Expert Opinion

Controlled release of heparin-binding growth factors using heparin-containing particulate systems for tissue regeneration

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The strategy of growth factor delivery to specific sites for therapeutic applications has been considered an essential process in biomedical fields despite some obstacles, such as a non-controlled release with initial burst. This article focuses on particulate systems using heparin for the controlled delivery of heparin-binding growth factors (HBGFs), an emerging area in the tissue engineering field. Since heparin has been widely utilized for growth factor delivery due to its electrostatic nature and specific affinity with HBGFs, heparin-containing polymeric particulates can be utilized as functional carriers to deliver growth factors in a controlled manner. In particular, examples of the HBGF delivery systems containing heparin, perspectives and potential applications are described and discussed.

Keywords: controlled release, growth factor delivery, heparin, heparin-binding growth factor (HBGF), polymeric particulate, tissue regeneration

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1. Introduction

Biomedical applications require various delivery systems to carry and release potentially therapeutic drugs to specific sites of the human body via various routes. Among such systems, tissue engineering has focused on delivering growth factors to regulate cell behaviors, such as attachment, proliferation, migration and differentiation, along with transporting the cell to the defective tissue site. The aim of this review is to provide knowledge of recent advances in heparin-binding growth factor (HBGF) delivery using heparin-containing polymeric particulate systems. This article also focuses on particulate systems for HBGFs delivery, except for hydrogel systems. Section 2 introduces studies of important HBGF delivery using polymeric particles. In Sections 3 and 4, particulate systems with heparin and the controlled delivery of HBGFs from those systems are described, respectively. Finally, potential applications of HBGF delivery are introduced in Section 5. In addition, comparisons of heparin-containing systems with other systems, their advantages and perspectives for controlled delivery of HBGFs are discussed in the 'Expert opinion' section.

1.1 Delivery strategies of growth factors

Growth factor refers to a naturally occurring protein capable of stimulating cellular proliferation and differentiation, which is important for regulating a variety of cellular processes [1]. Growth factors can act as either up- or down-regulators of the expression of cellular receptors, depending on their physiological concentration.

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Table 1. Growth factors commonly used in therapeutic applications.

Growth factor	Abbreviation	Relevant known activities
Vascular endothelial growth factor	VEGF	Migration, proliferation and survival of ECs
Fibroblast growth factor	FGF	Migration, proliferation and survival of ECs and many other cell types
Bone morphogenetic protein	BMP	Differentiation of bone-forming cells
Platelet-derived growth factor	PDGF	Promotes the maturation of blood vessels by the recruitment of SMCs Proliferation of osteoblasts, active in all stages of healing process
Insulin-like growth factor	IGF	Stimulates proliferation of osteoblasts and the synthesis of bone matrix
Placental growth factor	PIGF	Stimulates angiogenesis
Epidermal growth factor	EGF	Mitogenic for keratinocytes
Nerve growth factor	NGF	Promotes the survival and neurite outgrowth of degenerating cholinergic neurons
Hepatocyte growth factor	HGF	Serves as a growth factor for mature parenchymal hepatocytes Stimulates motility of epithelial cells and keratinocytes
Transforming growth factor	TGF	Stabilizes new blood vessels by promoting matrix deposition Proliferation and differentiation of bone-forming cells Promotes keratinocyte migration, ECM synthesis and remodeling, and differentiation of epithelial cells

EC: Endothelial cell; ECM: Extracellular matrix; SMC: Smooth muscle cell.

Growth factors can be classified by cellular functions and type of related cells that are involved in the corresponding therapeutic applications, such as angiogenesis, bone regeneration, nerve regeneration and wound healing [2]. Growth factors that have been used for therapeutic applications are summarized in **Table 1**.

Growth factors are in general delivered in solution form to a target site in the body via a possible route for therapeutic applications [3-5]. In systemic administration, however, growth factors have a short half-life and poor bioactivity due to rapid secretion and conformational instability. Therefore, many researchers have investigated delivery systems that can improve the bioavailability of growth factors. For example, the controlled release of growth factor in a target site over an extended period can be achieved by incorporating a growth factor into a polymeric carrier. The incorporated growth factor can be protected from its own proteolysis for the prolonged retention of bioactivity *in vivo*. There have been many attempts to deliver various growth factors to target sites by using polymeric biomaterials. Among these attempts, one of the important issues is to modulate the release kinetics of growth factors in order to achieve the correct period and rate for the most effective action of growth factors. Moreover, localization of a growth factor around the site of action is important to minimize or inhibit adverse effects, such as progressive tumor growth and metastasis by angiogenesis, which involves in the formation of new blood vessels from the established vasculature [6-8]. The treatment of a specific growth factor during tissue morphogenesis not only induces diverse cellular responses, but also controls neo-tissue formation and organization. Thus, designing a delivery system for growth factors that meets the above criteria is required for advanced tissue engineering. Control over the cascade of events leading to tissue regeneration may be maintained using strategies for growth factor delivery, mimicking the natural biological environment (**Figure 1**).

