

Extra Special Delivery

► *In Vivo Delivery of Nanoparticles*

Of all its myriad applications, none has engendered more excitement than that of nanotechnology to solve biomedical problems. The ability to utilize nanometer-sized particles as an investigational tool in biomedical research is laying the foundation for a powerful armamentarium for diagnosing and treating disease.^{1,2} Researchers are choosing ALZET® Osmotic Pumps for *in vivo* delivery of these agents in a variety of research models that require controlled, continuous dosing.

Varying in size and structure from a few tens to a few hundreds of nanometers, nanoparticles include liposomes, dextrans, fullerenes, dendrimers, quantum dots, and PEGylated molecules (Table 1). Key attributes of nanoparticles include their high surface area to volume, their ability to absorb and carry compounds such as fluorescent probes and antibodies, and their biocompatibility, especially when applied to tissue engineering.



Besides offering the benefit of continuous and direct delivery of nanoparticles, ALZET pumps are themselves an embodiment of nanotechnology. With more than 35 years of research behind the technology, the pump's nanoporous, semipermeable membrane represents one of the most reliable approaches for continuous delivery of biological agents to small lab animals. Its small size, simple design, controlled delivery, and ease of use,

have made ALZET pumps the recognized standard for continuous dosing *in vivo*.

Liposomes were one of the first nanoparticles to be studied for medical applications, particularly cancer. As early as 1986, researchers at the University of Texas were using ALZET pumps to deliver liposome encapsulated anticancer agents in a murine fibrosarcoma model.³ The first commercially available liposome formulation was approved in 1995 for Kaposi's sarcoma. *Doxorubicin (Doxil™)* is a powerful and toxic chemotherapeutic made more safe and efficacious by encapsulation into liposomes. The drug has since been approved for the treatment of metastatic breast cancer and recurrent ovarian cancer.

While nanoparticles are often designed as a biomolecular carrier, a drug itself may be formulated at nanoscale to function as its own "carrier". Nanomedicine is making great strides in oncology, where the ability to concentrate and localize agents at or within the tumor site is the ultimate goal for either enhanced tumor treatment or improved diagnostic imaging with minimized side effects.

Two studies highlighted in this issue of *Special Delivery* show how ALZET pumps are especially well suited for intracranial nanoparticle delivery. Agrawal *et al.* used ALZET pumps for *in vivo* delivery of siRNA-carrying dendriworms in a glioblastoma mouse model.⁴ Uno *et al.* used ALZET pumps for intracranial delivery of a combination of high-density lipoprotein (HDL) as a second carrier with α -tocopherol-conjugated siRNA for possible use against incurable neurological diseases.⁵ Both studies describe novel approaches for *in vivo* delivery of functional, gene-silencing siRNAs as potential models for siRNA-based therapy.

¹ Sakamoto *et al.* *Pharmacological Research* 2010; 62: 57-89

² Jong, W.H., Borm, P.J.A *International Journal of Nanomedicine* 2008; 3(2): 133-149

³ Drath *et al.* *Immunopharmacology* 1986;12(2):117-126

⁴ Agrawal *et al.* *ACS Nano* 2009; (3)9: 2495-2504

⁵ Uno *et al.* *Human Gene Therapy* 2011; 22: 711-719

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Dendriworm Delivery of siRNA in Mice Using the Alzet Osmotic Pump

—by Charles Versaggi

Small interfering RNAs (siRNAs) are small pieces of RNAi, 20-25 nucleotides in length, used to suppress specific genes of interest for target validation, genetic studies, and for therapy of diseases rooted in aberrant gene expression such as cancer. However, the delivery of functional gene silencing siRNAs into the cytoplasm of target cells *in vivo* is restricted because they are negatively charged and cannot move freely into the cytoplasm of the target cells. This prevents them from being fully exploited as possible therapeutics.

