Review

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# Affinity-Based Drug Delivery Systems for Tissue Repair and Regeneration

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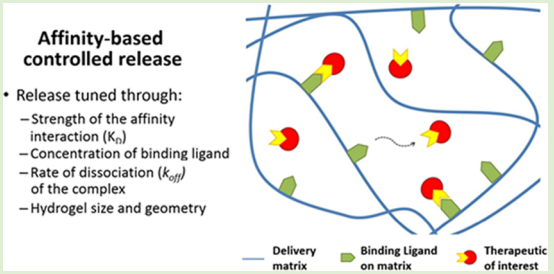
**ABSTRACT:** Affinity-based release systems use transient interactions to sustain and control the release of a therapeutic from a polymeric matrix. The most common affinity-based systems use heparin-based scaffolds to sustain the release of heparin-binding proteins, such as fibroblast growth factor-2 (FGF2) and vascular endothelial growth factor (VEGF). However, novel affinity-based systems based on, for example, protein−protein or DNA−protein interactions, are emerging to control the release of an expanding repertoire of therapeutics. Mathematical models of affinity-based systems have provided a thorough understanding of which parameters affect release rate from these systems, and how these release rates can be tuned. In this review, recent affinity-based release systems will be described, including an overview of the various types of affinity interactions used to modulate release, the mechanisms by which release from these systems is tuned, and the time scales of sustained release. This advanced drug delivery paradigm provides tunable and predictable release rates and has expanded the scope of deliverable therapeutics for tissue repair and regeneration.




## 1.0. INTRODUCTION

Local delivery strategies, which deliver therapeutics directly to the site of injury, are compelling because they minimize toxicity and undesirable side effects associated with conventional systemic administration. By targeting damaged/diseased cells, a lower dose can be delivered and treatment efficacy can be improved by circumventing metabolic catabolism and clearance associated with systemic delivery.[1] Since tissue repair occurs over long time frames (days/weeks), continuous dosing at a minimum effective dose is required.[2−4] Thus, sustained release is an important component of localized delivery systems.

**1.1. Strategies to Control Release.** Several different technologies have emerged to control release of therapeutic agents including: degradation-controlled polymeric matrices (bulk or surface erosion),[5] swelling-controlled hydrogels,[6] nano/microparticles,[7,8] and affinity-based delivery systems.

*1.1.1. Degrading Systems.* Degradation-controlled polymers physically encapsulate the therapeutic of interest within a degradable polymeric scaffold. Early systems of this nature were primarily hydrophilic (e.g., polyesters) and would degrade by bulk erosion. Drug delivery from these polymers is controlled primarily by diffusion, and thus, tunable release from these polymers is difficult to achieve because release is not ultimately controlled by the rate of polymer degradation.[9] Furthermore, fast diffusional release may increase drug concentration faster than intended (dose dumping) and lead to adverse side effects. To overcome these challenges, polymers with surface erosion properties were developed. These polymers entrap drug within a hydrophobic network, which is impenetrable by water (e.g., polyanhydrides, polyorthoesters, polyphosphazenes, and others).[1] Thus, polymer degradation always occurs at the polymer−water interface, analogous to the dissolution of a bar of soap.

*1.1.2. Particle-Based Systems.* Nano- and microparticles are solid particles comprised of polymers, such as poly(lactic-*co*-glycolic acid) (PLGA) particles or inorganic materials (e.g., silica-based particles). In these systems, drug is encapsulated within the polymer and slowly released as the polymer degrades, with surface bound drug giving rise to an initial burst release. The particles can be injected either alone[10] or dispersed in a hydrogel carrier at the site of injury.[11] These systems have been investigated exhaustively for the controlled delivery of numerous therapeutics.[12] Encapsulation and release of hydrophobic drugs are relatively facile because the small molecule hydrophobic drug sequesters into the hydrophobic polymer and is often unaffected by the formulation conditions; however, encapsulation and release of bioactive hydrophilic drugs, such as proteins and peptides, are more difficult. Protein therapeutics are frequently denatured during particle preparation when they are exposed to detergents, sonication, and organic solvents and do not readily sequester with the hydrophobic polymer phase.[13]

*1.1.3. Affinity-Based Systems.* Affinity-based systems were originally inspired by the controlled release mechanisms found in our own extracellular matrix (ECM), such as the binding of heparin to numerous proteins to preserve and regulate activity. These systems use transient interactions, with strengths ranging from $10^{-4}$ to $10^{-9}$ M, between complementary binding partners to slow diffusional release of a therapeutic from a polymer

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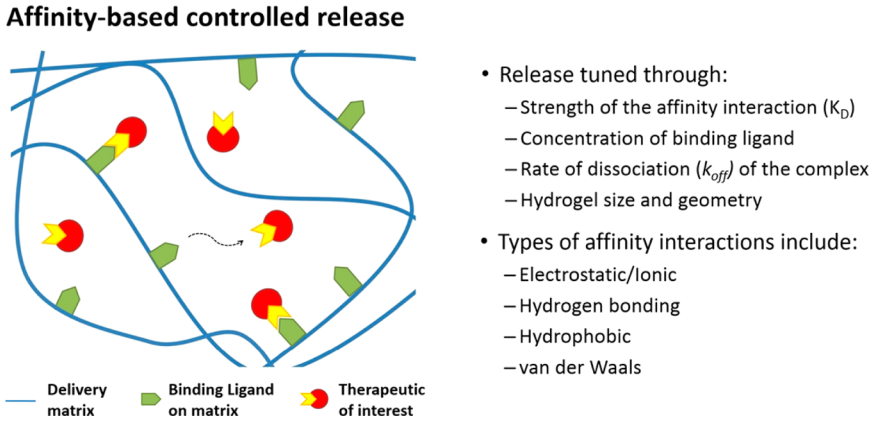


**Figure 1.** Schematic representation of a single species affinity-based release system. Release of a therapeutic of interest from a polymeric delivery matrix is attenuated by reversible interactions with the matrix-immobilized binding ligand. In some cases, the matrix itself may act as the binding ligand. The elements that can be used to tune the therapeutic release rate are summarized. The chemical nature of the interaction between the binding ligand and protein of interest can be one of (or a combination of) electrostatic/ionic, hydrogen bonding, hydrophobic, or van der Waals forces. It is important to note that the density of the binding ligand and therapeutic are not representative of all affinity-based systems.

network. Occasionally, this type of release is combined with bulk or surface eroding polymers to provide an additional mechanism of tuning release and as a mechanism to remove the material after delivery.

Affinity-based systems have emerged as attractive systems for delivery of therapeutics by circumventing common challenges of the aforementioned controlled release systems. This development has been particularly important for the delivery of hydrophilic protein therapeutics, which are fragile to the processing conditions used in formulation of other controlled release systems (e.g., organic solvents, lyophilization).[14] Affinity-based systems can prevent large burst release while providing tunable release profiles by attenuating diffusional release through transient interactions with the delivery matrix. Many affinity systems exploit the natural affinity of therapeutics (e.g., heparin-binding proteins for heparin, hydrophobic antibiotics for cyclodextrins), while other affinity systems have shown that the addition of an affinity group to the therapeutic of interest has not detrimentally affected the bioactivity of the therapeutic.[15,16] Moreover, mathematical simulations can predict release from these systems, preventing the need for trial-and-error experimentation to achieve a desired release profile.

