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Editorial

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Viral Nanoparticles in Drug Delivery and Imaging

Advances in nanotechnology, the exploitation of matter on the nanometer-size scale, have opened the door to the development of next-generation imaging agents and targeted therapies with increased sensitivity of contrast agents and higher efficacies of chemotherapies. Nanoparticles have many favorable properties for applications in medicine, including their ability to carry large payloads of drugs or contrast agents, and the ease with which targeting ligands can be added so that the payload is delivered to specific sites.

Diverse classes of nanomaterial-based carrier systems are currently undergoing scientific development and (pre)clinical testing; this includes dendrimers, polymers, metallic nanoparticles, and virus-based vectors and nanoparticles. Each system has its advantages and disadvantages regarding biocompatibility, pharmacokinetics, and specificity for the target tissue. This special *Molecular Pharmaceutics* issue highlights recent advances in the development and application of virus-based carrier systems in biomedical imaging and drug delivery.

The development and application of virus-derived materials in the medical sector is becoming a growing field of interest and impact. There are many novel types of viruses in development, including viruses from plants, bacteria, and mammals. Viral nanoparticles are genetically encoded and self-assemble into discrete and monodisperse structures of precise shape and size. For many virus-based systems, the structures are known to atomic resolution and can be tailored at the atomic level. This level of quality control and structural engineering cannot yet be achieved with synthetic nanoparticles. Viruses have naturally evolved to deliver cargoes to specific cells and tissues—a property that we as biomedical engineers, materials scientists, and chemists seek to mimic. Viruses can be tailored for desired applications using at least three approaches: (i) bioconjugate chemistries can be applied to link contrast agents, drugs, or targeting ligands to the exterior or interior capsid shell; (ii) dis- and reassembly protocols facilitate the encapsulation of artificial cargoes, i.e., drugs or contrast agents; (iii) genetic engineering allows the introduction of precise and reproducible modifications so that large quantities of identical particles can be manufactured, displaying targeting ligands or unique ligation handles for further modification through bioconjugation.

This issue covers a diverse range of platforms and topics and includes development and testing of icosahedrons of various sizes and structural features, elongated, high-aspect ratio filaments, and enveloped virus structures, from bacteriophages, plant viruses, and mammalian viruses. Studies include the development of virus-based scaffolds as contrast agents for applications in PET and MR imaging and their application as carrier systems to deliver drugs or siRNA for treatment of disease. Whereas some studies focus on devising methods for cargo loading or application of bioconjugate chemistries for use of fluorogenic viruses in sensing applications, others focus on the fundamental understanding of the biodistribution and fate of virus-based materials in preclinical studies and tissue culture—an important stepping stone toward potential translation to the clinic of this emerging field.

Drs. Qian Wang and Andrew Lee (University of South Carolina) report the application of cowpea mosaic virus (CPMV), a 30 nm sized icosahedron, as a macrofluorogenic probe for applications in cell labeling and sensing. The authors made use of the multivalency of the CPMV nanoparticle and its ability to carry a large payload of fluorophores leading to fluorescence enhancement and signal amplification.

The team led by Dr. Trevor Douglas (Montana State University) turned toward development of phage P22 for applications as a contrast agent in MR imaging. What makes P22 a particularly interesting platform for such applications is its whiffleball structure. The wiffleball is a 64 nm sized fenestrated capsid with 10 nm sized pores for efficient water exchange between bulk medium and capsid interior. The authors report the chemical tailoring of P22 with an oligomer network coupled to Gd-based contrast agents at the interior capsid surface, yielding a formulation with ionic relaxivities 6× higher than the free contrast agent.

Also focusing on the application of virus-based imaging agents, work carried out in Dr. Matt Francis's Lab (University of California, Berkeley) is targeted toward application of 30 nm sized MS2 phage carrying multiple copies of radionuclide copper-64 for PET-CT imaging of tumors. Biodistribution data indicate that the long circulating contrast agents home to tumors, and that remaining particles are cleared via spleen and liver, as expected for a protein-based macromolecular carrier system.

Dr. Nicole Steinmetz's Lab (Case Western Reserve University) started the preclinical evaluation of plant viruses of varying shapes: Using human tumor xenografts (fibrosarcoma, squamous sarcoma, and colon cancer), it was found that filamentous PVX (515 × 13 nm) exhibits higher tumor uptake compared to icosahedral CPMV (30 nm), particularly in the core of the tumor. Further, differences in the biodistribution profiles were apparent, with CPMV being preferentially cleared by the liver, whereas potato virus X (PVX) homes to the spleen. The shape of the nanocarriers impacts their *in vivo* fate and tumor homing properties, with increased tumor homing properties being observed for elongated materials; data indicate that this is mirrored by high aspect ratio, filamentous viruses also.

