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PERSPECTIVE

Local Affinity Release

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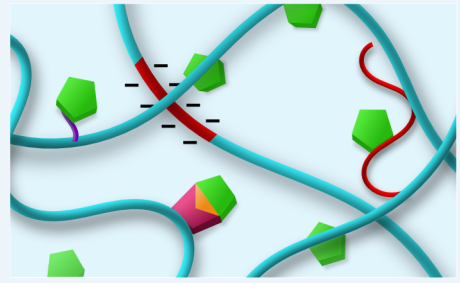
ABSTRACT: The use of hydrogels for therapeutic delivery is a burgeoning area of investigation. These water-swollen polymer matrices are ideal platforms for localized drug delivery that can be further combined with specific ligands or nanotechnologies to advance the controlled release of small-molecule drugs and proteins. Due to the advantage of hydrophobic, electrostatic, or specific extracellular matrix interactions, affinity-based strategies can overcome burst release and challenges associated with encapsulation. Future studies will provide innovative binding tools, truly stimuli-responsive systems, and original combinations of emerging technologies to control the release of therapeutics spatially and temporally. Local drug delivery can be achieved by directly injecting a therapeutic to its site of action and is advantageous because off-target effects associated with systemic delivery can be minimized. For prolonged benefit, a vehicle that provides sustained drug release is required. Hydrogels are versatile platforms for localized drug release, owing to the large library of biocompatible building blocks from which they can be formed. Injectable hydrogel formulations that gel quickly *in situ* and provide sustained release of therapeutics are particularly advantageous to minimize invasiveness. The incorporation of polymers, ligands or nanoparticles that have an affinity for the therapeutic of interest improve control over the release of small-molecule drugs and proteins from hydrogels, enabling spatial and temporal control over the delivery. Such affinity-based strategies can overcome drug burst release and challenges associated with protein instability, allowing more effective therapeutic molecule delivery for a range of applications from therapeutic contact lenses to ischemic tissue regeneration.



It is often necessary to have continuous, long-term release of small-molecule drugs in diseased tissues to obtain the desired therapeutic effects. While various techniques (*e.g.*, nanoprecipitation and emulsion–solvent evaporation) can be employed to encapsulate drugs in particles for systemic injections, local delivery requires an additional support material, such as a hydrogel, to keep the drugs at the site of injection. Affinity-based delivery strategies have emerged as an alternative to encapsulation, where the inherent or engineered affinity between the drug and the delivery vehicle is manipulated to control release. As many small-molecule drugs are poorly water-soluble, hydrophobic interactions offer an interesting alternative to prolong the diffusion of a drug out of a scaffold. For example, in this issue of *ACS Nano*, Huang *et al.* take advantage of the hydrophobic and $\pi-\pi$ stacking interactions of a hydrophobic drug with graphene oxide to achieve prolonged, local drug delivery to the eye.[1] The authors added graphene oxide nanosheets to chitosan-based hydrogel contact lenses to act as both crosslinkers and drug carriers for the delivery of voriconazole and demonstrated a significant therapeutic effect on fungal keratitis. This highlights the potential of affinity release strategies in the emerging field of therapeutic contact lenses.

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An alternative approach to achieve affinity-based release of small-molecule drugs consists of tunable host–guest interactions that rely on a stereoelectronic arrangement of binding sites. For example, the structured hydrophobic pockets of cyclodextrins have attracted much attention for small-molecule inclusion and sustained release. Cyclodextrin-functionalized polymers have also been widely used for the synthesis of supramolecular hydrogels. Burdick *et al.* recently demonstrated that the release of various small molecules (doxycycline, doxorubicin, and two model peptides) could be sustained for up to 21 days *in vitro*, without altering the self-assembly and mechanical properties of a hyaluronic acid-based cyclodextrin supramolecular hydrogel.[2] The release rate of a model peptide

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from this hydrogel was tuned by incorporating different amounts of tryptophan into the peptide, as this amino acid has a specific affinity for cyclodextrin. A similarly tunable system used the photoinducible isomerization property of azobenzene to release peptides on demand. The low affinity of *cis*-azobenzene to cyclodextrin compared to that of *trans*-azobenzene allows the phototriggered release of peptides upon irradiation with UV light.[3] Together, these findings establish a basis for tunable delivery of peptide therapeutics from cyclodextrin-modified hydrogels.