Another review of affinity-based release gives an in-depth review on the delivery of small molecule therapeutics using molecularly imprinted polymers and cyclodextrin-based systems, as well as release of some heparin-binding proteins.[17] Our review provides both a fundamental understanding of the kinetic basis of affinity-based release and an overview of the different types of affinity-based systems used to deliver proteins, peptides, and small molecule therapeutics. We have organized the paper based on major classes of affinity interactions and we have focused on systems where release profiles were shown, thereby providing perspective on the time scale of release for each system (e.g., hours, days, or months). Detailed reviews for other types of controlled release have been recently reported elsewhere.[5,18,19]

## 2.0. MODULATING RELEASE FROM AFFINITY-BASED SYSTEMS

**2.1. Single Species Affinity Systems.** Affinity-based systems control the release of a protein or small molecule therapeutic by attenuating its diffusion through a polymeric delivery matrix by reversible association−dissociation reactions between the therapeutic and a binding ligand immobilized on the matrix, forming a temporary immobile complex (Figure 1). The dissociation kinetics of this reaction are shown in eq 1.1, where the dissociation constant ($K_D$) is proportional to the association and dissociation rates of the complex ($k_{on}$ and $k_{off}$) and the equilibrium concentrations of each species ($C_{ligand}$, $C_{therapeutic}$, $C_{complex}$).

$$\text{binding ligand} + \text{therapeutic} \rightleftharpoons \text{complex}$$

$$K_D = \frac{k_{off}}{k_{on}} = \frac{C_{ligand} \cdot C_{therapeutic}}{C_{complex}} \quad (1.1)$$

The most intuitive mechanism by which release can be tuned from this type of system is by changing the strength of the affinity interaction ($K_D$). However, several other factors also affect the time scale of protein release from affinity systems.[16,20−24]

The characteristic time scale of release ($t_{final}$) of a therapeutic diffusing from a polymeric matrix is the quotient of the square of diffusion path length ($L$, meters), divided by protein diffusivity through the polymeric matrix ($D$, meters$^2$/second), as shown in eq 1.2.

$$t_{final} = \frac{L^2}{D} \quad (1.2)$$

The manner in which the time scale of therapeutic release can be modulated within affinity systems depends on the balance of several system parameters. For example, if diffusion of the therapeutic through the polymeric matrix ($t_{final}$) is much faster than dissociation of the complex ($k_{off}$), therapeutic release will be biphasic. First, any unbound protein will be released over a time scale of $L^2/D$. Next, the remaining bound protein will be released as it unbinds from the matrix over a time scale of $1/k_{off}$.[16,24] In this type of affinity system, the amount of therapeutic released during the initial diffusional burst may be tuned by varying the amount of free therapeutic at equilibrium, that is, by either changing the $K_D$ of the binding pair or the starting concentrations of binding ligand and therapeutic. The subsequent phase of release is dictated by the dissociation of the therapeutic-binding ligand complex. Changing the nature of

an affinity interaction (i.e., the $k_{off}$) is far more involved than modulating individual system parameters, such as $K_D$ and concentration. For this reason, it is usually more desirable to use a system in which protein diffusion through the polymer matrix ($t_{final}$) is slower than the unbinding rate ($k_{off}$) so that the overall protein release is controlled by the affinity interaction. Most affinity systems can be described by the single time scale of release shown in eq 1.3. For these systems, diffusional release is attenuated by the term $(1 + C_{bindingligand}/K_D)$, which means the concentration of therapeutic itself is irrelevant to the rate of therapeutic release. Rather, it is the ratio of the concentration of binding ligand ($C_{ligand}$) to the $K_D$, which is important.

$$t_{final} = \frac{L^2\left(1 + \frac{C_{ligand}}{K_D}\right)}{D} \quad (1.3)$$

In less common cases, when $C_{therapeutic} \gg C_{ligand}$, a biphasic release profile is obtained where the diffusional release is initially attenuated by the term $(1 + C_{bindingligand}/C_{protein})$ until the therapeutic concentration drops to $(k_{off}/k_{on})$ and release is attenuated by the term $(1 + C_{bindingligand}/K_D)$. In these systems, the concentration of therapeutic in the system can be modulated to tune the first phase of release, but is again irrelevant in the second phase of release. We have previously provided a comprehensive discussion of the characteristic regimes of therapeutic release from affinity-based systems including general guiding equations.[16]

It is important to note that for all affinity-based systems, the concentration of the binding ligand must exceed the $K_D$ to achieve controlled release. If the concentration of the binding ligand is below that of the $K_D$, there is no driving force to form an immobilized complex and therapeutic release will be diffusional ($L^2/D$). This must be considered when using weak affinity binding pairs or when the amount of binding ligand immobilized to the matrix is low. Finally, when all other system parameters are held constant, the release rate will change if the overall geometry of the polymeric matrix is changed (e.g., from cylindrical to conical or spherical where the diffusive path length is maintained). A scenario such as this one may arise when affinity systems are used in vivo versus in vitro, thereby potentially providing some insight into the discord often observed between in vivo and in vitro results.

**2.2. Multiple Species Affinity Systems.** Another affinity system, such as the one utilized by Sakiyama-Elbert and co-workers, has a more complex, multicomponent equilibrium. This dual affinity system has also been mathematically modeled to improve our understanding of which parameters modulate release.[25−29] In their system, heparin-binding peptides (HBP) immobilized to a fibrin matrix bind to soluble heparin (HP), which in turn binds soluble heparin-binding proteins (HBPro, Figure 2).

Four reversible reactions are possible in this multicomponent system, forming three immobilized species and one freely diffusible species:

$$HBP + HP \rightleftharpoons HBP{\cdot}HP_{immobilized}$$

$$HBP{\cdot}HP + HBPro \rightleftharpoons HBP{\cdot}HP{\cdot}HPro_{immobilized}$$

$$HP + HPro \rightleftharpoons HP{\cdot}HPro_{diffusible}$$

$$HBP + HP{\cdot}HBPro \rightleftharpoons HBP{\cdot}HP{\cdot}HPro_{immobilized}$$

HBPros are diffusible in two forms: alone, or bound to heparin. A mathematical model developed by Taylor and co-workers

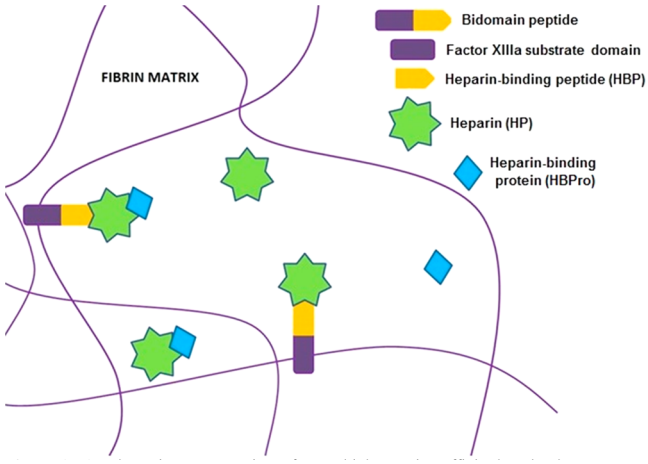


**Figure 2.** Schematic representation of a multiple species affinity-based release system. Heparin-binding peptide (HBP) is immobilized to a fibrin matrix through a Factor XIIIa substrate domain. The immobilized HBPs reversibly bind soluble heparin (HP), which in turn reversibly binds heparin-binding proteins (HBPro) to control protein release from the fibrin matrix. Adapted from ref 26, Copyright 2000, with permission from Elsevier.

was used to determine the optimal ratio of heparin to heparin-binding protein (in this case, neurotrophin-3, NT-3), which minimized the amount of freely diffusible protein in the system.[28] Figure 3 shows the biphasic nature of total bound NT-3 in this dual-affinity system. In this simulation, peptide and NT-3 concentrations were held constant and heparin concentration was varied. In this figure, NT-3 is completely

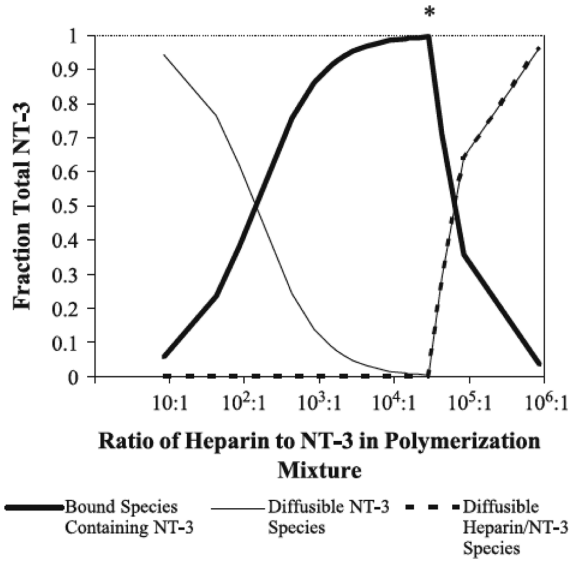


**Figure 3.** Bound therapeutic in multiple species affinity system exhibits a biphasic nature. The fraction of total therapeutic (NT-3) that is bound (thick black line) is compared to freely diffusible NT-3 (thin black line) and freely diffusible heparin-NT-3 complex (dotted black line). For a constant amount of NT-3 and heparin-binding peptide, the amount of immobilized NT-3 increases until the heparin/heparin-binding peptide concentration is equal. Once the concentration of heparin exceeds that of the immobilized heparin-binding peptides, the excess free heparin competes with heparin-NT-3 for binding sites and a diffusible heparin-NT-3 species is formed. Reprinted from ref 28, Copyright 2004, with permission from Elsevier.