1.2 Heparin and heparin-binding growth factors

Heparin, a sulfated polysaccharide belonging to the family of glycosaminoglycans (GAGs), has numerous important biological activities involved in the interaction with diverse proteins. **Figure 2** shows the structure of heparin, which can be divided as two kinds of disaccharide unit. Heparin is widely used as an anticoagulant based on its ability to accelerate the rate at which antithrombin III (AT III) inhibits serine proteases in the blood coagulation cascade, derived from the specific interaction of heparin with AT III [9]. All the therapeutic actions of heparin known to date are involved in the specific interaction with various kinds of protein. Heparin-binding proteins contain some growth factors with specific cellular functions, termed heparin-binding growth factors (HBGFs). The heparin/HBGF complex by specific recognition is beneficial for the long-term release of HBGFs and the retention of HBGFs' bioactivity.

2. Delivery of important HBGFs

The most promising systems for protein delivery are accompanied by encapsulation or entrapment processes of

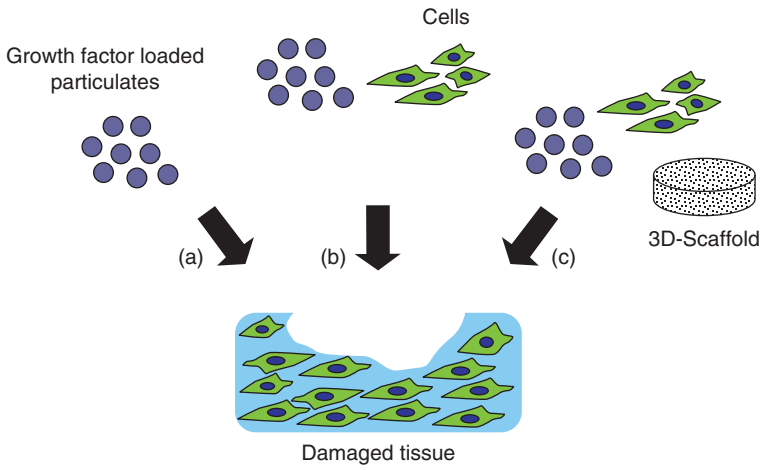

Figure 1. Growth factor delivery strategies for tissue regeneration. (a) Direct injection of growth factor loaded particulate; (b) injection with cultured cells; and (c) implantation of 3D-scaffold embedded with particulates and cells.

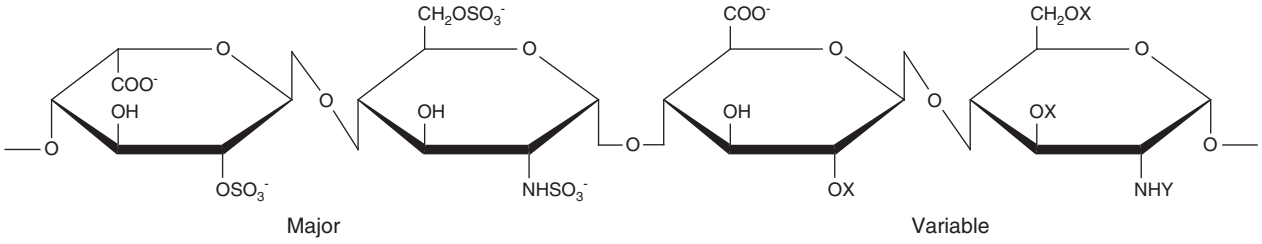

Figure 2. Major and minor disaccharide repeating units in heparin (X = H or SO^{3-}, Y = Ac, SO^{3-}, or H).

proteins into various polymeric systems [10]. Polymeric particulate systems may provide a great opportunity for achieving controlled release of an encapsulated protein as compared to polymeric hydrogel systems. In this section, four important kinds of HBGFs and polymeric particles for their delivery are introduced.

2.1 Transforming growth factor β 1

Transforming growth factor β1 (TGF-β1) has been studied as a potential induction factor for bone tissue engineering [11]. TGF-β1 plays a significant role in regulating bone formation at a fracture callus. One report demonstrated that TGF-β1 increased osteoblast proliferation, osteopontin and osteonectin production, as well as alkaline phosphatase activity during fracture healing in a rat femur model [12]. Mikos *et al.* prepared TGF-β1-loaded biodegradable microparticles of poly(D, L-lactic-*co*-glycolic acid) (PLGA) and poly(ethylene glycol) (PEG) [13,14]. The blend microparticles were fabricated using a double-emulsion ([water-in-oil]-in-water) solvent-extraction technique [15]. The effects of PEG content, buffer pH and loading densities of TGF-β1 on the protein release kinetics and the degradation of PLGA were determined *in vitro* for up to 28 days. After 8 days, the cumulative mass of released TGF-β1 reached a plateau, and there was negligible growth factor released during the remaining time.