To address this challenge, Agrawal *et al.* report the synthesis of a modular, nanoparticle-based, siRNA delivery vehicle that both maximizes the amount of siRNA that enters cells, and the amount that escapes into the cytoplasm for therapeutic gene-silencing.¹ The synthetic carriers, called “dendriworms”, were tested in a transgenic murine model of glioblastoma, where they were shown to be non-toxic and allow flexible siRNA loading (Refer to “Dendriworms” box on page 3). The versatile delivery vehicle was used to block expression of oncogenic epidermal growth factor receptor (EGFR) variant III, which leads to spontaneous glioblastoma tumor development. Since biodistribution testing of systemically delivered, fluorescently labeled dendriworms showed them to accumulate in several organs and tissues, the researchers delivered siRNA-dendriworms directly to the brain of healthy and disease-model mice using ALZET Osmotic Pumps.

To confirm lack of carrier-induced toxicity from dendriworms in healthy mice, the researchers first determined the biodistribution of dendriworms within CNS tissues of healthy CD-1 outbred mice following intracranial ALZET pump infusion. The pumps were filled with a solution containing 0.5 mg/mL fluorescently-labeled dendriworms with 0.115 mg/L siRNAs, connected to brain cannula, and implanted into mice. After 3 days or 7 days, the animals were sacrificed and the brains were analyzed.

Dendriworm fluorescence was observed in tissue sections from both posterior and anterior to the infusion site for both delivery durations. Fluorescence data revealed that dendriworms dispersed

through brain parenchyma for up to 5 mm from the infusion site in a distance-dependent manner, with the highest dendriworm concentration near the infusion area (Fig. 1). The investigators found no discernable pathology at the dendriworm concentration used.

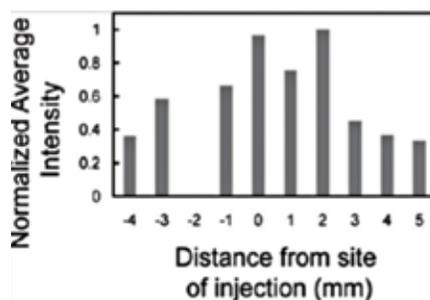


Figure 1. Biodistribution of nanoparticles in the mouse brain after 3 days of ALZET pump delivery. Reprinted with permission from Agrawal *et al.* ACS Nano 2009;3(9):2495-2504. Copyright 2009 American Chemical Society.

Subsequently, the researchers initiated *in vivo* efficacy studies in the EGFR variant III glioblastoma mouse model. ALZET Pumps (Model 1007D) were implanted 20 days post-tumor induction delivering (1) Vivo-Tag-680 dye-labeled dendriworms carrying an EGFR siRNA, or (2) green fluorescent protein-labeled (GFP) siRNA, or (3) aminated nanoworms carrying EGFR siRNA. All three groups received 7 days of their siRNA combination at 0.5 mg/mL of dendriworm and 0.115 mg/mL of siRNA (11 µg total siRNA). The animals were sacrificed and immunofluorescence was performed on the tumor sections to analyze EGFR knock down.

The images and results from quantification of EGFR expression showed that the dendriworms were able to penetrate the tumors, deliver their siRNA payload into the malignant cells, and silence the oncogenic gene expression of EGFR. Compared to dendrimers and nanoworms alone, “enrichment of polymers along the nanoworm backbone promotes improved proton sponge effect and endosomal escape, resulting in better siRNA silencing” (p. 2501).¹

The ALZET Osmotic Pump played an integral role in the success of these studies by enabling site-specific delivery of siRNA-conjugated dendriworms into brain tumors. The researchers concluded, “this paradigm can be applied to other gene delivery platforms to design polyvalent, nanoparticle-based siRNA delivery agents that are effective across various cell lines and amenable to multimodal *in vivo* detection and tracking.” Moreover, “the combination of delivery, imaging, and targeting capabilities within dendriworms offers a versatile and powerful agent to combat malignant glioma” (p. 2501).¹

For a list of references on the use of ALZET pumps for the delivery of siRNA or nanoparticles to the CNS, please contact ALZET Technical Support by email at alzet@direct.com.