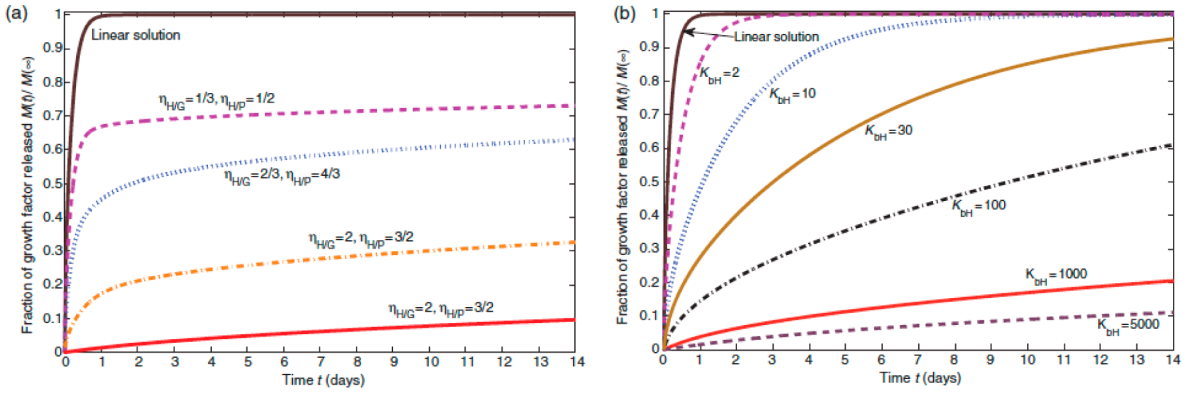


**Figure 4.** Tunable release from a multicomponent affinity release system is dependent on the relative concentrations of each species. (a) Concentrations of HBP and HP exceed the $K_D$ of the HBP/HP and HP/HBPro, respectively. In this scenario, small changes in the ratios of HP to HBP ($\eta_{H/P}$) and HP to HBPro ($\eta_{H/G}$) greatly impact the observed release rate. (b) Only the concentration of HBP exceeds the $K_D$ of the HBP/HP. In this scenario, large excesses of protein are required to tune release where $K_{bH}$ is the quotient of the initial concentration of HP in the system and the $K_D$ of the HP/HBPro interaction ($C_{heparin,0}/K_{D\ HP/HBPro}$). Reprinted from ref 29, Copyright 2012, by permission of Oxford University Press.

immobilized when the fraction of total NT-3 is 1 (indicated by * in Figure 3). This corresponds to a heparin-binding peptide to heparin ratio of 1:1, and in this case, a heparin to NT-3 ratio of 30000:1. Once the concentration of heparin begins to exceed the concentration of heparin-binding peptide, free heparin competes with the heparin-NT-3 complex to bind peptide, and NT-3 becomes freely diffusible in the form of a heparin-NT-3 complex. This relationship was verified for peptides with weaker binding affinities.[27]

In 2012, Vo and Meere used dimensionless and asymptotic analysis to model this complex system and achieve a broader understanding of the predominant factors governing release. Both affinity interactions within this system are very strong ($K_{D\ heparin/heparin\text{-}binding\ peptide} = 8 \times 10^{-8}$ M and $K_{D\ heparin/heparin\text{-}binding\ proteins} \sim 10^{-6}$–$10^{-8}$ M), and protein diffusion (as opposed to complex dissociation) is usually the limiting factor from these systems. Two characteristic scenarios can describe passive release from this system:

(1) When the concentration of HBP and HP is greater than the $K_D$ of the HBP-HP and HP-HBPro interactions, large excesses of HBP are not required to achieve tunable release (Figure 4a).

(2) When the concentration of immobilized HBP is greater than the $K_D$ of the HBP-HP interaction, but the concentration of HP is *not* greater than the $K_D$ of the HBPro interaction, and the ratio of HP to HBPro $\gg 1$, release can be controlled using high excesses of HP (Figure 4b).

Additionally, Vo and Meere cautioned that it is important to consider the possibility that not all of the heparin-binding peptide present in the system is immobilized through polymerization. In this case, any free HBP in solution that is bound to HP and HBPros will increase the rate of protein release.

In summary, affinity controlled release systems offer many versatile methods for tunable release. Therapeutic release can be kinetically (e.g., by $k_{off}$) or diffusion-controlled, and can be mono- or biphasic in nature. Two or three component affinity systems are equally tunable by modulating the $K_D$ or $k_{off}$ of the affinity interaction, the overall geometry of the system, $C_{ligand}$ and in select circumstances $C_{therapeutic}$ within the system. Mathematical modeling of these systems has provided important guiding insights for researchers in this field.

## 3.0. AFFINITY-BASED DELIVERY SYSTEMS

**3.1. Electrostatic Interactions: Heparin-Based, Heparin-Mimetic, and Other Nonheparin Systems.** Many affinity-based systems utilize electrostatic interactions to control release, such as heparin-based, heparin-mimetic, and other nonheparin systems. Seminal work in this area used heparin to control the release of heparin-binding proteins given that heparin, a highly sulfated glycosaminoglycan (GAG), is known to bind and stabilize numerous protein therapeutics with medium to high affinity ($K_D$ of $10^{-6}$–$10^{-9}$ M). This area of research is rapidly growing and in-depth discussion of heparin-based systems can be found in other recent reviews.[30,31] Herein we highlight the pivotal heparin research followed by a description of several other heparin-based and then other electrostatic systems.

*3.1.1. Heparin-Based Systems.* Heparin-based systems are inspired by the natural extracellular matrix (ECM), which sequesters and controls the release of numerous proteins in vivo. Initially, it was thought that proteins bind heparin via nonspecific electrostatic interactions; however, recently it was discovered that specific carbohydrate units within heparin are responsible for protein binding and regulation. Similarly, proteins that bind heparin have consensus sequences comprised primarily of cationic (e.g., lysine and arginine) and neutral (either hydrophilic or hydrophobic) residues.[32] This binding interaction has been exploited by either directly or indirectly (e.g., through physical interactions with heparin-binding peptides) conjugating heparin to a polymeric delivery matrix. We will describe a variety of recent heparin-based systems, focusing on systems where the release of therapeutics was explored.

An early affinity-based delivery system was conceived to stabilize a newly discovered protein, basic fibroblast growth factor (bFGF or FGF-2), which has shown beneficial effects for tissue repair by stimulating cell growth. Growth factors are generally extremely potent, but also extremely fragile, degrading rapidly in vivo. To effectively preserve and control release of this potent mitogen, Edelman et al.[33] first used ethylene-vinyl acetate copolymer (EVAc) matrices. However, when cast in EVAc matrices, 99% of bFGF bioactivity was lost. Protein adhesion to glass or synthetic polymer vessels was partly to blame for this; however, it was found that heparin was also required to preserve bFGF activity. Edelman and co-workers