LOCAL PROTEIN DELIVERY

While small-molecule hydrophobic drugs have dominated pharmaceutical sciences for numerous years, protein therapeutics have profound effects in a myriad of diseases, including diabetes and growth deficiency, where insulin and human growth hormone, respectively, have had significant benefits to patients. Yet, the delivery of proteins is significantly more challenging than that of small-molecule drugs because of their tertiary structure, making them inherently more fragile, with the potential consequent loss of bioactivity.

The strategies designed for small-molecule drug delivery do not always work well for protein delivery. Conventional delivery strategies, such as encapsulation in polymeric nanospheres, often result in low protein loading, protein degradation or denaturation, and limited bioactivity due to exposure to organic solvents and shear forces, which are inherent to the process. To overcome these limitations, innovative strategies for local protein delivery have emerged.

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Mimicking Natural Interactions. Many proteins have natural affinities for extracellular matrix (ECM) components, such as glycosaminoglycans (GAGs, *i.e.*, heparin) and structural proteins (*e.g.*, fibrin and collagen). There is an equilibrium between bound and unbound proteins in the ECM that regulates their activity in the cellular microenvironment. Thus, by mimicking the ECM, local delivery of proteins can be manipulated and this has resulted in the development of affinity-based release systems (Figure 1). Sakiyama-Elbert and Hubbell first showed that neurotrophic factor release could be sustained by the incorporation of low molecular weight heparin in fibrin gels. In their initial system, the use of a bidomain peptide enabled the immobilization of heparin in fibrin matrices to prolong the delivery of heparin-binding factors such as basic fibroblast growth factor, glial cell-derived neurotrophic factor or neurotrophin-3, and promote functional nerve regeneration.[4,5] A recent study focused on protein interactions with other GAGs (*e.g.*, chondroitin sulfate A and B), showing that the release rate of proteins from hydrogels containing GAGs could be tuned as a function of the GAG used, its natural binding affinity (low, medium, or high) to the protein, and the cross-linking density of the matrix.[6] We anticipate that the ECM/protein interaction will continue to be exploited but that specific binding sequences will replace the full ECM molecule to achieve a better-defined system for improved controlled release.[7]

While low molar mass GAGs can be covalently bound to hydrogels using various chemical reactions (*e.g.*, disulfide bonds, azide−alkyne Huisgen cycloadditions), it was recently shown that fibrinogen itself possesses heparin-like binding domains.[8] This discovery allows the sequestration of soluble factors within fibrin matrices with no further modification and was used in the design of a hydrogel/nanoparticle composite material to overcome the burst release often observed in conventional encapsulation strategies. With this strategy, it was possible to sustain the release of stromal cell-derived factor-1α (SDF-1α), a promising molecule to promote tissue regeneration after brain injury, from a fibrin gel.[9] Interestingly, as fibrin plays a key role in wound healing, the combination of controlled protein release and fibrin-based wound care opens up exciting opportunities.

Sequential Multiprotein Delivery. Depending on the application, it may be necessary to deliver multiple therapeutics at different rates, requiring innovations in sequential, multi-protein release strategies. For example, a sequential, affinity-based release system was designed to promote angiogenesis in ischemic tissues.[10] Alginate sulfate is capable of binding many heparin-binding proteins, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor-BB (PDGF-BB), and transforming growth factor-β1 (TGF-β1), with equilibrium binding constants close to those observed with heparin. This binding affinity was used to immobilize these three angiogenic factors on alginate sulfate scaffolds and achieve sequential release into tissue when implanted subcutaneously in rats. *In vitro*, VEGF was released first and VEGF and PDGF-BB reached a plateau by 6 days, whereas TGF-β1 was released steadily until the end of the 8 day study and showed a trend toward continued release past this end point. Tissue analysis at 1 and 3 months after implantation of the factor-immobilized scaffolds demonstrated increased blood vessel density and percentage of mature vessels when compared to scaffolds comprising alginate alone or alginate with physically adsorbed factors. While this study provides a basis for affinity interactions for *in vivo* sequential release, these interactions are strong, resulting in only a fraction of the loaded protein being released. The release sequence is also dictated by the natural affinities, with the strongest binder released more slowly. There is a need for binding partners with tunable affinities and higher specificity to release more of the immobilized factors and to improve control.