2.2 Basic fibroblast growth factor

Fibroblast growth factors (FGFs) play the role of key regulators in several developmental processes in which cell fate and differentiation to various tissue lineages are determined on numerous cell types, such as fibroblasts, endothelial cells, smooth muscle cells and keratinocytes. These effects encompass stimulation of growth, proliferation, migration and differentiation and are transmitted by an elaborate FGF signaling system. The importance of the proper spatial and temporal regulation of FGF signals is evident from human and mouse genetic studies which show that mutations leading to the dysregulation of FGF signals cause a variety of developmental disorders, including dominant skeletal diseases and cancer [16]. Basic FGF (bFGF) was applied to enhancing vascularization using porous alginate scaffolds and microparticles [17] and curing cavernous tissue in the diabetes mellitus (DM) [18].

Perets *et al.* described the construction of a novel porous alginate scaffold that incorporates tiny PLGA microparticles, enabling the release of bFGF to be controlled. bFGF- or bovine serum albumin (BSA)-containing microparticles existed

in an integral part of the solid alginate matrix. The results revealed that the media collected from the matrix containing bFGF over 12 days stimulated the proliferation of cardiac fibroblasts *in vitro*. The HE staining of cross-sections of the scaffolds at this time point showed minimal host tissue penetration into the perimeter of the scaffolds.

Diabetes mellitus (DM) has been recognized as a major risk factor for erectile dysfunction (ED) and cavernous tissue regeneration is a novel approach for the treatment of ED. There has been some experimental success in ED treatment using growth factors, such as vascular endothelial growth factor [19], bFGF [20] and insulin-like growth factor [21]. Hisasue *et al.* tried to develop bFGF incorporating gelatin microparticles to preserve erectile function in a diabetic rat model. Although the i.v. pressure response was significantly lower in the DM group (diabetic rats that received gelatin microparticles with saline) than the control group (non-diabetic rats), the pressure in the bFGF group (diabetic rats that received gelatin microparticles with bFGF) was maintained at the normal level found in controls.

2.3 Vascular endothelial growth factor

The appropriate regulation of angiogenesis is a critical step for the incorporation of vascular networks into a viable engineered tissue. The vascular endothelial growth factor (VEGF) family is the most extensively studied angiogenic growth factors. VEGF is an endothelial cell, as well as a cell-specific mitogen and is the major angiogenic factor involved in physiological and pathological angiogenesis [22-25]. Preclinical models of therapeutic angiogenesis using either recombinant VEGF protein or gene therapy encoding VEGF have been successful and the data also exist for the use of VEGF in human clinical trials [26-28]. However, controlled delivery systems are still required for efficient delivery of VEGF because of the short biological half-life of VEGF and its tumorigenic potential [29]. PLGA-based microparticles for VEGF delivery provided a clue to solve those problems [30-33].

In a study, VEGF and BSA were co-encapsulated into microparticles fabricated with PEG and 50/50 PLGA [30]. The initial burst of VEGF/BSA release from the microparticles was observed. The released concentration of VEGF over 8 – 28 days was in the physiologically active range [34,35]. The bioactivity of the VEGF released from the microparticles over time was assessed using an *in vitro* HUVEC proliferation assay. The cells exposed to exogenous VEGF or supernatant from VEGF-loaded microparticles showed an increased rate of proliferation, as indicated by an increased number of cells compared to the negative control. In a study by Faranesh and co-workers [32], PLGA microparticles containing VEGF were fabricated and the *in vitro* release kinetics of VEGF from the PLGA microparticles was investigated. In the release kinetics of VEGF from the PLGA microparticle, sustained release behaviors were observed after the initial burst. In the proliferation assay of HUVEC, no significant difference was found between the cell counts for the VEGF only and VEGF-incorporated microparticles, indicating that the VEGF released from the microparticles was as bioactive as the un-encapsulated VEGF. Reichert *et al.* compared the dexamethasone (Dex) and VEGF release kinetics from microparticles and hydrogels with the microparticle [33]. Drug release kinetics from the microparticle exhibited an initial burst followed by sustained release during one week. Embedding microparticles in hydrogels might result in attenuated release of drugs. The release profiles of VEGF and Dex from microparticles within hydrogels were similar to those from free microparticles.

2.4 Nerve growth factor

Local delivery of nerve growth factor (NGF) is required in neurotrophic factor therapy to enhance axonal regeneration following injury to the central nervous system (CNS), such as spinal cord injury. However, various systemic delivery systems were unsatisfactory because they caused undesirable activities in the CNS and in non-neuronal tissues, due to a wide receptor distribution [36]. To carry out NGF delivery into the specific subpopulation of responsive neurons, a few groups have previously developed PLGA biodegradable microparticles and investigated a controlled release of the growth factors [37-39].

Péan *et al.* [40] prepared NGF-loaded PLGA microparticles to assess the *in vivo* activity, comparing it with unloaded microparticles. In another study, an animal study and quantification of acetylcholinesterase (AChE)-positive neurons and cholineacethyltransferase (ChAT)-positive neurons were also carried out [41]. In non-treated rats, AChE staining showed a marked disappearance of cholinergic neurons on the lesioned side. In contrast, a significant protection of AChE-containing neurons was observed at two and six weeks after the implantation of NGF-loaded microparticles.

