[88] H. Lin, Y. Zhao, W. Sun, B. Chen, J. Zhang, W. Zhao, Z. Xiao, J. Dai, The effect of crosslinking heparin to demineralized bone matrix on mechanical strength and specific binding to human bone morphogenetic protein, Biomater. 2 (2008) 29, https://doi.org/10.1016/j.biomaterials.2007.11.032.
[89] S. Kim, Z.K. Cui, P.J. Kim, L.Y. Jung, M. Lee, Design of hydrogels to stabilize and enhance bone morphogenetic protein activity by heparin mimetics, Acta Biomater. 72 (2018) 45–54, https://doi.org/10.1016/j.actbio.2018.03.034.

[90] Y.J. Choi, J.Y. Lee, J.H. Park, J.B. Park, J.S. Suh, Y.S. Choi, S.J. Lee, C.P. Chung, Y.J. Park, The identification of a heparin binding domain peptide from bone morphogenetic protein-4 and its role on osteogenesis, Biomaterials 31 (2010) 7226–7238, https://doi.org/10.1016/j.biomaterials.2010.05.022.

[91] S. Kim, Z.K. Cui, P.J. Kim, L.Y. Jung, M. Lee, Design of hydrogels to stabilize and enhance bone morphogenetic protein activity by heparin mimetics, Acta Biomater. 72 (2018) 45–54, https://doi.org/10.1016/j.actbio.2018.03.034.

[92] I. Kim, S.S. Lee, S. Bae, H. Lee, N.S. Hwang, Heparin functionalized injectable cryogel with rapid shape-recovery property for neovascularization, Biomacromolecules 19 (2018), https://doi.org/10.1021/acs.biomac.8b00331.

[93] S.J. Paluck, T.H. Nguyen, H.D. Maynard, Heparin-Mimicking polymers: synthesis and biological applications, Biomacromolecules 17 (2016) 3417–3440, https://doi.org/10.1021/acs.biomac.6b01147.

[94] S. Tabasum, A. Noreen, A. Kanwal, M. Zuber, M.N. Anjum, K.M. Zia, Glycoproteins functionalized natural and synthetic polymers for prospective biomedical applications: a review, Int. J. Biol. Macromol. 98 (2017) 748–776, https://doi.org/10.1016/j.ijbiomac.2017.01.078.

[95] S.U. Hansen, G.J. Miller, M.J. Cliff, G.C. Jayson, J.M. Gardiner, Making the longest sugars: a chemical synthesis of heparin-related [4]n oligosaccharides from 16-mer to 40-mer, Chem. Sci. 6 (2015) 6158–6164, https://doi.org/10.1039/c5sc02091c.

[96] J. Hirsh, S.S. Anand, J.L. Halperin, V. Fuster, Mechanism of action and pharmacology of unfractionated heparin, Arterioscler. Thromb. Vasc. Biol. 21 (2001) 1094–1096, https://doi.org/10.1161/hq0701.093686.

[97] Y. Tang, X. Wang, Z. Li, Z. He, X. Yang, X. Cheng, Y. Peng, Q. Xue, Y. Bai, R. Zhang, K. Zhao, F. Liang, X. Xiao, U. Andersson, H. Wang, T.R. Billiar, B. Lu, Heparin prevents caspase-11-dependent septic lethality independent of anticoagulant properties, Immunity 54 (2021) 454–467.e6, https://doi.org/10.1016/j.immuni.2021.01.007.

[98] A. Al Nahain, V. Ignjatovic, P. Monagle, J. Tsanaktsidis, V. Ferro, Heparin mimetics with anticoagulant activity, Med. Res. Rev. 38 (2018) 1582–1613, https://doi.org/10.1002/med.21489.

[99] H.A. Braz-de-Melo, S.S. Faria, G. Pasquarelli-do-Nascimento, I. de O. Santos, G. P. Kobinger, K.G. Magalhães, The use of the anticoagulant heparin and corticosteroid dexamethasone as prominent treatments for COVID-19, Front. Med. 8 (2021), 615333.

[100] F. Wu, T. Xu, G. Zhao, S. Meng, M. Wan, B. Chi, C. Mao, J. Shen, Mesoporous silica nanoparticles-encapsulated agarose and heparin as anticoagulant and resisting bacterial adhesion coating for biomedical silicone, Langmuir 33 (2017) 5245–5252.

[101] D.F. Williams, Specifications for innovative, enabling biomaterials based on the principles of biocompatibility mechanisms, Front. Bioeng. Biotechnol. 7 (2019) 255, https://doi.org/10.3389/fbioe.2019.00255.

[102] L. Ghasemi-Mobarakeh, D. Kolahreez, S. Ramakrishna, D. Williams, Key terminology in biomaterials and biocompatibility, Curr. Opin. Biomed. Eng. 10 (2019) 45–50, https://doi.org/10.1016/j.cobme.2019.02.004.

[103] S. Naahidi, M. Jafari, M. Logan, Y. Wang, Y. Yuan, H. Bae, B. Dixon, P. Chen, Biocompatibility of hydrogel-based scaffolds for tissue engineering applications, Biotechnol. Adv. 35 (2017) 530–544, https://doi.org/10.1016/j.biotechadv.2017.05.006.

[104] G. Schmalz, K.M. Galler, Biocompatibility of biomaterials – lessons learned and considerations for the design of novel materials, Dent. Mater. 33 (2017) 382–393, https://doi.org/10.1016/j.dental.2017.01.011.

[105] M. Bernard, E. Jubeli, M.D. Pungente, N. Yagoubi, Biocompatibility of polymer-based biomaterials and medical devices-regulations,: in vitro screening and risk-management, Biomater. Sci. 6 (2018) 2025–2053, https://doi.org/10.1039/c8bm00518d.

[106] R. Li, Y. Li, Y. Wu, Y. Zhao, H. Chen, Y. Yuan, K. Xu, H. Zhang, Y. Lu, J. Wang, X. Li, X. Jia, J. Xiao, Heparin-poloxamer thermosensitive hydrogel loaded with bFGF and NGF enhances peripheral nerve regeneration in diabetic rats, Biomaterials 168 (2018) 24–37, https://doi.org/10.1016/j.biomaterials.2018.03.044.

[107] S. Tabasum, A. Noreen, A. Kanwal, M. Zuber, M.N. Anjum, K.M. Zia, Glycoproteins functionalized natural and synthetic polymers for prospective biomedical applications: a review, Int. J. Biol. Macromol. 98 (2017) 748–776, https://doi.org/10.1016/j.ijbiomac.2017.01.078.

[108] M. Goh, Y. Kim, K. Gwon, K. Min, Y.M. Hwang, G. Tae, In situ formation of injectable and porous heparin-based hydrogel, Carbohydr. Polym. 174 (2017) 990–998, https://doi.org/10.1016/j.carbpol.2017.06.126.

[109] S.H. Kim, T. Thambi, J.S. Lym, V.H. Giang Phan, D.S. Lee, Tunable engineering of heparinized injectable hydrogels for affinity-based sustained delivery of bioactive factors, Macromol. Mater. Eng. 304 (2019), https://doi.org/10.1002/mame.201900279.

[110] R. Li, Y. Li, Y. Wu, Y. Zhao, H. Chen, Y. Yuan, K. Xu, H. Zhang, Y. Lu, J. Wang, X. Li, X. Jia, J. Xiao, Heparin-poloxamer thermosensitive hydrogel loaded with bFGF and NGF enhances peripheral nerve regeneration in diabetic rats, Biomaterials 168 (2018) 24–37, https://doi.org/10.1016/j.biomaterials.2018.03.044.

[111] K. Jin, B. Li, L. Lou, Y. Xu, X. Ye, K. Yao, J. Ye, C. Gao, In vivo vascularization of MSC-loaded porous hydroxyapatite constructs coated with VEGF-functionalized collagen/heparin multilayers, Sci. Rep. 6 (2016), https://doi.org/10.1038/srep19871.

[112] F. Mokhtari-Jafari, G. Amoabediny, M.M. Dehghan, Role of biomechanics in vascularization of tissue-engineered bones, J. Biomech. 110 (2020), 109920, https://doi.org/10.1016/j.jbiomech.2020.109920.