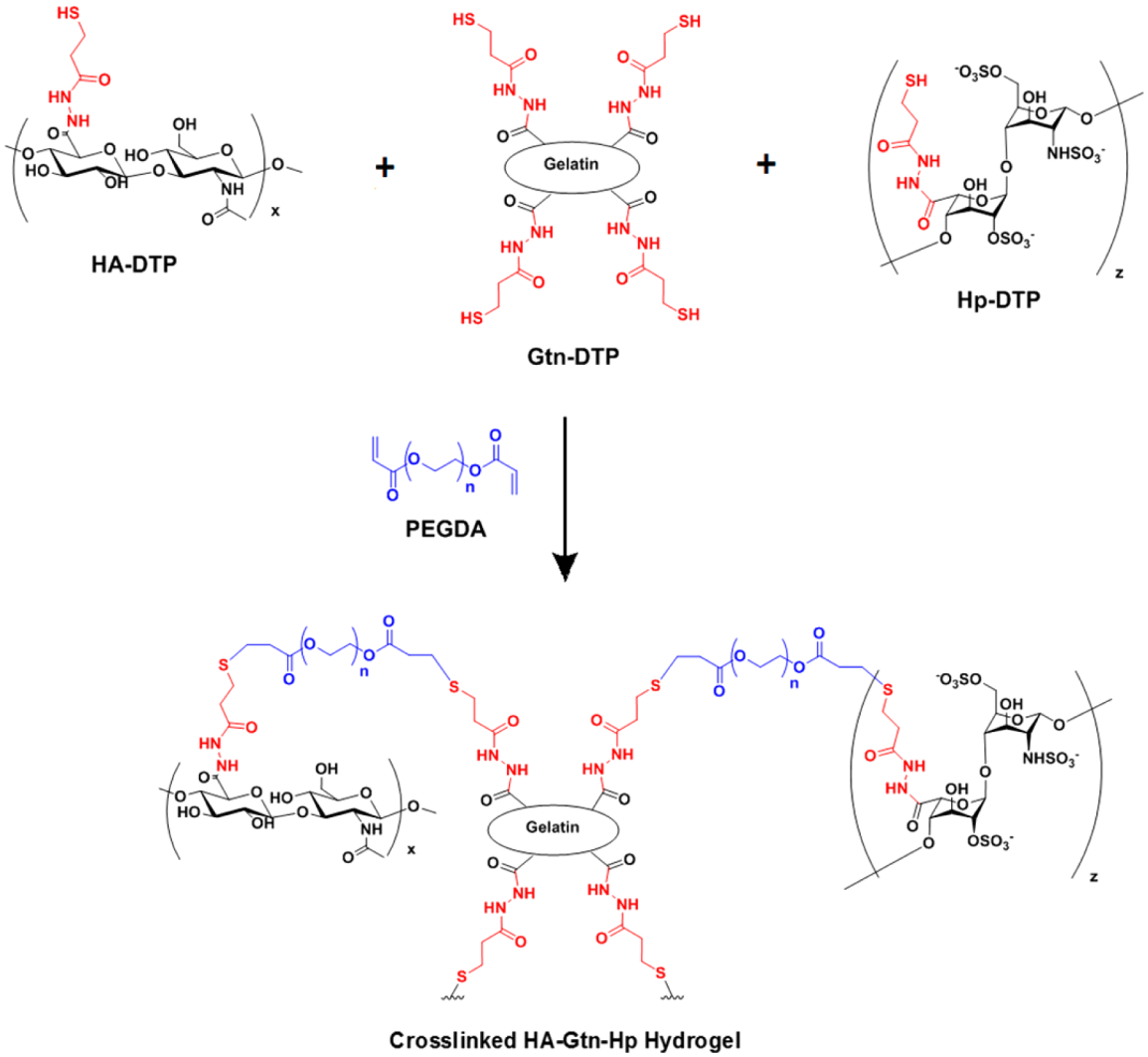


**Figure 5.** Hyaluronan-gelatin-heparin (HA-Gtn-Hp) cross-linked hydrogels can be used to sustain the release of heparin-binding proteins. Each monomer unit is functionalized with thiols and cross-linking occurs via conjugate addition to PEG-diacrylate (PEDGA).[36,38] Adapted from ref 38, Copyright 2010, with permission from Elsevier.

made the important observation that this growth factor was degraded (4% remained active) after exposure to dichloromethane vapors used during scaffold processing, even in the presence of heparin. This motivated the use of a biocompatible polymer for the delivery of protein therapeutics. EVAc was replaced with heparin-functionalized Sepharose beads encapsulated in alginate microspheres, which were able to sustain delivery of bFGF for 24 days. Release could be modulated by adding either heparinase, to cleave heparin from the solid bead support, or by increasing the ionic strength of the buffer, which weakened the heparin−bFGF interaction and released bFGF. Both methods used to tune release were external, as opposed to internal mechanisms of tunability.

The Prestwich lab expanded the repertoire of deliverable HBPros and also explored how release is tuned by modulating properties within the system. In a preliminary study, two GAG components of the natural ECM, hyaluronan (HA), and chondroitin sulfate (CS), along with heparin, were cross-linked via Michael addition to a polyethylene glycol diacrylate (PEGDA), to sustain the release of bFGF for ultimate applications in tissue regeneration.[34] Carboxylate groups of HA, CS, and heparin were activated with 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDCI) and conjugated to a linker containing reactive thiols for further conjugation. Various combinations of these thiolated components were then polymerized with PEGDA to form bFGF-loaded hydrogel matrices. All of the hydrogels cross-linked with heparin reduced the release of bFGF compared to nonheparin-functionalized controls and bFGF release was sustained for more than 35 days. As expected, as the amount of immobilized heparin increased, the release rate decreased. To confirm that the release of bFGF was attenuated by its affinity for matrix-bound heparin, hyaluronidase was added to all gels. Only in the control group, HA-PEG, did the rate of release increase, which confirmed that bFGF affinity for matrix-bound heparin (and not interaction with HA) was responsible for attenuated release from heparin gels. However, diffusional release of bFGF from CS-PEG gels and HA-PEG gels took 28 days, which suggested that other factors, such as steric hindrance or small pore size, also impeded diffusional release from the matrix and were contributing factors to the observed release profile. The authors cautioned that growth factors containing free thiols might be unintentionally immobilized to the delivery matrix during polymer cross-linking with PEGDA. Nevertheless, when

Table 1. Summary of Heparin-Based Affinity Release Systems That Control the Release of Heparin-Binding Proteins

| matrix | protein delivered | time of release | reference |
|---|---|---|---|
| ethylene-vinyl acetate copolymer (EVAc) or alginate microspheres embedded with heparin-sepharose beads | bFGF | 24 days | Edelman et al.[33] |
| hyaluronan/PEG/heparin | bFGF | 35 days | Cai et al.[34] |
| hyaluronan/PEG/heparin/gelatin | bFGF, VEGF | 42 days | Pike et al.[35] |
| | VEGF, bFGF, TGF$\beta$, KGF, Ang1, PDGF | 42 days | Peattie et al.[36] |
| PLGA/heparin | bFGF | 30 days | Chung et al.[39] |
| | VEGF | 40 days | Chung et al.[40] |
| PEG/heparin | bFGF | 7 days | Nie et al.[43] |
| | VEGF | 20 days | Tae et al.[41] |
| PEG/PCL/heparin | bFGF | 56 days | Lee et al.[42] |
| PCL nanofibers/heparin | VEGF | 15 days | Lu et al.[44] |
| peptide amphiphile/heparin | bFGF | 10 days | Rajangam et al.[45] |
| chitosan/alginate/heparin | bFGF | 3 days | Ho et al.[46] |
| demineralized bone matrix/heparin | VEGF | 3 days | Chen et al.[47] |
| fibrin/heparin-binding peptides/heparin | NGF | 14 days | Sakiyama-Elbert, Hubbell[48] |
| | | 7 days | Wood, Sakiyama-Elbert[27] |
| | GDNF | 14 days | Wood et al.[51] |
| | NT-3 | 14 days | Taylor et al.[28,53] |
| | NT-3, PDGF, SHH | 9 days | Willerth et al.[53] |
| fibrin/heparin-binding peptides/heparin and PLGA nanofibers | PDGF | 7 days | Manning et al.[49] |

examined in a subcutaneous mouse model of wound healing, gels containing heparin and bFGF stimulated angiogenesis more efficiently than gels without bFGF and/or heparin.

The next generation of this system investigated whether lower amounts of heparin (0.03, 0.3, and 3%) could attenuate release of bFGF or vascular endothelial growth factor (VEGF), another HBPro.[35] Thiolated derivatives of HA, gelatin (Gtn), and heparin were copolymerized with PEGDA (Figure 5) and growth factor release was studied over 42 days. Upon completion of the study, VEGF release from HA-PEG and HA-gelatin-PEG gels, and bFGF release from HA-PEG gels only, reached 50%. The addition of gelatin to HA-PEG gels resulted in complete bFGF release in 42 days. This was hypothesized to be due to the decreased steric interaction from gelatin versus HA. A trend was observed where, as the heparin concentration increased, the release of VEGF or bFGF decreased. The 0.3% heparin-containing gels were selected to investigate the release of other angiogenic growth factors, including transforming growth factor $\beta$ (TGF$\beta$), keratinocyte growth factor (KGF), angiopoietin-1 (Ang1), and platelet-derived growth factor (PDGF).[36] Each protein had a characteristic release profile and the total amount of protein released at the end of 42 days varied greatly between growth factors (e.g., 90% for bFGF and 1.8% for PDGF). Curiously, there was no trend based on molecular weight or affinity for heparin. This may be due to perturbations in the ability of heparin to bind growth factors after cross-linking.[37] It may also be due to nonspecific or specific adsorption of the growth factors to HA. Ideally, the polymeric matrix would be inert and cross-linking chemistry would be bio-orthogonal to avoid denaturing or immobilizing the deliverable therapeutic.