Tatard *et al.* developed pharmacologically active microcarriers (PAM) that were prepared with PLGA and coated with adhesion molecules, which may serve as a matrix for cell culture and may be used as cell carriers presenting a controlled delivery of active protein [42]. The microparticles were coated with poly(D-lysine) (PDL) in a CO_2 incubator. *In vitro* release behaviors were evaluated with the coated microparticles, after encapsulation of radiolabeled ^{125}I-NGF. That was characterized by a regular and continuous NGF release during the initial 2 weeks.

Shoichet *et al.* proposed polymeric microparticles composed of one of four polymers: PLGA 50/50, PLGA 85/15, PCL and a blend of PCL/PLGA 50/50 (1:1, w/w) [43,44]. The release profile of the NGF, co-encapsulated with ovalbumin (OVA), was tailored from biodegradable polymeric microparticles using both degradation rate and protein loading amount. Moreover, they also developed the NGF-incorporated poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) P(HEMA-co-MMA) nerve guidance channels (NGCs). According to their results, most of the NGF was released within 15 days, with a large initial burst.

Wang *et al.* tried to develop polyphosphoester (PPE) microparticles as a carrier for the sustained release of the NGF from the NGCs, a therapeutic device used to repair injured nerves [45,46]. One of the PPE polymers, poly(BHET-EOP/TC), is highly compatible with the nervous system, showing no toxicity against various types of cultured neurons and a mild *in vivo* tissue response [47]. In that study, poly(BHET-EOP/TC) and poly(DAPG-EOP), were used to deliver the NGF by the microparticle formulation. The release behavior of the NGF was observed over 10 weeks. The effect of NGF released from PPE microparticles on the peripheral nervous system was investigated using a silicone NGC model in rats. The implantation of NGC with NGF-loaded microparticle induced the formation of axons at the distal end of regenerated cables within the NGC.

3. Heparin-containing particulate systems for HBGF delivery

A variety of heparin-containing biomaterials has been developed in many research fields such as anticoagulation, tissue regeneration and drug delivery. The main type of such biomaterials was the hydrogel combining with other functional materials which form either covalently or physically crosslinked matrices. Thus, many of these reviews have already been reported. However, recently a number of particulate systems containing heparin have been successively reported due to increasing interest in HBGFs and their delivery. In this section, various types of heparin-containing polymeric particulates for HBGF delivery are summarized.

The use of biodegradable polymers has been constantly highlighted in many applications including tissue engineering, gene therapy, novel drug delivery systems and implantable devices. Conjugating heparin to such biodegradable polymers provides biomaterials for sustained release of common drugs as well as growth factors due to their biodegradable nature. Park and colleagues have investigated heparin-conjugated linear and star-shaped PLAs, although the conjugates were not fabricated as particulate forms for drug delivery [48,49]. Jeon *et al.* reported on heparin-conjugated PLGA nanospheres for long-term and zero-order release of bFGF [50]. They prepared the conjugates by coupling heparin to amino-terminated PLGA, followed by preparing nanospheres via oil/water emulsion and evaporation–extraction method. A different type of heparin-conjugated particles was investigated by Byun and co-workers. They prepared the deoxycholic acid-conjugated heparin derivative, which can form a nanoparticle due to its amphiphilic nature, and investigated the heparin conjugates for various applications, such as oral delivery [51], anticoagulation [52] and anticancer therapy [53].

Thermosensitive amphiphilic copolymers with self-assembling potential can form a micelle applicable for drug carriers. Heparin can be also conjugated to these polymers for the placement of heparin on the surface. Park and colleagues prepared a heparin-conjugated Tetronic-poly(caprolactone) (PCL) copolymer for protein delivery, the chemical structure of which is shown in **Figure 3A**. In their report, they demonstrated that the activity of heparin bound to the copolymer was approximately 46% of intact heparin by activated partial thromboplastin time (APTT) assay [54]. In a further paper, they investigated the sustained long-term delivery of bFGF with heparin-conjugated Tetronic–PCL micelle, of which the proposed structure is shown in **Figure 3B** [55]. Through successive studies, they investigated the properties and potential of the micelle for controlled release of a dual drug, bFGF and indometacin [56]. A more interesting concept for heparin-conjugated vesicle-type particles was suggested by Park and colleagues. They recently reported a thermally reversible Pluronic/heparin nanocapsule showing large volume transition [57]. The nanocapsule was fabricated with activated Pluronic and modified heparin by their modified emulsification/solvent evaporation method. In detail, nanocapsules of activated Pluronic in oil droplets under aqueous solution was prepared and conjugated with the aminated heparin, followed by evaporating the organic solvent. As another functional particle bearing heparin, a fluorescent, magnetic composite microsphere was fabricated to incorporate heparin [58]. In this method, styrene and maleic anhydride were successively polymerized on the fluorescent, magnetite colloid particles that were treated via a few procedures followed by conjugating heparin to the particles. The heparin-conjugated microspheres have diameters of 0.15 – 0.7 μm and a core–shell structure. Anticoagulation assay evaluated the bioactivity of heparin conjugated to the composite microsphere. Passirani *et al.* suggested a novel nanoparticle containing heparin conjugated to poly(methyl methacrylate) to investigate the inhibition of complement activation by the particles [59]. The nanoparticle was prepared by heating to 70°C after the polymerization of methyl methacrylate along with heparin in aqueous suspension. According to their results, the prepared nanoparticles retained the inhibition property similar to that of soluble heparin. Yuk *et al.* suggested a facile method that produces a heparin-functionalized nanoparticle for controlled release of growth factors [60]. Their nanoparticles were prepared by a spontaneous emulsion solvent diffusion method. The PLGA dissolved in an organic solvent and aqueous solution of Pluronic and heparin was mixed and stirred, followed by high speed centrifugation and resuspension. Consequently, a hydrophobic core and hydrophilic outer layer were composed of PLGA and Pluronic, respectively, and heparin was physically entrapped into the PLGA matrix of nanoparticle. Sahli and co-workers fabricated a heparin-coated liposome and investigated its stability in plasma and the effect on coagulation [61]. A liposome was fabricated by the removal of detergent from lipid-based micelles using the dialysis method. An amino group-conjugated heparin was coated on the liposome to improve the poor stability of liposomes. A peculiar polymeric microsphere for stem cell therapy was