[113] M.D.P. Rodriguez-Torres, L.S. Acosta-Torres, L.A. Diaz-Torres, Heparin-based nanoparticles: an overview of their applications, J. Nanomater. 2018 (2018), https://doi.org/10.1155/2018/9780489.

[114] K. Jin, B. Li, L. Lou, Y. Xu, X. Ye, K. Yao, J. Ye, C. Gao, In vivo vascularization of MSC-loaded porous hydroxyapatite constructs coated with VEGF-functionalized collagen/heparin multilayers, Sci. Rep. 6 (2016), https://doi.org/10.1038/srep19871.

[115] S. Aslani, M. Kabiri, S. HosseinZadeh, H. Hanaee-Ahvaz, E.S. Taherzadeh, M. Soleimani, The applications of heparin in vascular tissue engineering, Microvasc. Res. 131 (2020), 104027, https://doi.org/10.1016/j.mvr.2020.104027.

[116] K. Gwon, E. Kim, G. Tae, Heparin-hyaluronic acid hydrogel in support of cellular activities of 3D encapsulated adipose derived stem cells, Acta Biomater. 49 (2017) 284–295, https://doi.org/10.1016/j.actbio.2016.12.001.

[117] S.N. Ma, Z.X. Mao, Y. Wu, M.X. Liang, D.D. Wang, X. Chen, P. an Chang, W. Zhang, J.H. Tang, The anti-cancer properties of heparin and its derivatives: a review and prospect, Cell Adhes. Migrat. 14 (2020) 118–128, https://doi.org/10.1080/19336918.2020.1767489.

[118] J. wen, Q. Chen, L. Ye, H. Zhang, A. Zhang, Z. Feng, The preparation of pH and GSH dual responsive thiolated heparin/DOX complex and its application as drug carrier, Carbohydr. Polym. 230 (2020), https://doi.org/10.1016/j.carbpol.2019.115592.

[119] D.D. Akolpoğlu Başaran, U. Gündüz, A. Tezcaner, D. Keskin, Topical delivery of heparin from PLGA nanoparticles entrapped in nanofibers of sericin/gelatin scaffolds for wound healing, Int. J. Pharm. 597 (2021), 120207, https://doi.org/10.1016/j.ijpharm.2021.120207.

[120] L. Ghasemi-Mobarakeh, D. Kolahreez, S. Ramakrishna, D. Williams, Key terminology in biomaterials and biocompatibility, Curr. Opin. Biomed. Eng. 10 (2019) 45–50, https://doi.org/10.1016/j.cobme.2019.02.004.

[121] F. Raeisi, E. Raeisi, F.R.E. Raeisi, Mini review of polysaccharide nanoparticles and drug delivery process, J. Adv. Appl. NanoBio Tech. 1 (2020) 33–44, https://doi.org/10.47277/AANBT/1(2)44.

[122] H. Kuang, Y. Wang, Y. Shi, W. Yao, X. He, X. Liu, X. Mo, S. Lu, P. Zhang, Construction and performance evaluation of Hep/silk-PLCL composite nanofiber small-caliber artificial blood vessel graft, Biomaterials 259 (2020), 120288, https://doi.org/10.1016/j.biomaterials.2020.120288.

[123] P. Fabbri, M. Messori, 5 - surface modification of polymers: chemical, physical, and biological routes, in: C.F. Jasso-Gastinel, J.M. Kenny (Eds.), Modification of Polymer Properties, William Andrew Publishing, 2017, pp. 109–130, https://doi.org/10.1016/B978-0-323-44353-1.00005-1.

[124] H.D.M. Follmann, A.F. Martins, A.P. Gerola, T.A.L. Burgo, C.V. Nakamura, A. F. Rubira, E.C. Muniz, Antiadhesive and antibacterial multilayer films via layer-by-layer assembly of TMC/heparin complexes, Biomacromolecules 13 (2012) 3711–3722, https://doi.org/10.1021/bm3011962.

[125] Y. Chen, J. Wang, J. Dou, H. Yu, C. Chen, Layer by layer assembled chitosan (TiO2)-heparin composite coatings on MAO-coated Mg alloys, Mater. Lett. 281 (2020), 128640, https://doi.org/10.1016/j.matlet.2020.128640.

[126] M. Alehosseini, N. Golafshan, M. Kharaziha, M. Fathi, H. Edris, Hemocompatible and bioactive heparin-loaded PCL-α-TCP fibrous membranes for bone tissue engineering, Macromol. Biosci. 18 (2018), https://doi.org/10.1002/mabi.201800020.

[127] C. Shi, W. Tingting, J.P. Li, M.A. Sullivan, C. Wang, H. Wang, B. Deng, Y. Zhang, Comprehensive landscape of heparin therapy for COVID-19, Carbohydr. Polym. 254 (2021), https://doi.org/10.1016/j.carbpol.2020.117232.

[128] M. Alehosseini, N. Golafshan, M. Kharaziha, M. Fathi, H. Edris, Hemocompatible and bioactive heparin-loaded PCL-α-TCP fibrous membranes for bone tissue engineering, Macromol. Biosci. 18 (2018), https://doi.org/10.1002/mabi.201800020.

[129] P. Ghelich, M. Kazemzadeh-Narbat, A. Hassani Najafabadi, M. Samandari, A. Memić, A. Tamayol, (Bio) manufactured solutions for treatment of bone defects with an emphasis on US-FDA regulatory science perspective, Adv. Nanobiomed. Res. 2 (2022), 2100073.

[130] J. Calvet, D. Khorsandi, L. Tío, J. Monfort, Evaluation of a single-shot of a high-density viscoelastic solution of hyaluronic acid in patients with symptomatic primary knee osteoarthritis: the no-dolor study, BMC Muscoskel. Disord. 23 (2022) 1–10.

[131] P. Zhao, H. Gu, H. Mi, C. Rao, J. Fu, L. sheng Turng, Fabrication of scaffolds in tissue engineering: a review, Front. Mech. Eng. 13 (2018) 107–119, https://doi.org/10.1007/s11465-018-0496-8.

[132] T. Agarwal, G.M. Fortunato, S.Y. Hann, B. Ayan, K.Y. Vajanthri, D. Presutti, H. Cui, A.H.P. Chan, M. Costantini, V. Onesto, C. Di Natale, N.F. Huang, P. Makvandi, M. Shabani, T.K. Maiti, L.G. Zhang, C. De Maria, Recent advances in bioprinting technologies for engineering cardiac tissue, Mater. Sci. Eng. C 124 (2021), 112057, https://doi.org/10.1016/j.msec.2021.112057.

[133] T. Agarwal, B. Subramanian, T.K. Maiti, Liver tissue engineering: challenges and opportunities, ACS Biomater. Sci. Eng. 5 (2019) 4167–4182, https://doi.org/10.1021/acsbiomaterials.9b00745.

[134] K. Sharma, M.A. Mujawar, A. Kaushik, State-of-Art functional biomaterials for tissue engineering, Front. Mater. 6 (2019) 1–10, https://doi.org/10.3389/fmats.2019.00172.

[135] T. Agarwal, I. Chiesa, D. Presutti, V. Irawan, K.Y. Vajanthri, M. Costantini, Y. Nakagawa, S.-A. Tan, P. Makvandi, E.N. Zare, E. Sharifi, C. De Maria, T. Ikoma, T.K. Maiti, Recent advances in bioprinting technologies for engineering different cartilage-based tissues, Mater. Sci. Eng. C 123 (2021), 112005, https://doi.org/10.1016/j.msec.2021.112005.