Biocompatible synthetic polymer matrices have also been functionalized with heparin for protein delivery. Similar EDCI coupling chemistry was used by Chung et al. to immobilize heparin to PLGA microparticles.[39,40] After particle formation, terminal carboxylic acids on PLGA were functionalized with an amine linker, which subsequently was covalently bound to the carboxylic acids of heparin, and both reactions were completed via EDC/*N*-hydroxysuccinimide (NHS) chemistry. Nanoparticles were loaded with either VEGF or bFGF using a "solution dipping method". Cumulative VEGF release was linear, reaching approximately 90% over 40 days. In accordance with the guiding principle that the concentration of the therapeutic does not affect the observed rate of release (discussed in section 2.1), the concentration of VEGF delivered did not impact its release rate. Heparin-functionalized nanoparticles also achieved five times higher drug loading compared to nonfunctionalized nanoparticles and effectively decreased the burst release from 60 to 40%. Synthetic polymers, such as polyethylene glycol (PEG)-based matrices, have also been used to deliver HBPros such as VEGF and bFGF. Hydrazide-functionalized heparin was cross-linked with bis-NHS activated PEG and provided near zero order release of VEGF.[41]

By modulating the amount of immobilized heparin to polymeric micelles of Tetronic (block copolymers of poly(ethylene) oxide and poly(propylene) oxide) functionalized with polycaprolactone (PCL) and heparin, tunable release of bFGF was achieved.[42] Star-PEG matrices were functionalized with heparin using maleimide−thiol chemistry to deliver bFGF in a controlled manner.[43] Burst release was minimized and release was tuned by increasing the concentration of heparin within the system. Other materials such as peptide amphiphile nanofibers,[44,45] chitosan-alginate polyelectrolyte complexes,[46] and decellularized scaffolds[47] have also been functionalized with heparin to achieve controlled release. Tunable release was primarily achieved by varying the concentration of heparin because modulating the binding kinetics of the heparin−HBPro interaction is difficult. It is important to note that the type of covalent bond formed between heparin and the scaffold affects HBPro binding affinity.[37] Many groups have used heparin to maintain the activity of therapeutic proteins mostly in applications for tissue regeneration and repair; however,

many in vitro reports are either missing the proper controls or use incompletely defined experiments. An ideal system would be well-defined where unintended determinants of release are excluded, such as inadvertent protein immobilization or adsorption and steric hindrance to diffusion.

Another type of heparin-based system is the three-component, dual affinity system developed by Shelly Sakiyama-Elbert and Jeffrey Hubbell, as previously mentioned in section 2.2.[48] This complex system uses two affinity interactions to control the release of heparin-binding proteins from fibrin gels: a fibrin matrix functionalized with heparin-binding peptides binds heparin which in turn binds heparin-binding proteins.[25,27,28,48−51] Controlled release for over 14 days was first demonstrated with nerve growth factor (NGF).[48] To provide a mechanism with which to tune release of NGF, a library of heparin-binding peptides with a range of heparin-binding strengths was synthesized. Peptides with stronger affinities for heparin gave slower rates of release.[25] Furthermore, a biphasic effect due to the ratio of heparin to heparin-binding peptide was observed[27] due to the dual affinity interaction of the system, as described in section 2.2.

Another way to tune release is by varying heparin-binding peptide to heparin ratios. Tunable, controlled delivery of other factors such as glial-derived neurotrophic factor (GDNF), NT-3, PDGF, and sonic hedgehog (SHH) were achieved this way. In another example, the fibrin matrix was combined with a PLGA nanofiber scaffold to provide support, and PDGF was released for use in tendon repair.[49] An inherent limitation is the difficulty associated with using heparin itself and ability to only deliver heparin-binding proteins; however, since numerous proteins bind heparin, an opportunity for future research would be concomitant delivery of multiple HBPros from the system. The dual affinity system has been used extensively for in vitro cell studies and in vivo for applications in spinal cord regeneration or tendon repair. Encouragingly, no harmful side effects from the use of heparin, such as heparin-induced thrombocytopenia,[52] have been observed. Table 1 summarizes the work presented in this section.

*3.1.2. Heparin-Mimetic Systems.* Heparin-mimetic materials also aim to mimic the native ECM by presenting heparin-mimetic sulfate groups to bind heparin-binding proteins. For example, Mammadov and co-workers used heparin-mimetic peptides, which self-assemble to form an amphiphile nanofiber hydrogel, to deliver VEGF for 7 days.[54] Release was tuned by changing the chemical functionality (sulfonate vs carboxylic acid) of the heparin-mimetic peptides, which modulated binding affinity to VEGF. In another approach, Freeman and co-workers sulfated an alginate matrix to bind heparin-binding proteins. Surface plasmon resonance (SPR) was used to confirm the proteins bound the new material. Interestingly, the binding constants for numerous proteins were similar to those for heparin and sometimes the proteins bound more strongly to sulfated-alginate than to heparin.[55,56] Another interesting observation was that the concentration of bFGF had an impact on the release rate from sulfated alginate microspheres.[56] Based on the understanding of affinity release (presented in section 2.1), this suggests that an additional interaction is affecting release. This is further supported by the fact that these release profiles were not characteristic of Fickian diffusion.[57] Freeman codelivered three factors from sulfated alginate hydrogels: VEGF, PDGF, and TGF$\beta$1, each of which had a characteristic release profile from the gel. They observed that the delivery rate of VEGF was the same whether delivered separately or concomitantly with PDGF and TGF$\beta$1.[55] Release of hepatocyte growth factor (HGF) was also achieved using sulfated-alginate matrices for 7 days.[58]

Others have used sulfated glycosaminoglycans to control the release of chemokines such as RANTES or stromal cell derived factor 1$\alpha$ (SDF-1$\alpha$). A human immunodeficiency virus (HIV) therapeutic 5P12-RANTES is a chemokine ligand (CCL5) that binds the HIV receptor CCR5, leading to internalization that effectively prevents HIV binding and infections. This chemokine was delivered using heparin or chondroitin sulfate based hydrogels via the interaction of RANTES (CCL5) to these sulfated polymers.[59] Characteristic release from each hydrogel was determined by the strength of the affinity interaction. Release was also tuned by increasing the heparin content of the hydrogels, which slowed the release of 5P12-RANTES through the hydrogel. The Burdick group incorporated sulfated HA macromers through radical-mediated cross-links to form sulfated HA hydrogels, from which they were able to control release of stromal cell derived factor 1$\alpha$ (SDF-1$\alpha$).[60] While sulfated materials are relatively simple to produce and provide an effective means to control release, achieving tunable release from these matrices is generally challenging because it is difficult to control factors that impart tunability, such as ligand concentration, $K_D$ or $k_{off}$. These systems are summarized in Table 2.

**Table 2. Affinity-Based Release Systems Mimic Heparin to Control the Release of Heparin-Binding Proteins**

| matrix | protein delivered | time of release | reference |
|---|---|---|---|
| sulfated alginate | bFGF | 5 days | Freeman, Cohen[56] |
| | VEGF, PDGF, TGF$\beta$1 | 8 days | Freeman et al.[55] |
| | HGF | 7 days | Ruvinov et al.[58] |
| peptide amphiphile nanofiber hydrogel | VEGF | 7 days | Mammadov et al.[54] |
| heparin or chondroitin sulfate | 5P12-RANTES | 25 days | Wang et al.[59] |
| sulfated hyaluronan hydrogel | SDF-1$\alpha$ | 12 days | Purcell et al.[60] |

*3.1.3. Other Electrostatic Interactions.* Frequently, recombinant proteins are expressed with a Histidine tag (6xHis) for purification from cell lysate using Nickel affinity chromatography. Lin and Metters developed a drug delivery system based on this interaction. Because most proteins are expressed with His tags, no further modification to protein drugs is required. In the presence of transition metal ions, such as $Ni^{2+}$, $Cu^{2+}$, and $Zn^{2+}$, iminodiacetic acid (IDA) will bind His tags with an affinity of $\sim 10^{-4}-10^{-6}$ M. The polymer delivery matrix of PEG diacrylate was cophotopolymerized with glycidyl methacrylate-iminodiacetic acid (GMIDA) to form a hydrogel capable of stabilizing encapsulated proteins during release (preventing conjugation to the hydrogel) and subsequently controlled their release. This was first investigated using the model protein, bovine serum albumin (BSA), which is known to bind GMIDA in the presence of copper. The release of BSA was tunable over 24 h by varying the concentration of copper[61] and the GMIDA-*co*-PEG hydrogel (with $Ni^{2+}$, $Zn^{2+}$, or $Cu^{2+}$ ion chelators) increased the total amount of BSA released. Controlled and tunable release of the model protein, His-green fluorescent protein (His-GFP), was extended to 48 h, compared to diffusive release in less than 8 h. Release was tunable by