Nucleic acid aptamers have been manipulated for on-demand release of multiple protein drugs from a hydrogel/microparticle system. Aptamers have a high degree of specificity and high binding affinities for their protein targets but are also able to hybridize with complementary oligonucleotide sequences. Polystyrene microparticles were chemically modified with two aptamers, one known to bind specifically to VEGF and another to PDGF-BB. Then VEGF and PDGF-BB were simultaneously sequestered into an agarose hydrogel containing the modified microparticles. Due to the advantage of the competitive nature of specific binding partners, the release of each growth factor was triggered only upon addition of the associated complementary sequence as a competing substitute.[11] Due to the large number of possible aptamer sequences, this approach is not limited to controlling the release of only two proteins but could be augmented for the delivery of multiple factors.

Another method to control the release of multiple factors is exploiting the interaction of a well-known intracellular protein, Src homology 3 (SH3), with its binding peptides. Specifically, by expressing fusion proteins of SH3 with the protein of interest and modifying a hyaluronan−methylcellulose (HAMC) hydrogel with the corresponding binding peptide, the release can be computationally predicted and designed.[12,13] This

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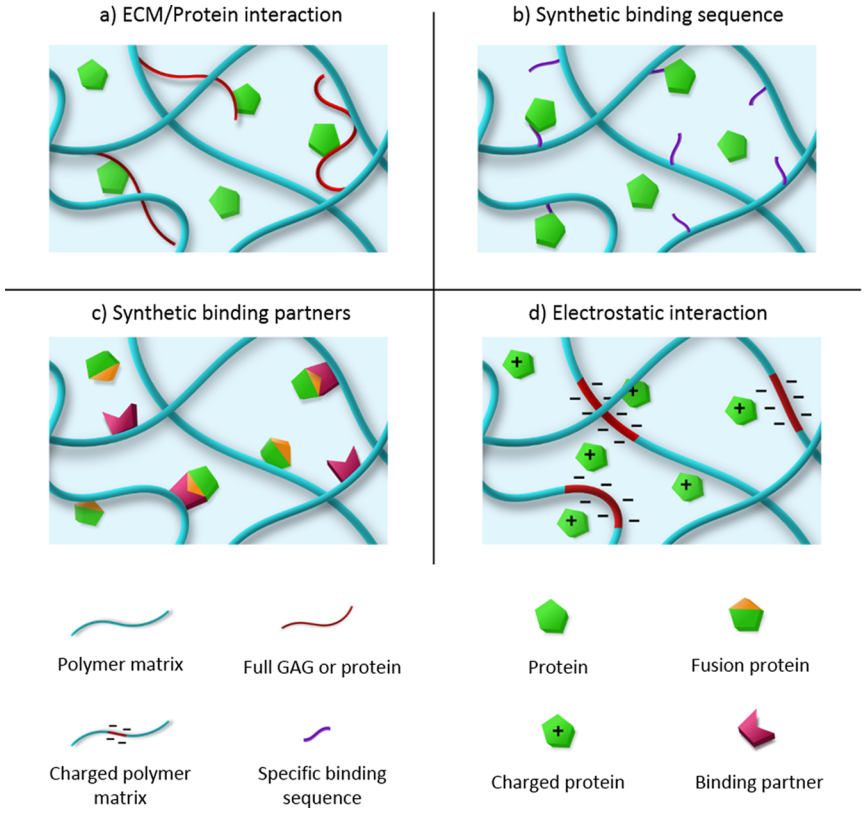

Figure 1. Affinity-based release strategies for the controlled delivery of proteins: (a) full ECM molecule (glycosaminoglycan, GAG, or protein) is incorporated into the support material; (b) specific bioactive sequence is grafted to the support material; (c) both material and protein are modified to interact specifically *via* binding partners; and (d) support material is designed for charge-based interaction with the protein (the material could be either positively or negatively charged, depending on the charge of the protein).

