Aptamers can bind a wide range of biomedically relevant proteins with affinities and specificities that have therapeutic utility. Although aptamers are susceptible to nuclease-mediated degradation and cannot easily cross biological barriers, specific aptamer modification can feasibly solve these problems. To address these obstacles, Lau et al. developed a general strategy for generating natural packaging and transport vehicles for targeting agents, such as aptamers and their small-molecule ligands, by using virus-like particles (VLPs) assembled from the recombinant expression of the bacteriophage Φ21 coat protein. Since RNA and DNA molecules are susceptible to nuclease-mediated degradation, it is important that Φ21 VLPs protect their encapsulated aptamers from nuclease-mediated degradation and enhance their permeability. Moreover, if self-assembled using natural proteins, VLPs can guarantee the biocompatibility and biodegradability of modified aptamers in therapeutic applications. Therefore, this Perspective explores the outlook for such aptamer modification strategies for nanodrug preparation and delivery applications and the challenges that lie ahead.

Table 1, aptamers can be easily assembled on the surface of carbon nanotubes, quantum dots, and metallic or silica nanoparticles by noncovalent physical adsorption or through covalent interactions. Such modifications of nucleotides can both stabilize aptamers against nuclease-mediated degradation and increase their solubility and binding affinity. Encapsulation-based aptamer protection and delivery using silica, polymers, or gels is another way to prevent enzymatic degradation while being delivered across cell membranes. However, limitations, such as cell toxicity, low biocompatibility, and biophysical and chemical instability, have prevented the full realization of aptamer delivery in vivo. The ideal delivery vehicle should protect aptamers from the physiological environment and minimize metabolism and degradation during transit. It should also be biocompatible, biodegradable, stable, and able to be engineered such that aptamer molecules are concentrated at the desired site of action to increase efficacy while, at the same time, decreasing unwanted side effects.

ABSTRACT

Aptamers can bind a wide range of biomedically relevant proteins with affinities and specificities that have therapeutic utility. Although aptamers are susceptible to nuclease-mediated degradation and cannot easily cross biological barriers, specific aptamer modification can feasibly solve these problems. To address these obstacles, Lau et al. developed a general strategy for generating natural packaging and transport vehicles for targeting agents, such as aptamers and their small-molecule ligands, by using virus-like particles (VLPs) assembled from the recombinant expression of the bacteriophage Φ21 coat protein. Since RNA and DNA molecules are susceptible to nuclease-mediated degradation, it is important that Φ21 VLPs protect their encapsulated aptamers from nuclease-mediated degradation and enhance their permeability. Moreover, if self-assembled using natural proteins, VLPs can guarantee the biocompatibility and biodegradability of modified aptamers in therapeutic applications. Therefore, this Perspective explores the outlook for such aptamer modification strategies for nanodrug preparation and delivery applications and the challenges that lie ahead.

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TABLE 1. Typical Aptamer-Modified Nanodrug Delivery Systems

<table>
<thead>
<tr>
<th>nanoparticles</th>
<th>typical assembly techniques</th>
<th>an example application</th>
</tr>
</thead>
<tbody>
<tr>
<td>single-stranded DNA (ssDNA)-single-walled carbon nanotubes (SWNTs)</td>
<td>noncovalent modification by aromatic interactions between nucleotide bases and carbon nanotube side walls</td>
<td>protecting single-stranded DNA during cellular delivery⁶</td>
</tr>
<tr>
<td>aptamer—quantum dots (QDs)</td>
<td>covalent conjugation between carbonyl-modified QDs and amine-labeled aptamer</td>
<td>a drug delivery vehicle for tumor cells⁷</td>
</tr>
<tr>
<td>aptamer—gold nanoparticles (GNPs)</td>
<td>covalent interaction by Au—S bond</td>
<td>a drug carrier in tumor treatment⁸</td>
</tr>
<tr>
<td>aptamer—silver nanoparticles (AgNPs)</td>
<td>affinity of biotin-aptamer and streptavidin stable AgNPs</td>
<td>intracellular protein imaging⁹</td>
</tr>
<tr>
<td>aptamer—silica nanoparticles (SiNPs)</td>
<td>covalent conjugation between carbonyl-terminated SiNPs and amine-labeled aptamer</td>
<td>targeted drug delivery¹⁰</td>
</tr>
</tbody>
</table>

The ideal delivery vehicle should protect aptamers from the physiological environment and minimize metabolism and degradation during transit.

Natural biomaterials, including lipids, proteins, and oligosaccharides, have been used to construct delivery platforms by encapsulating their cargos into diverse nanocontainers,¹² such as cages, microspheres, nanoparticles, hydrogels, minirods, and minipellets. This type of development has especially focused on protein-based delivery platforms due to the biocompatibility, biodegradability, and low toxicity of some natural proteins. A variety of such proteins have been used and characterized, including viral capsids,¹³,¹⁴ ferritin/apoferritin,¹⁵,¹⁶ albumin¹⁷ and collagen¹⁸,¹⁹ (Table 2). In the accompanying article in this issue of ACS Nano, Lau et al.²⁰ describe a general strategy for generating natural packaging and transport vehicles for nanodrug delivery by using virus-like particles (VLPs) assembled from the recombinant expression of the bacteriophage Qβ coat protein (Figure 1).