[136] Y. Liang, K.L. Kiick, Heparin-functionalized polymeric biomaterials in tissue engineering and drug delivery applications, Acta Biomater. 10 (2014) 1588–1600, https://doi.org/10.1016/j.actbio.2013.07.031.
[137] S. Aslani, M. Kabiri, S. HosseinZadeh, H. Hanaee-Ahvaz, E.S. Taherzadeh, M. Soleimani, The applications of heparin in vascular tissue engineering, Microvasc. Res. 131 (2020), 104027, https://doi.org/10.1016/j.mvr.2020.104027.
[138] C. Cheng, S. Sun, C. Zhao, Progress in heparin and heparin-like/mimicking polymer-functionalized biomedical membranes, J. Mater. Chem. B 2 (2014) 7649–7672, https://doi.org/10.1039/c4tb01390e.
[139] H. Cho, J. Kim, S. Kim, Y.C. Jung, Y. Wang, B.-J. Kang, K. Kim, Dual delivery of stem cells and insulin-like growth factor-1 in coacervate-embedded composite hydrogels for enhanced cartilage regeneration in osteochondral defects, J. Contr. Release 327 (2020) 284–295, https://doi.org/10.1016/j.jconrel.2020.08.002.
[140] J. Wu, J. Ye, J. Zhu, Z. Xiao, C. He, H. Shi, Y. Wang, C. Lin, H. Zhang, Y. Zhao, X. Fu, H. Chen, X. Li, L. Li, J. Zheng, J. Xiao, Heparin-based coacervate of FGF2 improves dermal regeneration by asserting a synergistic role with cell proliferation and endogenous facilitated VEGF for cutaneous wound healing, Biomacromolecules 17 (2016) 2168–2177, https://doi.org/10.1021/acs.biomac.6b00398.
[141] R. Subbiah, A. Cheng, M.A. Ruehle, M.H. Hettiaratchi, L.E. Bertassoni, R. E. Guldberg, Effects of controlled dual growth factor delivery on bone regeneration following composite bone-muscle injury, Acta Biomater. 114 (2020) 63–75, https://doi.org/10.1016/j.actbio.2020.07.026.
[142] Y. Xu, P.A. Patsis, S. Hauser, D. Voigt, R. Rothe, M. Günther, M. Cui, X. Yang, R. Wieduwild, K. Eckert, C. Neinhuis, T.F. Akbar, I.R. Minev, J. Pietzsch, Y. Zhang, Cytocompatible, injectable, and electroconductive soft adhesives with hybrid covalent/noncovalent dynamic network, Adv. Sci. 6 (2019), 1802077, https://doi.org/10.1002/advs.201802077.
[143] J. Zhang, D. Wang, X. Jiang, L. He, L. Fu, Y. Zhao, Y. Wang, H. Mo, J. Shen, Multistructured vascular patches constructed via layer-by-layer self-assembly of heparin and chitosan for vascular tissue engineering applications, Chem. Eng. J. 370 (2019) 1057–1067, https://doi.org/10.1016/j.cej.2019.03.270.
[144] D. Wang, X. Wang, X. Li, L. Jiang, Z. Chang, Q. Li, Biologically responsive, long-term release nanocoating on an electrospun scaffold for vascular endothelialization and anticoagulation, Mater. Sci. Eng. C 107 (2020), 110212, https://doi.org/10.1016/j.msec.2019.110212.
[145] Y. Xu, P.A. Patsis, S. Hauser, D. Voigt, R. Rothe, M. Günther, M. Cui, X. Yang, R. Wieduwild, K. Eckert, C. Neinhuis, T.F. Akbar, I.R. Minev, J. Pietzsch, Y. Zhang, Cytocompatible, injectable, and electroconductive soft adhesives with hybrid covalent/noncovalent dynamic network, Adv. Sci. 6 (2019), 1802077, https://doi.org/10.1002/advs.201802077.
[146] D. Wang, X. Wang, X. Li, L. Jiang, Z. Chang, Q. Li, Biologically responsive, long-term release nanocoating on an electrospun scaffold for vascular endothelialization and anticoagulation, Mater. Sci. Eng. C 107 (2020), 110212, https://doi.org/10.1016/j.msec.2019.110212.
[147] R.A. Hoshi, R. Van Lith, M.C. Jen, J.B. Allen, K.A. Lapidos, G. Ameer, The blood and vascular cell compatibility of heparin-modified ePTFE vascular grafts, Biomaterials 34 (2013) 30–41, https://doi.org/10.1016/j.biomaterials.2012.09.046.
[148] N. Kasoju, A. Pátíková, E. Wawrzynska, A. Vojtíšková, T. Sedlačík, M. Kumorek, O. Pop-Georgievski, E. Sticová, J. Kříž, D. Kubies, Bioengineering a pre-vascularized pouch for subsequent islet transplantation using VEGF-loaded polylactide capsules, Biomater. Sci. 8 (2020) 631–647, https://doi.org/10.1039/C9BM01280J.
[149] J. Zhu, D. Chen, J. Du, X. Chen, J. Wang, H. Zhang, S. Chen, J. Wu, T. Zhu, X. Mo, Mechanical matching nanofibrous vascular scaffold with effective anticoagulation for vascular tissue engineering, Compos. B Eng. 186 (2020), 107788, https://doi.org/10.1016/j.compositesb.2020.107788.
[150] C. Levinson, M. Lee, L.A. Applegate, M. Zenobi-Wong, An injectable heparin-conjugated hyaluronan scaffold for local delivery of transforming growth factor β1 promotes successful chondrogenesis, Acta Biomater. 99 (2019) 168–180, https://doi.org/10.1016/j.actbio.2019.09.017.
[151] H.M. Weber, M.V. Tsurkan, V. Magno, U. Freudenberg, C. Werner, Heparin-based hydrogels induce human renal tubulogenesis in vitro, Acta Biomater. 57 (2017) 59–69, https://doi.org/10.1016/j.actbio.2017.05.035.
[152] J. Zhu, D. Chen, J. Du, X. Chen, J. Wang, H. Zhang, S. Chen, J. Wu, T. Zhu, X. Mo, Mechanical matching nanofibrous vascular scaffold with effective anticoagulation for vascular tissue engineering, Compos. B Eng. 186 (2020), 107788, https://doi.org/10.1016/j.compositesb.2020.107788.
[153] C. Levinson, M. Lee, L.A. Applegate, M. Zenobi-Wong, An injectable heparin-conjugated hyaluronan scaffold for local delivery of transforming growth factor β1 promotes successful chondrogenesis, Acta Biomater. 99 (2019) 168–180, https://doi.org/10.1016/j.actbio.2019.09.017.
[154] J. Chen, A. Chin, A.J. Almarza, J.M. Taboas, Hydrogel to guide chondrogenesis versus osteogenesis of mesenchymal stem cells for fabrication of cartilaginous tissues, Biomed. Mater. 15 (2020), 045006, https://doi.org/10.1088/1748-605X/ab401f.
[155] E. Hesse, U. Freudenberg, T. Niemietz, C. Greth, M. Weisser, S. Hagmann, M. Binner, C. Werner, W. Richter, Peptide-functionalized starPEG/heparin hydrogels direct mitogenicity, cell morphology and cartilage matrix distribution in vitro and in vivo, J. Tissue Eng. Regen. Med. 12 (2018) 229–239, https://doi.org/10.1002/term.2404.
[156] H. Kim, H. Park, J.W. Lee, K.Y. Lee, Magnetic field-responsive release of transforming growth factor beta 1 from heparin-modified alginate ferrogels, Carbohydr. Polym. 151 (2016) 467–473, https://doi.org/10.1016/j.carbpol.2016.05.090.