affinity-based release system has also been investigated for combined drug and cell delivery, where it is postulated that survival of transplanted cells is enhanced by pro-survival factors within the gel. For example, HAMC was modified with SH3-binding peptide to control the release of SH3-insulin-like growth factor-1 fusion protein through affinity while simultaneously enhancing survival of retinal pigment epithelium cells within the gel for ultimate use in the treatment of blindness.[14] By reversing the modifications such that HAMC contains immobilized SH3 and the fusion protein includes the SH3-binding peptide, multiple proteins can be released at different rates. Alternatively, multiple, orthogonal binding partners can be manipulated to control protein release. The sequential delivery of multiple factors from hydrogel systems opens up new avenues to address the time-sensitive aspects of disease modulation while preserving the benefits of local delivery.

Although these systems enable greater control than natural interactions such as heparin, they often require protein manipulations that could have undesirable effects. An alternative strategy to control release is by charge-based interactions between polymeric delivery vehicles and proteins.

Protein–Polymer Electrostatic-Based Release. Electrostatic interactions between a polymeric delivery vehicle and protein therapeutic is a facile and innovative way to control release. For example, Song *et al.* designed a negatively charged diblock copolypeptide hydrogel to control the delivery of positively charged proteins, such as nerve growth factor, to the central nervous system.[15] Electrostatic interactions were also used to manipulate the release of positively charged proteins from negatively charged poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles dispersed within a hydrogel.[16] In this study, the release profile of proteins encapsulated in PLGA nanoparticles was unexpectedly similar to that of proteins mixed into the hydrogel with blank nanoparticles. Interestingly, this delivery system is self-regulated—as the PLGA nanoparticles degrade, acidic byproducts form and decrease the local pH, thereby protonating carboxylate anions to carboxylic acids. Consequently, the electrostatic interactions between the polymeric nanoparticles and proteins weaken, releasing the protein. By varying the nanoparticle size, the nanoparticle density, and the pH of the release environment, the release rates of positively charged proteins can easily be tuned to meet the requirements of the intended application. Future studies on nanoparticles with tunable surface charge will make this strategy suitable for a broader range of protein therapeutics.

The sequential delivery of multiple factors from hydrogel systems opens up new avenues to address the time-sensitive aspects of disease modulation while preserving the benefits of local delivery.

FUTURE CHALLENGES AND OPPORTUNITIES

By combining affinity release with stimuli-responsive materials, local, on-demand therapeutic delivery can be achieved. Significant advances can also come from unexplored combinations with other emerging technologies, such as three-

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dimensional printing or cell-based therapy.[17] For the delivery of small-molecule drugs, the drug itself can be manipulated to control its release, thereby incorporating delivery strategies into the drug discovery process. For the delivery of proteins, designing binding partners specific for each protein, using approaches similar to those in antibody engineering,[18] will enable affinity release of any protein with improved control and without potentially denaturing modification.

Truly responsive systems, such as one that responds to blood glucose levels for the treatment of diabetes, have thus far eluded the research community. Smart materials are often described as stimuli-responsive materials, but here the stimulus is usually pH or temperature, which results in a change in material shape and hence drug release. A truly responsive system will be inherently more complex and may integrate affinity interactions into smart materials to diagnose the microenvironment continuously and to release the appropriate factors in response. While proteins are the drugs of the present, cells may be the drugs of the future. Cells, such as islets or β-cells for the treatment of diabetes, are inherently stimuli-responsive, but cell delivery is complicated by the immune system. Successful cell delivery requires inventiveness in cell cloaking through both cellular and biomaterials-based approaches. The challenges of cell delivery into a host system may be overcome, in part, by providing cells with an environment that promotes survival/integration through co-delivery of relevant proteins and small molecules.[19] This is an active area of research that will greatly benefit from innovations in affinity release strategies.

Reversing the role of affinity systems to sequester molecules instead of delivering them presents an interesting opportunity for modulating the injury environment. For example, specific, strong affinities could draw toxic molecules out of affected areas and enhance the efficacy of subsequently delivered therapeutics. Ultimately, combinations of affinity interactions can aim to recapitulate the complex molecular exchanges of living tissues for therapeutic benefit and advanced tissue regeneration.

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Notes

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