Table 3. Summary of Affinity-Based Release Systems Discussed Which Use Ionic Interactions to Control Release

| affinity interaction | matrix | protein delivered | time scale | reference |
|---|---|---|---|---|
| GMIDA/BSA via $Cu^{2+}$ ion | PEG (diacrylate) | BSA | 1 day | Lin, Metters[61] |
| GMIDA/$Ni^{2+}$ or $Cu^{2+}$ ions | | His-GFP | 2 days | Lin, Metters[62] |
| GMIDA/His via $Ni^{2+}$, $Zn^{2+}$, or $Cu^{2+}$ ion and GMIDA/cationic protein | | lysozyme, His-GFP | 3 days and 10 days | Lin, Metters[63] |
| nonspecific electrostatic (±) | peptide hydrogel | human IgG, BSA, $\alpha$-lactalbumin | 30 days | Branco et al.[64] |
| mineral/protein | mineral-coated $\beta$-tricalcium phosphate granules | BSA, lysozyme | 28 days | Lee et al.[65] |
| | mineral-coated microparticles | VEGF, BMP-2 | 50 days | Yu et al.[66] |

Table 4. Summary of Cyclodextrin-Based Affinity Release Systems Which Use Hydrophobic Interactions to Control Release

| affinity interaction | matrix | therapeutic delivered | time scale | reference |
|---|---|---|---|---|
| cyclodextrin/hydrophobic interaction | cyclodextrin hydrogel | rifampin, novobiocin, vancomycin | 18 days[a] | Thatiparti et al.[68] |
| | | | 8 days[a] | Thatiparti et al.[67] |
| | | adamantane | 28 days[a] | Fu et al.[21] |
| | | rifampin, erythromycin vancomycin | 14 days[a] | Halpern et al.[69] |

[a]NB in vitro activity (zone of inhibition) assays show the active component was delivered for longer time scales.

increasing the concentration of chelating metal ions or increasing the affinity of the interaction ($Ni^{2+} \ll Cu^{2+}$).[62] In the absence of chelating ions, GMIDA can also bind cationic proteins via electrostatic interactions to delay release. Conversely, anionic proteins are released faster due to electrostatic repulsion. Both of these cases were observed when dual release of a cationic model protein, lysozyme, and His-GFP was investigated from GMIDA-*co*-PEG gels.[63] Release of lysozyme was attenuated in gels functionalized with GMIDA, and release of His-GFP was affected by both the electrostatic interaction (repulsion) and affinity interaction for GMIDA-$Ni^{2+}$. Control experiments had ethylenediaminetetraacetic acid (EDTA) added after gel formation to interrupt chelation, resulting in complete release of lysozyme. The release of His-GFP was not affected (~70% detected), which raised concerns that proteins may be inadvertently immobilized to the polymer matrix during photopolymerization. The competition and/or toxicity of endogenous Cu/Zn/Ni ions is also of concern when moving these systems to an in vivo application, and the bioactivity of therapeutic proteins after polymerization is yet to be investigated.

Other systems have used other electrostatic interactions to deliver various proteins. An electropositive fibrillar peptide hydrogel was used to control the release of negatively charged proteins.[64] Release from these gels was characteristically biphasic, consisting of a diffusional phase, and a second controlled phase dependent on electrostatic affinity. The release of positively charged and neutral proteins was not attenuated by electrostatic interactions, but was dependent on the steric interactions between the protein and polymer, as the mesh size of the network was on the same order of magnitude as the hydrodynamic diameter of the proteins. Electrostatic systems are limited to delivering only charged proteins and tunable release is difficult to achieve. Moreover, the swelling of these gels may be problematic for in vivo applications, especially when injected into confined volumes.

The Murphy group has investigated the use of mineral−protein interactions to control the release, over 28−50 days, of various proteins for bone regeneration purposes. Porous $\beta$-tricalcium phosphate granules were incubated with simulated body fluid to create a carbonate-substituted hydroxyapatite coating, which binds numerous proteins.[65] The protein release rate was tunable by changing the concentration of the mineral layer, either by changing the concentration of carbonate in the simulated body fluid or by modulating the incubation time. A similar method was employed to control the release of VEGF and BMP-2 from mineral-coated microparticles.[66] It is unclear how systems based on nonspecific electrostatic interaction will perform in vivo with competing soluble ligands. The presence of competing soluble ligands, which bind with greater affinity than the therapeutic of interest, would competitively bind to the affinity system, resulting in dose-dumping as opposed to controlled release. In summary, systems based on electrostatic interactions are inherently limited to delivering charged therapeutics. Nonetheless, these systems have proven efficacious in vivo when properly engineered. A summary of affinity-based systems using ionic interactions is detailed in Table 3.

**3.2. Hydrophobic Interactions: Cyclodextrins.** Cyclodextrins (CD) have been exhaustively investigated for their controlled release capabilities of hydrophobic small molecules.[21,67−69] Tunable release for numerous antibiotics, including rifampin, novobiocin, and vancomycin, was shown for more than 18 days using CDs, and the delivery of the model drug adamantane from CD-based gels was achieved for 28 days. More exciting, is the in vitro activity of these devices. A zone of inhibition assay was used to test the activity of released antibiotic from CD-coated metal screws or autoclaved CD-gels. CD-coated metal screws released active Rifampin and novobiocin for more than 40 days in this assay. Vancomycin and erythromycin were actively released from CD-based gels for 13 and 24 days, respectively, and rifampicin was active for more than 80 days when released from a CD-based gel.[69] Release was tuned by varying the concentration of the dextran binding unit and the affinity of the dextran (e.g., $\alpha$, $\beta$, or $\gamma$) for the hydrophobic drug of interest. Cyclodextrins have proven excellent as delivery vehicles for hydrophobic small molecules, especially antibiotics. A potential next step in this field would be the development of a CD-based system capable of delivering multiple antibiotics at the same time, which may be useful to prevent recurrent infections from antibiotic resistance. These systems are summarized in Table 4 above. Recent reviews provide in-depth discussion on cyclodextrins in drug delivery, including controlled release applications.[70,71]

Table 5. Summary of Affinity-Based Release Systems Discussed Which Use Protein−Protein Interactions to Control Release

| affinity interaction | matrix | protein delivered | time of release | reference |
|---|---|---|---|---|
| collagen/collagen-binding domain | collagen | NT-3 | NR[a] | Fan et al.[75] |
| | | PDGF | NR[a] | Lin et al.[76] |
| | | EGF | NR[a] | Yang et al.[77] |
| | | TGF-$\beta$[a] | NR[a] | Andrades et al.[78] |
| NGF-binding peptide/NGF | fibrin | NGF | 5 days | Willerth et al.[50] |
| bFGF-binding peptide/bFGF | PEG (diacrylate) | bFGF | 30 days | Lin, Anseth[79] |
| VEGF-binding peptide/VEGF | PEG microspheres | VEGF | 30 days | Impellitteri et al.[80] |
| | | VEGF | 48 days | Belair et al.[81] |
| SH3 domain/SH3-binding peptides | HAMC | bFGF | 10 days | Vulic, Shoichet[15] |
| | | chABC | 7 days | Pakulska et al.[82] |
| proline-rich domain/WW domain | MITCH (peptide-based hydrogel) | VEGF-mimetic peptide | 21 days | Mulyasasmita et al.[83] |
| heparin protein-binding domain/heparin binding protein | fibrin | BDNF, TGF$\beta$, BMP-2, IGFBP-5, bFGF, PGF | 7 days | Martino et al.[86] |
| peptide/protein (nonspecific electrostatic) | peptide hydrogel | bFGF, VEGF, BDNF | 2 days | Gelain et al.[84] |

[a]NR - not reported.