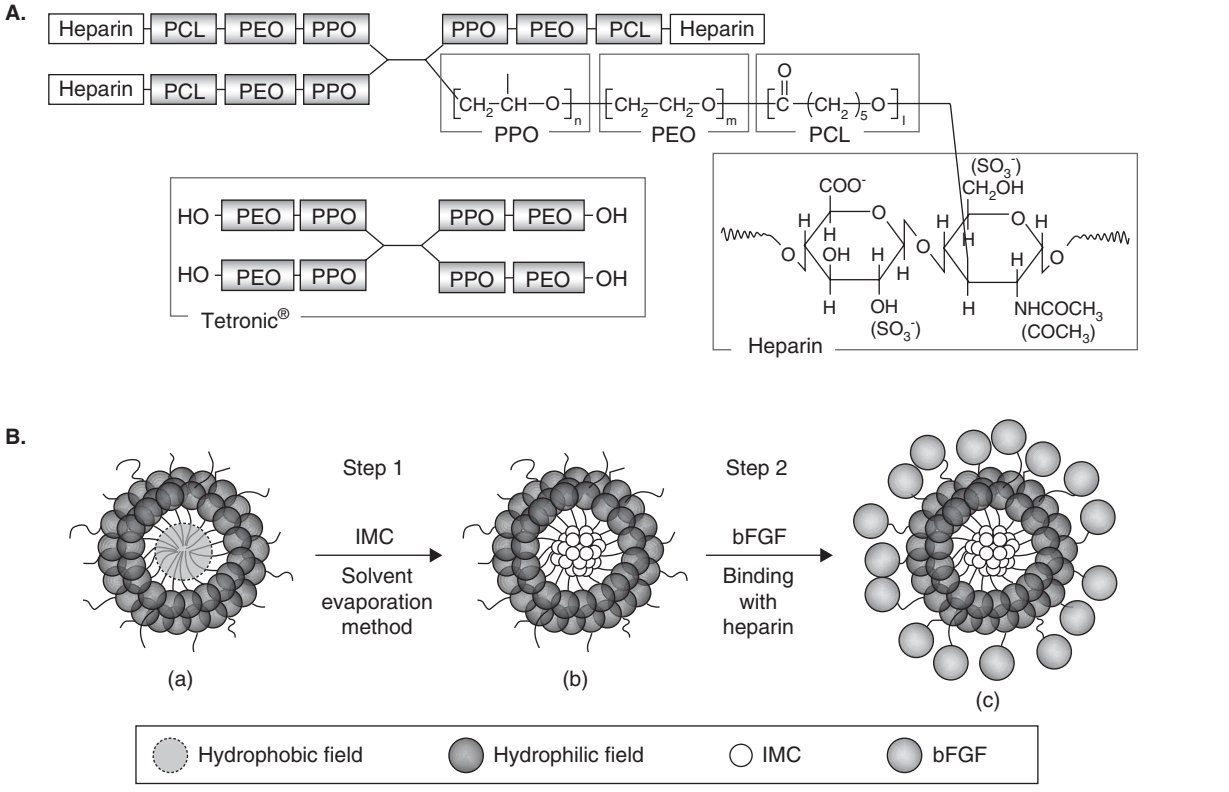

Figure 3. A. Chemical structures of Tetronic and Tetronic-PCL-heparin (n = 20, m = 62, l = 5). B. Schematic representation for the preparation of dual drug-loaded Tetronic–PCL–heparin micelles.