¹ Agrawal *et al.* ACS Nano 2009; (3)9: 2495-2504

² Tomalia *et al.* Polym. J. 1985; 17: 117-232

³ Kukowska-Latallo *et al.* Proc. Natl. Acad. Sci 1996; 93: 4897-4902

Dendriworms

Dendriworms are made of *dendrimers* conjugated to *nanoworms*. First reported nearly two decades ago, polyamidoamine (PAMAM) dendrimers are synthetic, branched multivalent macromolecules, typically on the order of 10 nm, and have been used to deliver genes and antisense oligonucleotides.²³ Nanoworms are magnetic and fluorescent, nanometer-sized spheres of iron oxide joined together like pop beads.

Previous studies of siRNA delivery with dendrimers and nanoworms have shown poor efficiencies. Since dendrimers buffer the endosome via their tertiary and secondary amines, Agrawal *et al.* hypothesized that polyvalent conjugation of lower generation dendrimers onto an elongated, magnetic nanoparticle host could generate a construct that would induce a high-proton sponge effect and enable efficient endosomal escape of siRNAs. The investigators were able to synthesize a magnetic, fluorescent dendrimer-nanoworm combination (Fig. 2). The resulting construct contained approximately 7 magnetic nanoparticles, 45-50 dendrimers, and 50 siRNA molecules. Stable under test conditions for up to 6 hours, the dendriworms enabled endosomal release of siRNA across a wide range of loading doses in cultured cells with no significant toxicity.

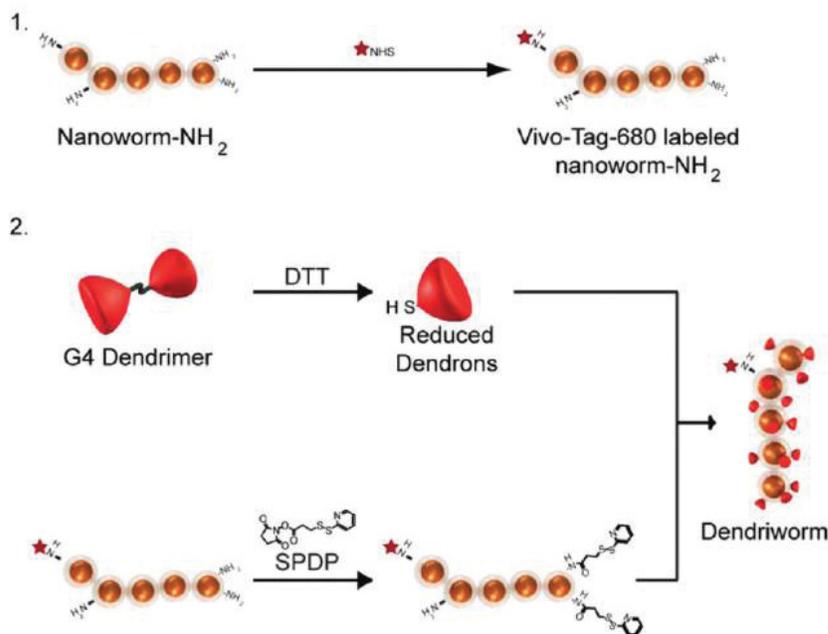


Figure 2. Dendriworm synthesis scheme. Reprinted with permission from Agrawal *et al.* *ACS Nano* 2009;3(9):2495-2504. Copyright 2009 American Chemical Society.

High-Density Lipoprotein Improves Delivery of α -Tocopherol-Conjugated siRNA to the Brain

—by Charles Versaggi and Jose Gadea

The least toxic of all vitamins, vitamin E (α -tocopherol) is a lipophilic natural molecule that shares physiological pathways from blood to the brain, as well as to the liver. Previous studies by Uno *et al.* have shown α -tocopherol can be used systemically as an *in vivo* carrier of siRNA to the liver.¹ These findings suggest a role for α -tocopherol in siRNA therapy for neurological disorders; however, the blood-brain barrier and the specialized metabolism of the brain have made therapeutic delivery to this organ a major challenge.