[157] X. Sun, J. Wang, Y. Wang, C. Huang, C. Yang, M. Chen, L. Chen, Q. Zhang, Scaffold with orientated microtubule structure containing polylysine-heparin sodium nanoparticles for the controlled release of TGF-β1 in cartilage tissue engineering, ACS Appl. Bio Mater. 1 (2018) 2030–2040, https://doi.org/10.1021/acsabm.8b00523.
[158] J. Crecente-Campo, E. Borrajo, A. Vidal, M. Garcia-Fuentes, New scaffolds encapsulating TGF-β3/BMP-7 combinations driving strong chondrogenic differentiation, Eur. J. Pharm. Biopharm. 114 (2017) 69–78, https://doi.org/10.1016/j.ejpb.2016.12.021.
[159] I.G. Kim, J. Ko, H.R. Lee, S.H. Do, K. Park, Mesenchymal cells condensation-inducible mesh scaffolds for cartilage tissue engineering, Biomaterials 85 (2016) 18–29, https://doi.org/10.1016/j.biomaterials.2016.01.048.
[160] H. Cho, J. Kim, S. Kim, Y.C. Jung, Y. Wang, B.-J. Kang, K. Kim, Dual delivery of stem cells and insulin-like growth factor-1 in coacervate-embedded composite hydrogels for enhanced cartilage regeneration in osteochondral defects, J. Contr. Release 327 (2020) 284–295, https://doi.org/10.1016/j.jconrel.2020.08.002.
[161] E. Kunisch, A.-K. Knauf, E. Hesse, U. Freudenberg, C. Werner, F. Bothe, S. Diederichs, W. Richter, StarPEG/heparin-hydrogel based in vivo engineering of stable bizonal cartilage with a calcified bottom layer, Biofabrication 11 (2018), 015001, https://doi.org/10.1088/1758-5090/aae75a.
[162] J. Zhu, K. Xia, W. Yu, Y. Wang, J. Hua, B. Liu, Z. Gong, J. Wang, A. Xu, Z. You, Q. Chen, F. Li, H. Tao, C. Liang, Sustained release of GDF5 from a designed coacervate attenuates disc degeneration in a rat model, Acta Biomater. 86 (2019) 300–311, https://doi.org/10.1016/j.actbio.2019.01.028.
[163] S.S. Lee, J.H. Kim, J. Jeong, S.H.L. Kim, R.H. Koh, I. Kim, S. Bae, H. Lee, N. S. Hwang, Sequential growth factor releasing double cryogel system for enhanced bone regeneration, Biomaterials 257 (2020), 120223, https://doi.org/10.1016/j.biomaterials.2020.120223.
[164] Y. Gong, Y. Zhang, Z. Cao, F. Ye, Z. Lin, Y. Li, Development of CaCO 3 microsphere-based composite hydrogel for dual delivery of growth factor and Ca to enhance bone regeneration, Biomater. Sci. 7 (2019) 3614–3626, https://doi.org/10.1039/C9BM00463G.
[165] F. Fahimipour, E. Dashtimoghadam, M. Mahdi Hasani-Sadrabadi, J. Vargas, D. Vashaee, D.C. Lobner, T.S. Jafarzadeh Kashi, B. Ghasemzadeh, L. Tayebi, Enhancing cell seeding and osteogenesis of MSCs on 3D printed scaffolds through injectable BMP2 immobilized ECM-Mimetic gel, Dent. Mater. 35 (2019) 990–1006, https://doi.org/10.1016/j.dental.2019.04.004.
[166] A.N. Rindone, B. Kachniarz, C.C. Achebe, R.C. Riddle, A.N. O'Sullivan, A. H. Dorafshar, W.L. Grayson, Heparin-conjugated decellularized bone particles promote enhanced osteogenic signaling of PDGF-BB to adipose-derived stem cells in tissue engineered bone grafts, Adv. Healthc. Mater. 8 (2019), 1801565, https://doi.org/10.1002/adhm.201801565.
[167] M. Guillot-Ferriols, J.C. Rodríguez-Hernández, D.M. Correia, S.A.C. Carabineiro, S. Lanceros-Méndez, J.L. Gómez Ribelles, G. Gallego Ferrer, Poly(vinylidene) fluoride membranes coated by heparin/collagen layer-by-layer, smart biomimetic approaches for mesenchymal stem cell culture, Mater. Sci. Eng. C 117 (2020), 111281, https://doi.org/10.1016/j.msec.2020.111281.
[168] G.I. Brierly, J. Ren, J. Baldwin, S. Saifzadeh, C. Theodoropoulos, M.V. Tsurkan, A. Lynham, E. Hsu, D. Nikolarakos, C. Werner, M.A. Woodruff, D.W. Hutmacher, L.J. Bray, Investigation of sustained BMP delivery in the prevention of medication-related osteonecrosis of the jaw (MRONJ) in a rat model, Macromol. Biosci. 19 (2019), 1900226, https://doi.org/10.1002/mabi.201900226.
[169] R. Subbiah, A. Cheng, M.A. Ruehle, M.H. Hettiaratchi, L.E. Bertassoni, R. E. Guldberg, Effects of controlled dual growth factor delivery on bone regeneration following composite bone-muscle injury, Acta Biomater. 114 (2020) 63–75, https://doi.org/10.1016/j.actbio.2020.07.026.
[170] H. Yi, S. Forsythe, Y. He, Q. Liu, G. Xiong, S. Wei, G. Li, A. Atala, A. Skardal, Y. Zhang, Tissue-specific extracellular matrix promotes myogenic differentiation of human muscle progenitor cells on gelatin and heparin conjugated alginate hydrogels, Acta Biomater. 62 (2017) 222–233, https://doi.org/10.1016/j.actbio.2017.08.022.
[171] Y.D.P. Limasale, P. Atallah, C. Werner, U. Freudenberg, R. Zimmermann, Tuning the local availability of VEGF within glycosaminoglycan-based hydrogels to modulate vascular endothelial cell morphogenesis, Adv. Funct. Mater. 30 (2020), 2000068, https://doi.org/10.1002/adfm.202000068.
[172] L. Schirmer, K. Chwalek, M.V. Tsurkan, U. Freudenberg, C. Werner, Glycosaminoglycan-based hydrogels with programmable host reactions, Biomaterials 228 (2020), 119557, https://doi.org/10.1016/j.biomaterials.2019.119557.
[173] Y. Matsuzaki, R. Iwaki, J.W. Reinhardt, Y.-C. Chang, S. Miyamoto, J. Kelly, J. Zbinden, K. Blum, G. Mirhaidari, A. Ulziibayar, T. Shoji, C.K. Breuer, T. Shinoka, The effect of pore diameter on neo-tissue formation in electrospun biodegradable tissue-engineered arterial grafts in a large animal model, Acta Biomater. 115 (2020) 176–184, https://doi.org/10.1016/j.actbio.2020.08.011.
[174] W. Yao, H. Gu, T. Hong, Y. Wang, S. Chen, X. Mo, W. Li, C. Wang, T. Zhu, S. Lu, A bi-layered tubular scaffold for effective anti-coagulant in vascular tissue engineering, Mater. Des. 194 (2020), 108943, https://doi.org/10.1016/j.matdes.2020.108943.
[175] H. Bai, Z. Wang, M. Li, Y. Liu, W. Wang, P. Sun, S. Wei, Z. Wang, J. Li, A. Dardik, Hyaluronic acid–heparin conjugated decellularized human great saphenous vein patches decrease neointimal thickness, J. Biomed. Mater. Res. B Appl. Biomater. 108 (2020) 2417–2425, https://doi.org/10.1002/jbm.b.34574.
[176] Z. Wang, C. Liu, D. Zhu, X. Gu, Y. Xu, Q. Qin, N. Dong, S. Zhang, J. Wang, Untangling the co-effects of oriented nanotopography and sustained