### 3.3. Multiple Interactions: Protein−Protein, DNA Aptamers, and Other Multiple Interaction Systems.

*3.3.1. Protein−Protein Interactions.* An emerging class of affinity-based systems utilizes protein−protein interactions to control release. Protein−protein interactions are ubiquitous and essential to numerous cellular processes such as signal transduction, enzymatic function, cell regulation, and the immune response, among others.[72] Kinetics may be weak and transient (e.g., within cellular signaling cascades, SH3 domains, and SH3-binding peptides, $K_D$ $10^{-5}$−$10^{-7}$ M[73]) or relatively strong (e.g., inhibition complexes, barnase-barstar, $K_D$ $10^{-12}$ M[74]) and binding may be specific or promiscuous. Researchers have recently used protein−protein interactions to control the release of therapeutic proteins (sometimes a chimeric protein) from polymeric delivery vehicles. In these systems, the drug of interest binds to a polymeric matrix via noncovalent interactions with either the matrix itself or to a polypeptide or protein covalently bound to the delivery matrix. Protein−protein association is driven by electrostatic interaction (long ranging forces), and the resultant protein−protein complexes are generally stabilized by hydrophobic interactions.[72] Thus, site-directed mutagenesis to relevant amino acids may tune binding kinetics and ultimately modulate release in this type of affinity-based system.

In the first example of this type of system, a polypeptide collagen-binding domain (CBD) was exploited to sequester and deliver numerous growth factors for tissue engineering applications. In all cases, the therapeutic growth factor of interest was recombinantly expressed from *Escherichia coli* (*E. coli*) with a CBD. While release profiles for most of these systems have not been reported, Scatchard analyses repeatedly suggest that CBD-GFs bind collagen better than native GFs and cellular assays show improved bioactivity from CBD-GFs.[75−78]

Specific growth-factor binding peptides, sometimes derived from receptor binding pockets, have also been used to control release. Sakiyama-Elbert's group screened a phage display library against NGF to create a library of NGF-binding peptides. When these peptides were incorporated into a fibrin matrix, the release of NGF was attenuated compared to fibrin controls in a predictable manner.[50] Lin and Anseth used a bFGF binding peptide to control the release of bFGF from PEG hydrogels.[79] They were able to tune release by modulating the amount of binding peptide within the PEG hydrogels; however, they noticed that the amount of binding peptide required was quite high for the $K_D$ of the interaction (122 nM). Thus, they postulated that covalent immobilization of binding peptides to the matrix affected the binding affinity. To test this hypothesis, they used a PEG-interpenetrating network (IPN) hydrogel where the binding peptide was preconjugated to a linear polymer chain, such that no further cross-linking of the peptide would occur during gel formation. This improved binding strength and a binding peptide excess of only 50× the amount of protein resulted in slower release than excesses of 1000× for the PEGDA hydrogels. These findings suggest that matrix bound peptides may show different affinity for their ligands and that tunable release is possible by varying binding ligand concentration. However, control experiments showing diffusive release of bFGF from PEG-IPN gels were not shown, which would confirm that the controlled release from PEG-IPN gels is due to improved affinity interactions and not changes in diffusivity.

The Murphy group has also used growth-factor specific binding peptides to control the release of VEGF. A VEGF-binding peptide derived from the VEGF-receptor-2 was used to deliver VEGF from PEG microspheres. A mutant peptide was modified with four D-amino acids to prevent proteolytic degradation and release was tuned by modulating the concentration of binding peptide bound to the polymeric matrix.[80,81] This study was the first to look at the effect of serum on release of VEGF.

The Shoichet lab created an affinity-based delivery platform by hijacking the interactions between the SH3 domain and SH3-binding peptides. Numerous SH3-binding peptides have been characterized that bind to SH3 with $K_D$s between $10^{-5}$ and $10^{-7}$ M.[73] Both bFGF and chondroitinase ABC were expressed as fusion proteins with the SH3 domain and delivered from methylcellulose matrices modified with SH3-binding peptides.[15,82] Tunable release was achieved by varying either the strength of the binding interaction or the ratio of peptide to protein. This was only achieved when the concentration of the immobilized ligand (binding peptide) was varied, as explained in section 2.1. This system is the first system capable of delivering any protein that can be expressed as a fusion protein with SH3, at a predictable and tunable rate.

The Heilshorn group used a similar approach to deliver a VEGF-mimetic peptide from mixing-induced two component hydrogels (MITCH). MITCH reversibly gels through binding of WW domains (named for two conserved tryptophan (W) residues) to proline-rich peptide sequences. A chimeric peptide consisting of a proline-rich binding sequence and the VEGF-mimetic peptide was incorporated into MITCH during gelation. The release rate was tuned using proline-peptides with different binding strengths.[83]

The advantage of affinity-based systems that use binding-peptides or proteins is the specificity of binding. It would be interesting to combine several of these systems into one to provide concomitant delivery of multiple proteins. Nonspecific affinity (electrostatic interactions) of peptide hydrogels for proteins has also been used to control release of brain-derived neurotrophic factor (BDNF), bFGF, and VEGF for 2 days.[84] Similarly, Hubbell and co-workers discovered a heparin-binding domain on fibrinogen that binds numerous heparin-binding proteins.[85] Controlled release of BDNF, TGF$\beta$, bone morphogenetic protein 2 (BMP-2), insulin-like growth factor binding protein (IGFBP-5), bFGF, and placental growth factor (PGF) from fibrin matrices were observed over a 7 day period.[86] Remarkably, no modification was required, highlighting that nascent matrix-protein interactions need to be studied and may be sufficient to control release. A limitation of this system is that tunable release would be difficult to achieve as release is dependent on inherent affinity of the protein for the fibrin matrix versus engineering this affinity into the system. In summary, many affinity-based systems rely on protein−protein interactions to attenuate release, with most systems capable of facile tunable release (Table 5).

*3.3.2. DNA Aptamers.* DNA aptamers are single-stranded oligonucleotides that can be selected to bind distinct targets with high affinity and selectivity.[87] Aptamers have previously been used as therapeutics themselves, or as targeting moieties to deliver cancer therapeutics.[87] More recently, they have been utilized as binding ligands for affinity-based release systems. DNA aptamers can be raised against a target molecule in vitro using a process called systematic evolution of ligands by exponential enrichment (SELEX). Aptamers will recognize the target sequence and create secondary structure by complementary base pairing to fit a binding site. Sometimes tertiary structures are also possible, allowing aptamers to bind via electrostatic interactions, hydrogen bonding, or van der Waals forces.[88]

Aptamer-based affinity systems are versatile and facile. These systems can be characterized as platform systems because aptamers can be synthesized to bind any therapeutic of interest with varying affinity and high specificity. The first aptamer-based affinity system was developed by Soontornworajit and co-workers in 2010 for the delivery of PDGF-BB.[89] Aptamers were functionalized at the 5′ end with an acrydite functional group and copolymerized into acrylamide gels. Aptamers of different binding strengths ($K_D$ of 220 or 25 nM) were used to tune release of PDGF-BB. Cumulative release was reduced from 90% for unmodified gels to 60 and 16% for each aptamer-functionalized gel, respectively, over 6 days. The versatility of DNA aptamers also allowed for in vitro on-demand release of PDGF-BB from aptamer-functionalized polystyrene microparticles embedded in an agarose hydrogel with soluble complementary oligonucleotide sequences added to the gel at defined intervals, competing for binding and displacing any bound protein.[90] In additional studies, an aptamer library of different binding strengths (11−300 nM) was created by varying tail sequences to achieve tunable protein release between 10 to 75% over 14 days from a poloxamer (block copolymer comprised of poly(ethylene oxide) and poly(propylene oxide)) hydrogel (Figure 6).[91] The specificity of

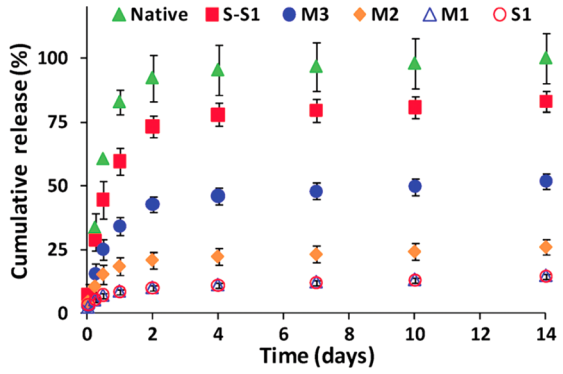


**Figure 6.** Affinity-based release of PDGF-BB from aptamer-based hydrogels. Release from aptamer-functionalized hydrogels containing either S1 (11.3 nM), M1 (27.6 nM), M2 (109 nM), M3 (354 nM), or a scrambled aptamer (S−S1) and release from a native gel are shown. As binding strength increases, the release rate of PDGF-BB from aptamer-based hydrogels decreases. Reproduced from ref 91. Copyright 2010, American Chemical Society.