reported by Na *et al.* [62]. The PLGA microspheres were coated with poly(ethylenimine) (PEI) to give a positively charged surface. Then a nanostructure was prepared by the polyionic complex formation between poly(L-lysine) and heparin. Finally, the prepared heparin-containing nanostructure was coated on to the cationic PEI-coated surface of PLGA microsphere. The heparinized microsphere had good viability with human mesenchymal stem cells (hMSC) and promoted chondrogenic differentiation. Chinen *et al.* prepared a microparticle of heparin and alginate crosslinked gel to stabilize bFGF and control angiogenesis. The heparin/alginate particles were fabricated by mechanically breaking a heparin/alginate hydrogel sheet. They reported that bFGF-adsorbed heparin/alginate microparticles released bFGF as much as stimulating the growth of human umbilical venous endothelial cells up to 5 weeks [63]. Andersson *et al.* fabricated a small particle composed of a complex between heparin and chitosan from water-in-oil microemulsion, which is applicable for the oral administration of pharmaceutical ingredients [64]. The formation of the nano-sized particles inside the water droplets was confirmed by various tools.

In addition, a heparin-binding nanostructure promoting growth of blood vessels was fabricated by Rajangam *et al.* [65]. The nanostructure could be formed in a few seconds by mixing two liquids, one liquid being a dilute aqueous solution of the peptide amphiphile and the second liquid an aqueous solution of heparin with or without angiogenic growth factors. The heparinized nanostructure was a rigid cylindrical scaffold composed of nanofibers, showing binding affinity with angiogenic growth factors.

4. Controlled release of HBGFs based on heparin affinity

The specific interactions between proteins and heparin are primarily ionic and based on the presence and appropriate positioning of sulfo and carboxyl groups. So clusters of positively charged basic amino acids on proteins form ion pairs with spatially defined and negatively charged sulfo or carboxyl groups on the heparin chain [9]. FGF-1 and FGF-2 were the first discovered members of HBGF families, and the thermodynamics and kinetics of their interaction with heparin have been extensively studied [66-68]. VEGF is an angiogenic factor consisting of four alternatively spliced forms, three of which interact with heparin [69]. HBGF binds to heparan sulfate to act as a tumor suppressor, morphogen and angiogenic factor [70,71]. Heparin can also modulate biological activities of the TGF-β1, which plays an important role in cell migration, proliferation and extracellular matrix synthesis, also involved in immune processes [72,73].

Other HBGFs include PDGF and the HB-EGF, etc [74,75]. Although particulate systems are developed for biomedical applications, their use for HBGF delivery is concentrated on tissue regeneration via local delivery of growth factors along with cells. In such applications, controlled or sustained delivery of HBGFs is essential to achieve the long-term repair of injured tissue. In the scope of this review, systems for the controlled delivery of HBGF can be classified into two classes of polymers containing conventional biodegradable polymers without heparin, and systems with heparin.

In the case of biodegradable systems, the release behaviors of most drugs from the matrices are dependent on diffusion, chemical reaction or solvent activation [76]. Behavior is also affected by the molecular weight or chemical composition of polymers and HBGFs, the hydrophobic/hydrophilic nature of the matrix, etc. The release profile of drugs from biodegradable particles mostly reveals a burst at the initial phase of the profile, which is caused by rapid diffusion of drugs located to the exterior of the particle matrix, resulting in inefficient controlled delivery of the drug. In HBGF delivery, however, utilizing the heparin/HBGF interaction provides a breakthrough for such inefficient delivery. Jeon *et al.* used a heparin-conjugated PLGA nanoparticle, loaded with bFGF, and dispersed in fibrin gel. The release period of bFGF from the heparinized PLGA nanoparticle was sustained for 21 days [50]. Moreover, the nanoparticles dispersed in fibrin gel released bFGF for a more prolonged period. In the release profiles, no initial burst of release was observed and long-term and almost zero-order release behavior was presented. Compared with the short-term release period of bFGF from fibrin gel, the release behavior was derived from the presence of heparin, which enables binding specifically with bFGF. Another example obviously supported the potential of heparin for sustained release due to the heparin/HBGFs interaction. In the study mentioned above in Section 3, Yuk *et al.* investigated the release behavior of lysozyme and VEGF from heparinized PLGA nanoparticles. In the release profiles of lysozyme, increases in heparin content induced sustained release of lysozyme from 6 days up to 20 days. In the release profiles of VEGF, however, the release periods were prolonged up to about 40 days and the release behavior was almost zero-order release mode. These results demonstrate that the release of HBGFs can be sustained due to the binding affinity of heparin. Park and co-workers developed a nanoparticle of Tetronic–PCL–heparin (TCH) conjugate as a type of self-assembled micelle for the sustained delivery of HBGFs with heparin-binding domain in tissue engineering fields [53]. The structure of Tetronic is very similar to Pluronic, which has poly(propylene oxide) and poly(ethylene oxide) [77]. Pluronic block copolymers have been widely studied for pharmaceutical and biomedical applications, as reviewed recently [78-80]; are water soluble and exhibit low toxicity; and certain molecular weights have been approved by the FDA for use in the human body [81]. As the TCH conjugate was dissolved in water, it spontaneously formed micelles through intermolecular hydrophobic interactions. The immobilization of heparin led to an increase in binding of bFGF more than twofold in comparison of Tetronic–PCL (TC). The bFGF release from the TCH micelle was sustained with zero-order over two months. Free bFGF, which is weakly bound onto the surface of micelles through non-specific interaction, is easily participated by protein aggregation mechanism [82]. Because of this phenomenon, free heparin was added to TC solution. As shown in **Figure 4A**, the bFGF that interacts with heparin had a more sustained release behavior (b) in comparison with free heparin–bFGF union (a). Park and colleagues investigated release profiles of another drug along with bFGF by perceiving the potential of the TCH micelle for incorporating hydrophobic drugs [55,56]. The spontaneous release profiles of both drugs from a matrix of TCH nanoparticle was investigated as shown in **Figure 4B**. The release profile of indometacin shows significant initial burst and a relatively faster rate, whereas that of bFGF exhibits no initial burst and a more sustained period. A similar trend of results was also shown in another study. A heparin-bound cylindrical nanostructure showed no initial burst and sustained release of FGF-2 as compared with a phosphate gel matrix [65].