anticoagulation in a biomimetic intima on neovessel remodeling, Biomaterials 231 (2020), 119654, https://doi.org/10.1016/j.biomaterials.2019.119654.
[177] D. Wang, X. Wang, Z. Zhang, L. Wang, X. Li, Y. Xu, C. Ren, Q. Li, L.-S. Turng, Programmed release of multimodal, cross-linked vascular endothelial growth factor and heparin layers on electrospun polycaprolactone vascular grafts, ACS Appl. Mater. Interfaces 11 (2019) 32533–32542, https://doi.org/10.1021/acsami.9b10621.
[178] J. Zhang, D. Wang, X. Jiang, L. He, L. Fu, Y. Zhao, Y. Wang, H. Mo, J. Shen, Multistructured vascular patches constructed via layer-by-layer self-assembly of heparin and chitosan for vascular tissue engineering applications, Chem. Eng. J. 370 (2019) 1057–1067, https://doi.org/10.1016/j.cej.2019.03.270.
[179] C.-H. Yao, K.-Y. Chen, M.-H. Cheng, Y.-S. Chen, C.-H. Huang, Effect of genipin crosslinked chitosan scaffolds containing SDF-1 on wound healing in a rat model, Mater. Sci. Eng. C 109 (2020), 110368, https://doi.org/10.1016/j.msec.2019.110368.
[180] D.J. Borg, P.B. Welzel, M. Grimmer, J. Friedrichs, M. Weigelt, C. Wilhelm, M. Prewitz, A. Stißel, A. Hommel, T. Kurth, U. Freudenberg, E. Bonifacio, C. Werner, Macroporous biohybrid cryogels for co-housing pancreatic islets with mesenchymal stromal cells, Acta Biomater. 44 (2016) 178–187, https://doi.org/10.1016/j.actbio.2016.08.007.
[181] Y.H. Kim, J.H. Ko, S. Lee, J.Y. Oh, G.S. Jeong, S.-N. Park, I.K. Shim, S.C. Kim, Long-term reversal of diabetes by subcutaneous transplantation of pancreatic islet cells and adipose-derived stem cell sheet using surface-immobilized heparin and engineered collagen scaffold, BMJ Open Diabetes Res. Care 8 (2020), e001128, https://doi.org/10.1136/bmjdrc-2019-001128.
[182] N. Kasoju, A. Pátíková, E. Wawrzynska, A. Vojtíšková, T. Sedlačík, M. Kumorek, O. Pop-Georgievski, E. Sticová, J. Kříž, D. Kubies, Bioengineering a pre-vascularized pouch for subsequent islet transplantation using VEGF-loaded polylactide capsules, Biomater. Sci. 8 (2020) 631–647, https://doi.org/10.1039/C9BM01280J.
[183] S.D. Gentile, A.P. Kourouklis, H. Ryoo, G.H. Underhill, Integration of hydrogel microparticles with three-dimensional liver progenitor cell spheroids, Front. Bioeng. Biotechnol. 8 (2020), https://doi.org/10.3389/fbioe.2020.00792.
[184] H.M. Weber, M.V. Tsurkan, V. Magno, U. Freudenberg, C. Werner, Heparin-based hydrogels induce human renal tubulogenesis in vitro, Acta Biomater. 57 (2017) 59–69, https://doi.org/10.1016/j.actbio.2017.05.035.
[185] M. Nowak, U. Freudenberg, M.V. Tsurkan, C. Werner, K.R. Levental, Modular GAG-matrices to promote mammary epithelial morphogenesis in vitro, Biomaterials 112 (2017) 20–30, https://doi.org/10.1016/j.biomaterials.2016.10.007.
[186] P. Atallah, L. Schirmer, M. Tsurkan, Y.D. Putra Limasale, R. Zimmermann, C. Werner, U. Freudenberg, in situ-forming, cell-instructive hydrogels based on glycosaminoglycans with varied sulfation patterns, Biomaterials 181 (2018) 227–239, https://doi.org/10.1016/j.biomaterials.2018.07.056.
[187] K. Chen, S. Wang, B. Chen, F. Sun, N. Li, Gene therapy in solid tumors: trends in trials in China and beyond, Drug Discov. Today 26 (2021) 296–300.
[188] A. Shahryari, M. Saghaeian Jazi, S. Mohammadi, H. Razavi Nikoo, Z. Nazari, E. S. Hosseini, I. Burtscher, S.J. Mowla, H. Lickert, Development and clinical translation of approved gene therapy products for genetic disorders, Front. Genet. 10 (2019) 868.
[189] C.-C. Ma, Z.-L. Wang, T. Xu, Z.-Y. He, Y.-Q. Wei, The approved gene therapy drugs worldwide: from 1998 to 2019, Biotechnol. Adv. 40 (2020), 107502.
[190] P. Verma, A. Srivastava, C. v Srikanth, A. Bajaj, Nanoparticle-mediated gene therapy strategies for mitigating inflammatory bowel disease, Biomater. Sci. 9 (2021) 1481–1502.
[191] M. Ashrafizade, M. Delfi, F. Hashemi, A. Zabolian, H. Saleki, M. Bagherian, N. Azami, M.V. Farahani, S. omid Sharifzadeh, S. Hamzehlou, Biomedical application of chitosan-based nanoscale delivery systems: potential usefulness in siRNA delivery for cancer therapy, Carbohydr. Polym. (2021), 117809.
[192] S. Mirzaei, M.H. Gholami, F. Hashemi, A. Zabolian, K. Hushmandi, V. Rahmanian, M. Entezari, Y.R. Girish, K.S.S. Kumar, A.R. Aref, Employing siRNA tool and its delivery platforms in suppressing cisplatin resistance: approaching to a new era of cancer chemotherapy, Life Sci. (2021), 119430.
[193] I. Serrano-Sevilla, A. Artiga, S.G. Mitchell, L. De Matteis, J.M. de la Fuente, Natural polysaccharides for siRNA delivery: nanocarriers based on chitosan, hyaluronic acid, and their derivatives, Molecules 24 (2019) 2570.
[194] M.S. Huh, E.J. Lee, H. Koo, J.Y. Yhee, K.S. Oh, S. Son, S. Lee, S.H. Kim, I.C. Kwon, K. Kim, Polysaccharide-based nanoparticles for gene delivery, Polym. Gene Deliv. Syst. (2017) 65–83.
[195] J.-J. Nie, W. Zhao, H. Hu, B. Yu, F.-J. Xu, Controllable heparin-based comb copolymers and their self-assembled nanoparticles for gene delivery, ACS Appl. Mater. Interfaces 8 (2016) 8376–8385.
[196] L. Song, X. Liang, S. Yang, N. Wang, T. He, Y. Wang, L. Zhang, Q. Wu, C. Gong, Novel polyethyleneimine-R8-heparin nanogel for high-efficiency gene delivery in vitro and in vivo, Drug Deliv. 25 (2018) 122–131.
[197] M. Kullberg, R. McCarthy, T.J. Anchordoquy, Systemic tumor-specific gene delivery, J. Contr. Release 172 (2013) 730–736.
[198] B. Chertok, R. Langer, D.G. Anderson, Spatial control of gene expression by nanocarriers using heparin masking and ultrasound-targeted microbubble destruction, ACS Nano 10 (2016) 7267–7278.
[199] A. Caporali, E. Pani, A.J.G. Horrevoets, N. Kraenkel, A. Oikawa, G.B. Sala-Newby, M. Meloni, B. Cristofaro, G. Graiani, A.S. Leroyer, Neurotrophin p75 receptor (p75NTR) promotes endothelial cell apoptosis and inhibits angiogenesis: implications for diabetes-induced impaired neovascularization in ischemic limb muscles, Circ. Res. 103 (2008) e15–e26.
[200] J.-K. Han, H.-S. Lee, H.-M. Yang, J. Hur, S.-I. Jun, J.-Y. Kim, C.-H. Cho, G.-Y. Koh, J.M. Peters, K.-W. Park, Peroxisome proliferator–activated receptor-δ agonist enhances vasculogenesis by regulating endothelial progenitor cells through genomic and nongenomic activations of the phosphatidylinositol 3-kinase/Akt pathway, Circulation 118 (2008) 1021–1033.
[201] D.D. Tu, Y.G. Chung, E.S. Gil, A. Seth, D. Franck, V. Cristofaro, M.P. Sullivan, D. di Vizio, P. Gomez III, R.M. Adam, Bladder tissue regeneration using acellular bi-layer silk scaffolds in a large animal model of augmentation cystoplasty, Biomaterials 34 (2013) 8681–8689.
[202] H.N. Yang, J.H. Choi, J.S. Park, S.Y. Jeon, K.D. Park, K.-H. Park, Differentiation of endothelial progenitor cells into endothelial cells by heparin-modified supramolecular pluronic nanogels encapsulating bFGF and complexed with VEGF165 genes, Biomaterials 35 (2014) 4716–4728.
[203] M. Ashrafizade, M. Delfi, F. Hashemi, A. Zabolian, H. Saleki, M. Bagherian, N. Azami, M.V. Farahani, S. omid Sharifzadeh, S. Hamzehlou, Biomedical application of chitosan-based nanoscale delivery systems: potential usefulness in siRNA delivery for cancer therapy, Carbohydr. Polym. (2021), 117809.
[204] I. Pilipenko, V. Korzhikov-Vlakh, V. Sharoyko, N. Zhang, M. Schäfer-Korting, E. Rühl, C. Zoschke, T. Tennikova, pH-sensitive chitosan–heparin nanoparticles for effective delivery of genetic drugs into epithelial cells, Pharmaceutics 11 (2019) 317.
[205] V. Korzhikov-Vlakh, I. Katernuk, I. Pilipenko, A. Lavrentieva, I. Guryanov, V. Sharoyko, A.A. Manshina, T.B. Tennikova, Photosensitive poly-l-lysine/heparin interpolyelectrolyte complexes for delivery of genetic drugs, Polymers (Basel) 12 (2020) 1077.
[206] A.H. Najafabadi, M. Abdouss, S. Faghihi, Preparation and characterization of PEGylated chitosan nanocapsules as a carrier for pharmaceutical application, J. Nanoparticle Res. 16 (2014) 1–14.
[207] M. Mansourian, K. Ghasemi, D. Khorsandi, G. Vaseghi, Comparative effectiveness of all available treatments for metastatic hormone-sensitive prostate cancer: a network meta-analysis, Am. J. Therapeut. 27 (2020) e541–e543.
[208] H.T. Liao, Y.-T. Lai, C.-Y. Kuo, J.-P. Chen, A bioactive multi-functional heparin-grafted aligned poly (lactide-co-glycolide)/curcumin nanofiber membrane to accelerate diabetic wound healing, Mater. Sci. Eng. C 120 (2021), 111689.
[209] M.C. Goh, Y. Hwang, G. Tae, Epidermal growth factor loaded heparin-based hydrogel sheet for skin wound healing, Carbohydr. Polym. 147 (2016) 251–260, https://doi.org/10.1016/j.carbpol.2016.03.072.
[210] S.H. Kim, T. Thambi, J.S. Lym, V.H. Giang Phan, D.S. Lee, Tunable engineering of heparinized injectable hydrogels for affinity-based sustained delivery of bioactive factors, Macromol. Mater. Eng. 304 (2019), https://doi.org/10.1002/mame.201900279.
[211] B. Patel, J. Rashid, N. Gupta, F. Ahsan, Low-molecular-weight heparin-coated and montelukast-filled inhalable particles: a dual-drug delivery system for combination therapy in asthma, J. Pharmaceut. Sci. 106 (2017) 1124–1135.
[212] S. Zhang, W.J. Cho, A.T. Jin, L.Y. Kok, Y. Shi, D.E. Heller, Y.L. Lee, Y. Zhou, X. Xie, J.R. Korzenik, Heparin-coated albumin nanoparticles for drug combination in targeting inflamed intestine, Adv. Healthc. Mater. 9 (2020), 2000536.
[213] Y. Yu, A. Williams, X. Zhang, L. Fu, K. Xia, Y. Xu, F. Zhang, J. Liu, M. Koffas, R. J. Linhardt, Specificity and action pattern of heparanase Bp, a β-glucuronidase from Burkholderia pseudomallei, Glycobiology 29 (2019) 572–581, https://doi.org/10.1093/glycob/cwz039.
[214] F.M. Hsu, M.H. Hu, Y.S. Jiang, B.Y. Lin, J.J. Hu, J.S. Jan, Antibacterial polypeptide/heparin composite hydrogels carrying growth factor for wound healing, Mater. Sci. Eng. C 112 (2020), https://doi.org/10.1016/j.msec.2020.110923.
[215] H. Lee, B. In, P.K. Mehta, M.Y.L.N. Kishore, K.H. Lee, Dual role of a fluorescent peptidyl probe based on self-assembly for the detection of heparin and for the inhibition of the heparin-digestive enzyme reaction, ACS Appl. Mater. Interfaces 10 (2018) 2282–2290, https://doi.org/10.1021/acsami.7b15411.
[216] M.S. Bae, N.R. Ko, S.J. Lee, J.B. Lee, D.N. Heo, W. Byun, B.J. Choi, H.B. Jeon, H. J. Jang, J.Y. Ahn, D.S. Hwang, B.Y. Jung, I.K. Kwon, Development of novel photopolymerizable hyaluronic acid/heparin-based hydrogel scaffolds with a controlled release of growth factors for enhanced bone regeneration, Macromol. Res. 24 (2016) 829–837, https://doi.org/10.1007/s13233-016-4112-7.
[217] F. Copes, P. Chevallier, C. Loy, D. Pezzoli, F. Boccafoschi, D. Mantovani, Heparin-modified collagen gels for controlled release of pleiotrophin: potential for vascular applications, Front. Bioeng. Biotechnol. 7 (2019) 74.
[218] J.S. McGonigle, G. Tae, P.S. Stayton, A.S. Hoffman, M. Scatena, Heparin-regulated delivery of osteoprotegerin promotes vascularization of implanted hydrogels, J. Biomater. Sci. Polym. Ed. 19 (2008) 1021–1034.
[219] Y. Qiao, J. Wan, L. Zhou, W. Ma, Y. Yang, W. Luo, Z. Yu, H. Wang, Stimuli-responsive nanotherapeutics for precision drug delivery and cancer therapy, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. vol. 11 (2019) e1527.
[220] H.X. Nguyen, E.A. O'Rear, Modified dextran, heparin-based triggered release microspheres for cardiovascular delivery of therapeutic drugs using protamine as a stimulus, J. Microencapsul. 34 (2017) 299–307.
[221] M. Ashrafizadeh, Z. Ahmadi, N.G. Kotla, E.G. Afshar, S. Samarghandian, A. Mandegary, A. Pardakhty, R. Mohammadinejad, G. Sethi, Nanoparticles targeting STATs in cancer therapy, Cells 8 (2019) 1158, https://doi.org/10.3390/cells8101158.
[222] J. Liao, H. Huang, Review on magnetic natural polymer constructed hydrogels as vehicles for drug delivery, Biomacromolecules 21 (2020) 2574–2594.
[223] Y. Peng, L.E. Tellier, J.S. Temenoff, Heparin-based hydrogels with tunable sulfation & degradation for anti-inflammatory small molecule delivery, Biomater. Sci. 4 (2016) 1371–1380.