aptamers was confirmed by releasing VEGF or PDGF-BB from gels functionalized with aptamers specific to the opposite protein (e.g., VEGF from anti-PDGF-BB aptamer functionalized hydrogels and vice versa). These experiments demonstrated that controlled release was aptamer-specific.[92]

A biocompatible PEG-diacrylate monomer was photopolymerized in the presence of acrylated oligonucleotides to form an affinity-based hydrogel that was able to sustain release of tetracycline, a small molecule antibiotic, for more than 3 days via specific interactions between the oligonucleotide and tetracycline.[93] Another group delivered the antibiotic neomycin from gold nanoparticles functionalized with aptamers for 1 day. Here, release was tuned by varying the temperature of the release experiments, which effectively changed the binding kinetics of the interaction. Release was faster at lower temperatures (4 °C) due to lower affinity compared to room temperature or body temperature.[94] DNA aptamers represent versatile and exciting new systems for controlled release (summarized in Table 6). Binding ligands are robust and easy to synthesize with varying binding affinity. One can envision that concomitant, tunable protein release from aptamer-based systems could readily be achieved. New systems capable of delivering multiple proteins at defined rates would be useful for the regeneration of complex tissues, requiring stimulation by multiple factors at specific times. Of potential concern is the immunogenicity of aptamers. Some have reported low immunogenicity and toxicity,[92] whereas others have reported complement activation and stimulation of the innate immune response.[95]

*3.3.3. Other Multiple Interaction Systems.* Other affinity systems also use multiple affinity interactions to deliver small molecule or protein therapeutics. This includes systems based on peptide−glycosaminoglycan interactions, antibody−antigen interactions, or molecularly imprinted polymers. As described in section 3.1.1, proteins are known to bind the extracellular matrix. Instead of using a glycosaminoglycan as the polymer

Table 6. Summary of Affinity-Based Release Systems That Use DNA Aptamers to Control Release

| affinity interaction | matrix | therapeutic delivered | time scale | reference |
|---|---|---|---|---|
| DNA aptamer/protein | polyacrylamide gel | PDGF-BB | 6 days | Soontornworajit et al.[89] |
| | aptamer-functionalized polystyrene microparticles embedded in a agarose hydrogel | PDGF-BB | 25 days | Soontornworajit et al.[90] |
| | pluronic F-127 (poloxamer) hydrogel, polystyrene microparticles coated with aptamer | PDGF-BB | 10 days | Soontornworajit et al.[96] |
| | pluronic F-127 (poloxamer) hydrogel, polystyrene microparticles coated with aptamer | PDGF-BB | 14 days | Soontornworajit et al.[91] |
| | aptamer-functionalized polystyrene microparticles | PDGF-BB and VEGF | 10 days | Battig et al.[92] |
| DNA aptamer/small molecule | PEG hydrogels | tetracycline | 3 days | Zhang et al.[93] |
| | gold nanoparticle | neomycin | 1 day | Sundaram et al.[94] |

Table 7. Summary of Other Multiple Interaction Affinity-Based Systems Used to Control Release

| affinity interaction | matrix | therapeutic delivered | time scale | reference |
|---|---|---|---|---|
| chondroitin sulfate-binding peptide/chondroitin sulfate/NGF | PEG/chondroitin sulfate matrix | NGF | 2 days | Butterfield et al.[97] |
| His-tag/antibody to His-tag | demineralized bone matrix | BMP-2 | 5.5 days | Zhao et al.[98] |
| molecular imprinted polymer (MIP) to target therapeutic | methacrylate polymer | citalopram | 40 h | Abdouss et al.[102] |

matrix, Butterfield and co-workers functionalized a PEG hydrogel with chondroitin sulfate binding peptides. These peptides are capable of binding soluble chondroitin sulfate, which has an affinity for various proteins, including NGF.[97] Through this multiple-species affinity interaction, controlled release of NGF from a PEG/chondroitin sulfate matrix was achieved, but only demonstrated for 2 days.

Others have used antibody−antigen interactions to control protein release. For example, the release of His-tagged bone morphogenetic protein-2 (BMP-2) from demineralized bone matrices (DBM) was attenuated by immobilizing a monoclonal antipolyhistidine antibody to the DBM.[98] This antibody−antigen affinity also increased total protein loading within the delivery matrix and effectively controlled release and minimized burst release. Since the affinity interaction of this system is independent of the protein delivered, there is potential to deliver many different proteins from this system so long as they can be expressed with a His-tag.

Molecularly imprinted polymers (MIPs), originally developed for use in analytical applications for separating and quantifying various small molecules[99] have also been investigated to control the release of therapeutics.[17,99−101] For example, the release of the antidepressant citalopram from an imprinted methacrylate polymer matrix was extended to 40 h compared to nonimprinted systems, which released citalopram within 15 h.[102] Release was tuned by varying the affinity of the polymer for citalopram, which was achieved by varying the pH of the release medium, which may be useful in vivo in those sites where pH is varied, but perhaps not a useful in vivo strategy for this antidepressant. Incomplete template removal and therapeutic instability during the imprinting process (polymerization) are also concerns that must be addressed before these systems are further evaluated as drug delivery devices.[103] A perspective on the use of MIPs in drug delivery was recently published.[101] Table 7 summarizes some of these other affinity-based release systems.

**3.4. Using Reversible Chemistry to Control Release.** In an interesting example, Koehler and co-workers used reversible Diels−Alder chemistry instead of a true affinity interaction to control release of an adhesive peptide, RGDS. These reaction kinetics are thermally controlled, thus varying the temperature between 37 and 80 °C modulates release, which is interesting, yet perhaps not useful under physiological conditions.[22] Similar to other affinity systems, the release rate can also be tuned by changing the concentration of the available maleimide moiety (the binding ligand) on the polymer matrix. Though release is tunable, it is currently limited to short time scales (36 h) and future work should advance greater time scales controlled release. This system is summarized in Table 8.

Table 8. Summary of Affinity-Based Release by Reversible Chemistry

| affinity interaction | matrix | therapeutic delivered | time scale | reference |
|---|---|---|---|---|
| Diels−Alder reversible reaction | PEG hydrogel | cell-adhesive peptide (RGDS) | 36 h | Koehler et al.[22] |

## 4.0. CONCLUSIONS AND FUTURE OUTLOOK

Affinity-based release has emerged as an effective means to sustain and control the release of various therapeutic proteins or small molecules from polymeric scaffolds, some of which cannot be adequately delivered using other methods due to their fragility (e.g., enzyme therapeutics). These systems are developed in vitro and later applied in vivo to treat injured or diseased tissues. Thus, many polymeric scaffolds are comprised of biocompatible or biologically derived hydrogels, which mimic the native extracellular matrix to facilitate integration and minimize immune response. The most common affinity-based strategy utilizes the affinity interaction between heparin and numerous proteins to both preserve bioactivity and sustain release. In the past decade, novel affinity interactions to control therapeutic release have been investigated. More advanced affinity-based systems have expanded the scope of deliverable therapeutics and enabled tunable and predictable release rates.

Mathematical modeling of affinity-based systems has provided a thorough understanding of which parameters affect release rate and how the release rate can be tuned. In some systems, unknown matrix-therapeutic interactions or the mesh/pore size of the matrix may influence the release rate in addition to the affinity interaction. Thus, it is important for researchers to properly control experimental variables to ensure that novel affinity-based systems are fully characterized and well under-

stood. Even with proper in vitro characterization, competitive binding by endogenous ligands in vivo may augment release rate.

This exciting delivery paradigm is still in its infancy. New systems exploiting novel affinity interactions are continually emerging, and many more are yet to be discovered. Most systems are developed to release a specific therapeutic and concomitant delivery of multiple therapeutics at independent rates is not frequently investigated. To achieve greater success in vivo, it has been shown that the delivery of multiple factors at independent rates is required. Thus, new systems should permit the simultaneous and independently controlled delivery of multiple therapeutics. For example, a polymeric matrix could be modified with several different protein-specific binding peptides to control the release of different protein therapeutics. Alternatively, one can envision functionalizing a delivery matrix with a library of DNA aptamers, each specific to a different therapeutic of interest. Affinity-based systems with well-defined and predictable release kinetics should prove to be an asset to in vivo therapy.

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### Notes

The authors declare no competing financial interest.

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