More specifically, the aptamer of interest is first modified with a RNA sequence from the Qβ genome that can form as a hairpin structure and bind to the Qβ coat protein. Next, the sequences of Qβ coat protein and modified aptamer are inserted into separate plasmids. The two plasmids are then cotransformed with Escherichia coli to yield an elevated level of Qβ coat proteins and modified aptamers. Finally, the modified RNA hairpin sequence promotes the encapsidation of aptamers via binding to the interior surface of the capsid shell in the process of Qβ coat proteins self-assembling into VLPs. Expression of Qβ coat protein and aptamer also can be performed with a single plasmid, as described by Lau et al.²⁰ Nevertheless, the authors thought that the dual-plasmid approach might have an advantage in maintaining stable copy numbers of plasmids because different plasmids contained different antibiotic-resistant genes and origins of replication. While VLPs ensure the biocompatibility and biodegradability of modified aptamers by using natural proteins, the Qβ capsid shell was demonstrated to be impermeable to enzymes, thus protecting encapsulated aptamers from degradation by nuclease and enhancing their permeability for nanodrug delivery. Importantly, the capsid provides channels for small-molecule ligands while allowing sufficient space for encapsulated aptamers to maintain their secondary and tertiary structures, as revealed by the unreduced affinities of aptamers when binding with their small-molecule ligands. In addition, VLPs are stable substrates for diverse chemical and genetic modifications, enabling decoration of their exterior surfaces with targeting, immunogenic, or labeling agents. Therefore, Lau et al. have offered a convenient functional aptamer encapsulation technique that holds great potential as a promising platform for RNA aptamer delivery.

OUTLOOK AND CHALLENGES

Lau et al.’s strategy suggests a general platform for the production of VLPs to encapsulate aptamers. Favorable biocompatibility and biodegradability and uniform size are the advantages of VLPs for the delivery of modified aptamers to their target release sites. It has been shown that VLP encapsulation protects aptamers and their small-molecule ligands from degradation by erosion and proteolyis. Furthermore, since the flow rate of small-molecule ligands is driven by the concentration difference between the interior and exterior of the VLPs, the release rate of molecules can also be regulated by VLP channel sizes to avoid the use of high doses of drugs. Virus-like particles are a major breakthrough for aptamer delivery in protein-based templates. Specifically, VLPs have improved the pharmacokinetics and biodistribution of aptamer delivery by improving the drug release mode.

Nonetheless, some challenges still confront VLP-based aptamer delivery systems. First, the properties and applications of VLPs are still in the development stage for aptamer delivery and therapeutic purposes. Most viruses developed for bionanotechnology applications have not yet been evaluated; hence, the use
of these viruses may be limited in aptamer delivery as a result of potential cytotoxicity. For example, human adenoviruses, which are typically used for gene delivery purposes, once resulted in severe hepatotoxicity in murine models. Second, for clinical aptamer delivery, the biodegradability and immune response of VLPs still need to be evaluated, especially if modifications are decorated on the exterior surface of VLPs, such as folic acid and antigenic peptides. Third, fundamental questions about pharmacokinetic profiles and therapeutic efficacy of VLP-based aptamer

<table>
<thead>
<tr>
<th>proteins</th>
<th>delivery system</th>
<th>description/characteristics</th>
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| collagen                        | microparticles, minirods         | 1. low toxicity, biodegradability, and immunogenicity  
2. structural protein with high content of glycine, hydroxyproline, and hydroxylysine  
3. contain carboxyl group and secondary amino groups with possible cross-linking function |
| elastin/elastin-like polypeptide (ELP) | ELP-Dox nanoparticles, hydrogel | 1. assembling into aggregate at phase transition for therapeutic application  
2. elastin-like polypeptide has a pentapeptide repeatable unit as Val-Pro-Gly-Xaa-Gly  
3. MW of ELP can be made by recombinant technology as precisely as possible  
4. genetic and chemical modification of ESP for versatile functionalities as well as control of biopolymer properties |
| ferritin/apoferritin             | protein cage                     | 1. assembled and disassembled size; container at different pH  
2. the first protein cage to be used  
3. three distinct features: exterior, interface, interior for different functionalities  
4. genetic and chemical modification for possible combination of biology and material science |
| albumin                         | nanoparticles                    | 1. bioconjugate hydrophilic drug  
2. extremely robust to various conditions  
3. binds to many drugs such as indole compound, penicillin, and benzodiazepins  
4. nontoxic, biodegradable, and immunogenic  
5. the most abundant plasma protein |
| viral capsid                    | protein cage                     | 1. modification of specific amino acid residues resulting in multifunctionalities  
2. viral diversity providing different sizes and shapes as well as catalyst functions  
3. three distinct features: interior, interface, and exterior for possible therapeutic applications  
4. assembled and disassembled biological container at different conditions |
| whey protein                    | hydrogel, whey protein beads, microspheres | 1. independent of pH change, easy to entrap hydrophilic drugs  
2. possibly form gel, foam, and emulsion  
3. biocompatible and biodegradable end product  
4. protect drugs from digestive enzymes |

Figure 1. Schematic of the structure of virus-like particle encapsidating aptamers and small-molecule ligands of aptamers. The capsid shell provides channels for small-molecule ligands, as well as sufficient space for encapsulated aptamers to maintain their secondary and tertiary structures.
delivery systems remain to be answered. Finally, future developments for VLP-based aptamer delivery should also focus on enhanced site-specific drug delivery by using newly developed receptor-targeting ligands, improving sustained drug release rates with enhanced permeability and retention time, minimizing damage to normal cells by directing drug molecules for site-avoidance delivery, and developing multiple drugs for sequential release.

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REFERENCES AND NOTES