[224] Y. Peng, L.E. Tellier, J.S. Temenoff, Heparin-based hydrogels with tunable sulfation & degradation for anti-inflammatory small molecule delivery, Biomater. Sci. 4 (2016) 1371–1380.
[225] X. Yang, H. Du, J. Liu, G. Zhai, Advanced nanocarriers based on heparin and its derivatives for cancer management, Biomacromolecules 16 (2015) 423–436.
[226] J. Atallah, H.H. Khachfe, J. Berro, H.I. Assi, The use of heparin and heparin-like molecules in cancer treatment: a review, Cancer Treat. Res. Commun. 24 (2020), 100192, https://doi.org/10.1016/j.ctarc.2020.100192.
[227] S.-N. Ma, Z.-X. Mao, Y. Wu, M.-X. Liang, D.-D. Wang, X. Chen, P. Chang, W. Zhang, J.-H. Tang, The anti-cancer properties of heparin and its derivatives: a review and prospect, Cell Adhes. Migrat. 14 (2020) 118–128.
[228] A.G. Liebsch, H. Schillers, Quantification of heparin's antimetastatic effect by single-cell force spectroscopy, J. Mol. Recogn 34 (2021), e2854, https://doi.org/10.1002/jmr.2854.
[229] T.A. Al-Hilal, S.W. Chung, J.U. Choi, F. Alam, J. Park, S.W. Kim, S.Y. Kim, F. Ahsan, I.S. Kim, Y. Byun, Targeting prion-like protein doppel selectively suppresses tumor angiogenesis, J. Clin. Invest. 126 (2016) 1251–1266, https://doi.org/10.1172/JCI83427.
[230] S.W. Chung, S.M. Bae, M. Lee, T.A. Al-Hilal, C.K. Lee, J.K. Kim, I.S. Kim, S.Y. Kim, Y. Byun, LHT7, a chemically modified heparin, inhibits multiple stages of angiogenesis by blocking VEGF, FGF2 and PDGF-B signaling pathways, Biomaterials 37 (2015) 271–278, https://doi.org/10.1016/j.biomaterials.2014.10.004.
[231] J.U. Choi, S.W. Chung, T.A. Al-Hilal, F. Alam, J. Park, F. Mahmud, J.H. Jeong, S. Y. Kim, Y. Byun, A heparin conjugate, LHbisD4, inhibits lymphangiogenesis and attenuates lymph node metastasis by blocking VEGF-C signaling pathway, Biomaterials 139 (2017) 56–66, https://doi.org/10.1016/j.biomaterials.2017.05.026.
[232] B. Kevane, K. Egan, S. Allen, P. Maguire, E. Neary, Á. Lennon, F. Ní Áinle, Endothelial barrier protective properties of low molecular weight heparin: a novel potential tool in the prevention of cancer metastasis? Res. Pract. Thromb. Haemost. 1 (2017) 23–32, https://doi.org/10.1002/rth2.12011.
[233] F. Alam, T.A. Al-Hilal, J. Park, J.U. Choi, F. Mahmud, J.H. Jeong, I.S. Kim, S. Y. Kim, S.R. Hwang, Y. Byun, Multi-stage inhibition in breast cancer metastasis by orally active triple conjugate, LHTD4 (low molecular weight heparin-taurocholate-tetrameric deoxycholate), Biomaterials 86 (2016) 56–67, https://doi.org/10.1016/j.biomaterials.2016.01.058.
[234] T.J. Kovacsovics, A. Mims, M.E. Salama, J. Pantin, N. Rao, K.M. Kosak, P. Ahorukomeye, M.J. Glenn, M.W.N. Deininger, K.M. Boucher, L.M. Bavisotto, G. Gutierrez-Sanchez, T.P. Kennedy, S.G. Marcus, P.J. Shami, Combination of the low anticoagulant heparin CX-01 with chemotherapy for the treatment of acute myeloid leukemia, Blood Adv. 2 (2018) 381–389, https://doi.org/10.1182/bloodadvances.2017013391.
[235] S.-C. Lin, C.-P. Wu, T. Tseng, Y. Jhang, S.-C. Lee, Role of syndecan-1 and exogenous heparin in hepatoma sphere formation, Biochem. Cell. Biol. 98 (2020) 112–119.
[236] R.S. Boothello, N.J. Patel, C. Sharon, E.I. Abdelfadiel, S. Morla, D.F. Brophy, H. Robert Lippman, U.R. Desai, B.B. Patel, A unique nonsaccharide mimetic of heparin hexasaccharide inhibits colon cancer stem cells via p38 MAP kinase activation, Mol. Cancer Therapeut. 18 (2019) 51–61, https://doi.org/10.1158/1535-7163.MCT-18-0104.
[237] G.M.T. Chang, H.M. Atkinson, L.R. Berry, A.K.C. Chan, Inhibition of plasmin generation in plasma by heparin, low molecular weight heparin, and a covalent antithrombin-heparin complex, Blood Coagul. Fibrinolysis 28 (2017) 431–437, https://doi.org/10.1097/MBC.0000000000000611.
[238] N.A. Afratis, K. Karamanou, Z. Piperigkou, D.H. Vynios, A.D. Theocharis, The role of heparins and nano-heparins as therapeutic tool in breast cancer, Glycoconj. J. 34 (2017) 299–307, https://doi.org/10.1007/s10719-016-9742-7.
[239] Y. Yu, Q. Lv, B. Zhang, F. Lan, Y. Dai, Adjuvant therapy with heparin in patients with lung cancer without indication for anticoagulants: a systematic review of the literature with meta-analysis, J. Cancer Res. Therapeut. 12 (2016) C37–C42, https://doi.org/10.4103/0973-1482.191627.
[240] H.J.M. Groen, E. van der Heijden, T.J. Klinkenberg, B. Biesma, J. Aerts, A. Verhagen, C. Kloosterziel, H.J.M. Smit, F. Schramel, V. van der Noort, H. van Tinteren, E.F. Smit, A.-M.C. Dingemans, Randomized phase III study of adjuvant chemotherapy with or without low-molecular weight heparin in completely resected non-small cell lung cancer patients: the NVALT-8 study, J. Clin. Oncol. 34 (2016) 8506, https://doi.org/10.1200/jco.2016.34.15_suppl.8506, 8506.
[241] A. Al Nahain, V. Ignjatovic, P. Monagle, J. Tsanaktsidis, G. Vamvounis, V. Ferro, Anticoagulant heparin mimetics via RAFT polymerization, Biomacromolecules 21 (2020) 1009–1021, https://doi.org/10.1021/acs.biomac.9b01688.
[242] A. Alekseeva, G. Mazzini, G. Giannini, A. Naggi, Structural features of heparanase-inhibiting non-anticoagulant heparin derivative Roneparstat, Carbohydr. Polym. 156 (2017) 470–480, https://doi.org/10.1016/j.carbpol.2016.09.032.
[243] M. Weissmann, U. Bhattacharya, S. Feld, E. Hammond, N. Ilan, I. Vlodavsky, The heparanase inhibitor PG545 is a potent anti-lymphoma drug: mode of action, Matrix Biol. 77 (2019) 58–72, https://doi.org/10.1016/j.matbio.2018.08.005.
[244] J. Emami, M. Kazemi, F. Hasanzadeh, M. Minaiyan, M. Mirian, A. Lavasanifar, Novel pH-triggered biocompatible polymeric micelles based on heparin–α-tocopherol conjugate for intracellular delivery of docetaxel in breast cancer, Pharmaceut. Dev. Technol. 25 (2020) 492–509, https://doi.org/10.1080/10837450.2019.1711395.
[245] A.T. Andrgie, Y.S. Birhan, T.W. Mekonnen, E.Y. Hanurry, H.F. Darge, R.H. Lee, H. Y. Chou, H.C. Tsai, Redox-responsive heparin-chlorambucil conjugate polymeric prodrug for improved anti-tumor activity, Polymers (Basel) 12 (2020) 43, https://doi.org/10.3390/polym12010043.