5. Potential applications

The delivery of growth factor for tissue regeneration would induce *in vitro* and *in vivo* attachment, proliferation and differentiation of various cells [83]. Released growth factors exert their action by binding to cell surface receptors as well as their diffusible gradients around the boundary of carrier [84]. Therefore, applications of growth factor delivery are dependent on cellular types and the site to be regenerated. In this section, three potential applications using growth factor delivery for tissue regeneration are described, containing regenerations of bone, nerve and neo-vasculature.

5.1 Bone regeneration

A primary goal in bone regeneration is to accelerate the process of bone repair. Naturally occurring bone repair generally proceeds via three successive phases: the inflammatory, chondrogenic and osteogenic phases [85]. The TGF family plays an important role in bone formation associated with the proliferation and differentiation of osteoblast. BMPs, members of the TGF superfamily, appear to act as differentiation factors, causing mesenchymal stem cells to differentiate into bone-forming cells. Other growth factors such as bFGF and PDGF are also involved in bone regeneration through the proliferation of osteoblasts [2]. Most applications focus on dose-dependent synergistic effects by the delivery of multiple growth factors. The success of clinical trials is significantly related to both the mode of growth factor delivery and the requirements for multiple signals to drive the regeneration process to completion [86]. Schmidmaier *et al.* reported that the combined treatment of IGF-I and TGF-β1 showed a

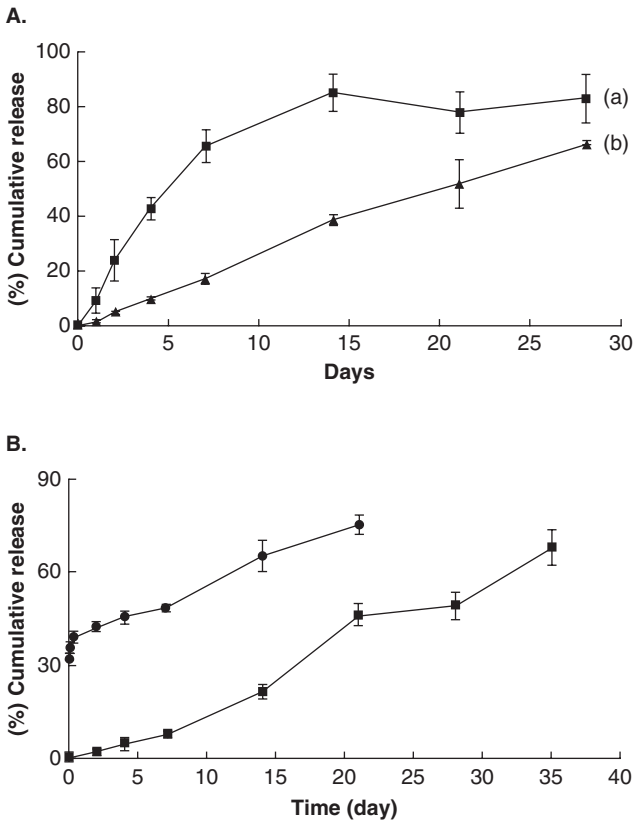

Figure 4. A. The cumulative release profile of bFGF is illustrated. The micelle solution was placed into a dialysis bag and then immerged in a large vial containing warmed PBS (pH 7.4, 37°C). Samples: (a) bFGF-loaded Tetronic-PCL micelle + Heparin; and (b) bFGF-loaded Tetronic–PCL–Heparin micelle. **B.** Cumulative total drug release profiles of dual drug-loaded TCH micelles. IMC (•) and bFGF (■) were released from dual drug loaded TCH micelles (n = 3, mean ± SD).

synergistic effect on fracture healing in a rat tibia fracture model [87]. Simmons *et al.* showed that dual delivery of BMP-2 and TGF-β3 could promote significant *in vivo* bone formation [88]. However, the combinative therapy of growth factors should be carefully chosen because it may cause an inhibitory effect on the healing process [89].