The work by Drs. Tuli Mukhopadhyay and Bogdan Dragnea (Indiana University) describes the packing of artificial cargoes, including RNA, DNA, proteins, fluorophores, and gold nanoparticles into the capsids of alphaviruses. These viruses have the advantage that they naturally differentiate between healthy and tumor cells and preferentially target tumor cells (this is in contrast to passive tumor homing reported by Francis and Steinmetz). Active receptor-targeting has potential advantages over passive tumor homing; and the development

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of viruses with natural tissue tropism toward tumors is thus an exciting new development. In a tissue culture experiment, the team confirmed cell targeting and uptake of engineered alphaviruses delivering cargoes (in this case gold nanoparticles).

In a different approach and collaborative effort between the laboratories led by Dr. David Evans (John Innes Centre, now University of Hull) and Dr. Nicole Steinmetz (Case Western Reserve University), CPMV was externally loaded with a chemotherapeutic cargo (here doxorubicin) using different bioconjugate chemistries. Drug release and cell killing profiles indicate that CPMV formulations carrying the drug via a covalent bond showed a time-delayed but greatly enhanced efficacy compared to free drug. Cell imaging data indicate that CPMV is targeted to the lysososomal compartment where the carrier is degraded and the drug released inducing cell killing.

This study goes hand-in-hand with work reported by Dr. Marianne Manchester (University of California, San Diego), who has pioneered the application of CPMV for cell and tissue targeting. Her laboratory previously showed that CPMV targets mammalian cells via the surface marker vimentin. Here, the author turned toward dissecting the trafficking and intracellular fate of CPMV. Data indicate that CPMV entry is mediated via a combination of caveolar endocytosis and macropinocytosis; CPMV then traffics through the early endosomes to the late lysosomal compartment. The understanding of the cellular fate is critical for further development of the platform for payload delivery to subcellular compartments.

Two studies published in the issue describe the use of engineered virus-based capsids to deliver payloads of siRNAs to cancer cells *in vitro* and *in vivo*. The first study, from Dr. Peter Stockley's group (University of Leeds), focuses on siRNA delivery using receptor-targeted MS2 phage. Loading of siRNA payloads into the viral carrier was accomplished making use of self-assembly mechanisms. Cell-specificity was achieved using a targeting ligand, transferrin. The authors show that although commercial cationic lipids deliver more siRNAs to cells, siRNA payload delivery by the virus-based system is more effective. This can be attributed to more efficient intracellular trafficking of the natural virus-based carrier system versus the synthetic system.

The second study, led by Dr. Hyung Jun Ahn (Korea Institute of Science and Technology), demonstrates the utility of siRNA delivery in a preclinical study. Specifically, hepatitis B virus coat proteins fused with p19 RNA binding protein were assembled and loaded with siRNAs; the capsid was then modified with RGD targeting ligands to introduce tumor specificity. The virus-based carrier system was shown to effectively protect the siRNA payload during circulation. Tissue-specific RNA interference was confirmed using tumor-bearing mice and optical imaging.

In summary, virus-based materials of all kinds of shapes and sizes, derived from various organisms, are currently being studied, developed, and tested by scientists and engineers throughout the world. Even though viruses have been around for a very long time, their application as contrast agents and drug delivery vehicles is still a novel and emerging discipline. The field of viral nanotechnology has grown out of its infancy. Basic engineering, genetic, and chemical modification protocols are in place, and new methods for tailoring are continuously being developed. Several laboratories have turned toward the study of virus-based nanomaterials in cells and preclinical animal models—a critical requirement for potential translation from bench to bedside.

In closing, I would also like to thank Dr. Gordon Amidon for devoting this issue to the emerging field of virus-based materials and their application in biomedical imaging and drug delivery. I would also like to thank Ms. Kim Barrett for her assistance throughout the process and help putting this issue together. A big thank you goes to all reviewers and colleagues for their comments and discussions. Finally, I would like to thank all authors for their contribution to the development and application of virus-based materials in biomedical research.

It remains exciting at the virus−biomedical interface!

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