[246] H. Wu, Y. Luo, D. Xu, X. Ke, T. Ci, Low molecular weight heparin modified bone targeting liposomes for orthotopic osteosarcoma and breast cancer bone metastatic tumors, Int. J. Biol. Macromol. 164 (2020) 2583–2597, https://doi.org/10.1016/j.ijbiomac.2020.08.068.
[247] W. Niu, Q. Xiao, X. Wang, J. Zhu, J. Li, X. Liang, Y. Peng, C. Wu, R. Lu, Y. Pan, A biomimetic drug delivery system by integrating grapefruit extracellular vesicles and doxorubicin-loaded heparin-based nanoparticles for glioma therapy, Nano Lett. 21 (2021) 1484–1492.
[248] Y. Chen, R. Wang, Y. Wang, W. Zhao, S. Sun, C. Zhao, Heparin-mimetic polyurethane hydrogels with anticoagulant, tunable mechanical property and controllable drug releasing behavior, Int. J. Biol. Macromol. 98 (2017) 1–11, https://doi.org/10.1016/j.ijbiomac.2017.01.102.
[249] B. Newland, C. Varricchio, Y. Körner, F. Hoppe, C. Taplan, H. Newland, D. Eigel, G. Tornillo, D. Pette, A. Brancale, P.B. Welzel, F.P. Seib, C. Werner, Focal drug administration via heparin-containing cryogel microcarriers reduces cancer growth and metastasis, Carbohydr. Polym. 245 (2020), 116504, https://doi.org/10.1016/j.carbpol.2020.116504.
[250] N. Menon, H.X. Dang, U.S. Datla, M. Moarefian, C.B. Lawrence, C.A. Maher, C. N. Jones, Heparin-based hydrogel scaffolding alters the transcriptomic profile and increases the chemoresistance of MDA-MB-231 triple-negative breast cancer cells, Biomater. Sci. 8 (2020) 2786–2796, https://doi.org/10.1039/c9bm01481k.
[251] C. He, Z.-Q. Shi, C. Cheng, C.-X. Nie, M. Zhou, L.-R. Wang, C.-S. Zhao, Highly swellable and biocompatible graphene/heparin-analogue hydrogels for implantable drug and protein delivery, RSC Adv. 6 (2016) 71893–71904.
[252] D. Cao, H. Li, Y. Luo, N. Feng, T. Ci, Heparin modified photosensitizer-loaded liposomes for tumor treatment and alleviating metastasis in phototherapy, Int. J. Biol. Macromol. 168 (2021) 526–536.
[253] P. Li, G.H. Lee, S.Y. Kim, S.Y. Kwon, H.R. Kim, S. Park, From diagnosis to treatment: recent advances in patient-friendly biosensors and implantable devices, ACS Nano 15 (2021) 1960–2004, https://doi.org/10.1021/acsnano.0c06688.
[254] Q.A. Phan, L.B. Truong, D. Medina-Cruz, C. Dincer, E. Mostafavi, CRISPR/Cas-powered nanobiosensors for diagnostics, Biosens. Bioelectron. 197 (2022), https://doi.org/10.1016/j.bios.2021.113732.
[255] A. Vasala, V.P. Hytönen, O.H. Laitinen, Modern tools for rapid diagnostics of antimicrobial resistance, Front. Cell. Infect. Microbiol. 10 (2020) 308, https://doi.org/10.3389/fcimb.2020.00308.
[256] M.A. Morales, J.M. Halpern, Guide to selecting a biorecognition element for biosensors, Bioconjugate Chem. 29 (2018) 3231–3239.
[257] A. Vernet-Crua, D. Medina-Cruz, E. Mostafavi, A. Benko, J.L. Cholula-Diaz, M. Saravanan, H. Vahidi, H. Barabadi, T.J. Webster, Nanobiosensors for theranostic applications, in: Handbook on Nanobiomaterials for Therapeutics and Diagnostic Applications, Elsevier, 2021, pp. 511–543.
[258] L.C. Rosales-Rivera, J.L. Acero-Sánchez, P. Lozano-Sánchez, I. Katakis, C. K. O'Sullivan, Amperometric immunosensor for the determination of IgA deficiency in human serum samples, Biosens. Bioelectron. 33 (2012) 134–138.
[259] Y. Chen, Y. Yang, Y. Tu, An electrochemical impedimetric immunosensor for ultrasensitive determination of ketamine hydrochloride, Sensor. Actuator. B Chem. 183 (2013) 150–156.
[260] T. Xu, B. Chi, M. Chu, Q. Zhang, S. Zhan, R. Shi, H. Xu, C. Mao, Hemocompatible ϵ-polylysine-heparin microparticles: a platform for detecting triglycerides in whole blood, Biosens. Bioelectron. 99 (2018) 571–577.
[261] I. Capila, R.J. Linhardt, Heparin–protein interactions, Angew. Chem. Int. Ed. 41 (2002) 390–412.
[262] D. Wasik, A. Mulchandani, M.V. Yates, A heparin-functionalized carbon nanotube-based affinity biosensor for dengue virus, Biosens. Bioelectron. 91 (2017) 811–816, https://doi.org/10.1016/j.bios.2017.01.017.
[263] D. Wasik, A. Mulchandani, M.V. Yates, Salivary detection of dengue virus NS1 protein with a label-free immunosensor for early dengue diagnosis, Sensors (Switzerland) 18 (2018) 2641, https://doi.org/10.3390/s18082645.
[264] D.A. Wasik, Development of Carbon Nanotube-Based Biosensors to Detect Dengue Virus, ProQuest Dissertations and Theses., 2017, p. 140.
[265] E.M.A. Pereira, A.F. Dario, R.F.O. França, B.A.L. Fonseca, D.F.S. Petri, Binding of dengue virus particles and dengue proteins onto solid surfaces, ACS Appl. Mater. Interfaces 2 (2010) 2602–2610.
[266] V.A. Kostyuchenko, Q. Zhang, J.L. Tan, T.-S. Ng, S.-M. Lok, Immature and mature dengue serotype 1 virus structures provide insight into the maturation process, J. Virol. 87 (2013) 7700–7707.
[267] J. Simon, E. Flahaut, M. Golzio, Overview of carbon nanotubes for biomedical applications, Materials 12 (2019) 624, https://doi.org/10.3390/ma12040624.
[268] H. Ehtesabi, Application of carbon nanomaterials in human virus detection, J. Sci.: Adv. Mater. Dev. 5 (2020) 436–450, https://doi.org/10.1016/j.jsamd.2020.09.005.
[269] T. Xu, B. Chi, M. Chu, Q. Zhang, S. Zhan, R. Shi, H. Xu, C. Mao, Hemocompatible ϵ-polylysine-heparin microparticles: a platform for detecting triglycerides in whole blood, Biosens. Bioelectron. 99 (2018) 571–577.
[270] G. Guan, J. Sha, D. Zhu, Heparin-MPA dual modified CdS quantum dots used as a simple and rapid label-free fluorescent sensor for protamine and hemin detection, Microchem. J. 133 (2017) 391–397.
[271] H. Chen, S. Shao, Y. Yu, Y. Huang, X. Zhu, S. Zhang, J. Fan, G.Y. Yin, B. Chi, M. Wan, A dual-responsive biosensor for blood lead detection, Anal. Chim. Acta 1093 (2020) 131–141.
[272] P.S. Sidhu, M.H. Abdel Aziz, A. Sarkar, A.Y. Mehta, Q. Zhou, U.R. Desai, Designing allosteric regulators of thrombin. Exosite 2 features multiple subsites