5.2 Angiogenesis

Angiogenesis, the development of new blood vessels out of existing ones, is a promising treatment strategy for various ischemic diseases such as cardiovascular diseases and stroke. Although a number of growth factors such as bFGF, HGF, PDGF and VEGF are known as an angiogenic factors, it is difficult to effectively modulate the growth factors due to the complex process of angiogenesis that is regulated by pro- and anti-angiogenic growth factors [8]. Among the growth factors, VEGF is highly specific to the endothelium, and thus *in vitro* and *in vivo* trials with VEGF have been constantly used to stimulate angiogenesis. Interestingly, dual growth factor delivery in angiogenesis can promote the formation of new blood vessels. PDGF promotes vessel maturation via the recruitment of smooth muscle cells to develop endothelium, while VEGF is able to initiate neo-vascularization [90].

5.3 Nerve regeneration

The process of nerve injury and regeneration involves many interactions between cellular elements and extracellular matrix (ECM) [91]. NGF as a neurotrophic factor influences the survival of neuronal cells and regeneration of peripheral nerves, including neurodegenerative diseases such as Alzheimer's disease or Parkinson's disease. Especially, NGF exerts biological effects on small, medium size primary sensory neurons and sympathetic neurons [92]. Although there are few studies on NGF delivery by polymeric particulates, there have been continuous efforts to recover and alleviate the function of impaired nerve. Nerve guidance channels (NGCs) have shown a little promise in promoting regeneration after spinal cord injury. Axonal regeneration through NGCs, tissue integration, and consequently functional recovery can be improved by the delivery of neurotrophic factors. However, the impact of neurotrophic factors when combined with NGCs has not been maximized due to the lack of an appropriate drug delivery system. Treating the NGF-loaded PLGA microspheres in the lumen of NGCs allows for localized and sustained delivery of NGF, which makes it possible to enhance the nerve repair [93].

6. Conclusions

Heparin-containing particulates have been developed in various types by different methods for the local delivery for tissue regeneration, as well as systemic delivery via i.v. administration for other diverse applications. Many examples given in this article suggested that utilizing heparin in particulate systems could accomplish controlled release of HBGFs containing a sustained long-term release period, zero-order release behavior, or no initial burst.

7. Expert opinion

Growth factor delivery is a therapeutically powerful approach that has been applied to regenerative applications, such as bone regeneration, angiogenesis, nerve regeneration and wound healing. The therapeutic success of the treatment of growth factors depends on the optimal concentration of a required growth factor at suitable stages and sites in the process of tissue regeneration. Although various delivery technologies are providing solutions, controlled and local delivery technology of growth factors using polymeric particulates may be chosen as just candidates in each case. The growth factor-containing particulate carrier can not only be directly administered to the site of action, but can also be embedded into a polymeric matrix for implantation.

In these applications, most researchers suggest that controlled delivery of growth factors is an indispensable strategy for continuously stimulating cell growth in the site to repair. Relating to this, heparin encouraged us to study controlled delivery of HBGFs based on their specific affinity. Most biodegradable materials developed to date for growth factor delivery have exhibited an initial burst and fast rate of release, as reported in many examples of this article. The release behavior of growth factors is decided by diffusion due to the water invasion and physical erosion of polymeric matrix. However, a few examples of heparin-containing materials showed opposite results, suggesting a clue to the solution of such problems. The release mechanism is not yet clear, although it was estimated to be derived from changes in physiological conditions, which should be supported by interdisciplinary research into heparin.

Tissue engineering is still maturing, and it may take more time to obtain the satisfactory therapeutic effects and safety that is required for clinical applications. Growth factor delivery strategies using polymeric carriers will be helpful to stimulate clinical trials. However, along with further studies related to local concentration and the activity around the tissue, the development of novel bioactive materials to deliver growth factors in a controlled manner will be also required. Embedding heparin in particulate systems accompanies chemical or conformational variation of the structure, resulting in a loss of the inherent activity of heparin. To minimize the loss of activity, physical interactions are more favorable than chemical conjugation, although those weaken binding affinity. The location of embedded heparin within the particulate matrices also affects the release behavior. These problems are dependent on the design, process and technology of heparin containing particulate formation. Therefore, novel particulate systems are required to efficiently encapsulate growth factors as well as to minimize loss of heparin activity. In addition, the stability of growth factors should also be evaluated after particulate formulation.

Various biological activities of heparin, such as anti-coagulant [94], anti-inflammatory [95], anti-adhesive [96], antimetastatic [97] or anti-allergic activities [98] are derived from the specific interactions with some designated proteins. Such proteins include adhesion molecules, chemokines, enzymes and cytotoxic mediators except growth factors. Utilizing heparin as anticoagulant has already been opened up and developed in many studies, even in clinical uses. As described in this article, many studies on growth factor delivery using heparin are now being actively performed. To our knowledge, however, applications involved in antimetastatic activity for cancer therapy has hardly been exploited, although some references in the initial stage were found. Our prospect for this emerging field is that this study is very promising and has significant potential for anticancer therapy.

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Declaration of interest

The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

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