that can be targeted by sulfated small molecules for inducing inhibition, J. Med. Chem. 56 (2013) 5059–5070.
[273] J.A. Huntington, Thrombin plasticity, Biochimica et Biophysica Acta (BBA), Proteins Proteomics 1824 (2012) 246–252.
[274] G. Jie, J. Yuan, Novel magnetic Fe3O4@ CdSe composite quantum dot-based electrochemiluminescence detection of thrombin by a multiple DNA cycle amplification strategy, Anal. Chem. 84 (2012) 2811–2817.
[275] J. Han, Y. Zhuo, Y. Chai, R. Yuan, Dual-responses for electrochemical and electrochemiluminescent detection based on a bifunctional probe, Chem. Commun. 50 (2014) 3367–3369.
[276] F. Li, H. Cui, A label-free electrochemiluminescence aptasensor for thrombin based on novel assembly strategy of oligonucleotide and luminol functionalized gold nanoparticles, Biosens. Bioelectron. 39 (2013) 261–267.
[277] N. Mohajeri, E. Mostafavi, N. Zarghami, The feasibility and usability of DNA-dot bioconjugation to antibody for targeted in vitro cancer cell fluorescence imaging, J. Photochem. Photobiol., B 209 (2020), https://doi.org/10.1016/j.jphotobiol.2020.111944.
[278] Q. Wen, P. Lu, P. Yang, A glassy carbon electrode modified with in-situ generated chromium-loaded CdS nanoprobes and heparin for ultrasensitive electrochemiluminescent determination of thrombin, Microchim. Acta 183 (2016) 123–132.
[279] N.H. Mudliar, P.M. Dongre, P.K. Singh, A Heparin based dual ratiometric sensor for Thrombin, Int. J. Biol. Macromol. 167 (2021) 1371–1378, https://doi.org/10.1016/j.ijbiomac.2020.11.091.
[280] T. Xu, B. Chi, J. Gao, M. Chu, W. Fan, M. Yi, H. Xu, C. Mao, Novel electrochemical immune sensor based on Hep-PGA-PPy nanoparticles for detection of α-Fetoprotein in whole blood, Anal. Chim. Acta 977 (2017) 36–43.
[281] U. Han, W. Kim, H. Cha, J.H. Park, J. Hong, Nano-structure of vitronectin/heparin on cell membrane for stimulating single cell in iPSC-derived embryoid body, iScience 24 (2021), 102297.
[282] A. Zarepour, S. Hooshmand, A. Gökmen, A. Zarrabi, E. Mostafavi, Spinal cord injury management through the combination of stem cells and implantable 3d bioprinted platforms, Cells 10 (2021), https://doi.org/10.3390/cells10113189.