In this study, the investigators show that ALZET pumps can be used effectively to overcome the blood-brain barrier and deliver novel siRNA nanoformulations directly to brain tissues.² High-density lipoprotein (HDL) was used as a second carrier to facilitate delivery of α -tocopherol-conjugated siRNA (Toc-siRNA) into cells since HDL-like particles in the brain have been shown to play a role in lipid transfer to neurons and glial

cells. The effectiveness of this vector system for *in vivo* delivery of siRNA against the β -site amyloid precursor protein cleaving enzyme 1 (BACE1) was evaluated in a mouse model of Alzheimer's disease.

ALZET pumps (Model 1007D) were filled with either free Toc-siBACE, Toc-siBACE/HDL, or vehicle (PBS) and connected to the ALZET Brain Infusion Kit 3 for direct infusion to the dorsal third ventricle (ICV) of mice. The investigators demonstrated that a 7 day continuous ICV infusion of Toc-siBACE/HDL leads to extensive and specific knock-down of the BACE1 target gene within brain tissues — particularly in the cerebral cortex and hippocampus. Free Toc-siBACE infusion resulted in a weaker transduction signal, indicating that HDL is required for effective siRNA delivery to brain cells. Furthermore, this vector system significantly lowered the dose of siRNA required for silencing the target mRNA. Only 3 nmol Toc-siRNA with HDL was enough to reduce BACE1 mRNA in

the parietal cortex by approximately 70%. Uno *et al.* reported they “could get a similar level of silencing effect in the brain with about a 1000-fold lower amount of siRNA with our method compared with ICV infused free siRNA or antisense oligonucleotides” (p.717).²

The authors speculate that the siRNA delivery efficiency can be attributed to their vector system utilizing the physiological receptor-mediated lipid metabolism pathway in the brain. In this study, ALZET pumps enabled direct delivery of the siRNA formulations to the CNS, whereas HDL facilitated the transport of α -tocopherol-conjugated siBACE into neuronal cells. This delivery approach points to a promising clinical application for treating other neurological diseases such as Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis.

¹ Nishina *et al.* *Mol. Ther.* 2008; 4: 734-740

² Uno *et al.* *Human Gene Therapy* 2011; 22: 711-719

Table 1: Nanoparticle classes delivered with ALZET pumps.

Nanoparticle	Description
Liposomes	Phospholipid “fatty” structures with targeting ligands attached to their surface used for delivering drugs and other agents.
Dextrans	Polysaccharides conjugated to a drug to increase blood residence time, reduce immunogenicity, or provide passive tumor targeting.
Fullerenes	Molecules composed entirely of carbon in the form of spheres, ellipsoids or tubes. Examples include buckminsterfullerenes, buckyballs, and carbon nanotubes.
Dendrimers	Synthetic, branched multivalent macromolecules, typically on the order of 10 nm, used to deliver genes and antisense oligonucleotides
PEGylated molecules	Molecules covalently attached to polyethylene glycol polymer in order to mask the agent from the immune system, increase the agent’s hydrodynamic size for improved bioavailability, or provide water solubility to hydrophobic drugs and proteins.
Peptoids	Protein-like synthetic polymers designed to mimic the structure and functionality of proteins. The synthetic flexibility and biocompatibility of peptoids provide a flexible and robust platform for integrating functionality into defined 2D nanostructures.



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Release Rates and Durations

ALZET pumps are available in 3 different sizes, durations ranging from 1 day to 42 days, and various release rates to meet your experimental research needs.



Pump Model	Reservoir Volume	Duration	Release Rate	Order #
1003D	100 µl	3 days	1.0 µl/hr	0000289
1007D	100 µl	1 week	0.5 µl/hr	0000290
1002	100 µl	2 weeks	0.25 µl/hr	0004317
1004	100 µl	4 weeks	0.11 µl/hr	0009922
2001D	200 µl	1 day	8.0 µl/hr	0000294
2001	200 µl	1 week	1.0 µl/hr	0000292
2002	200 µl	2 weeks	0.5 µl/hr	0000296
2004	200 µl	4 weeks	0.25 µl/hr	0000298
2006	200 µl	6 weeks	0.15 µl/hr	0007223
2ML1	2 ml	1 week	10 µl/hr	0000323
2ML2	2 ml	2 weeks	5.0 µl/hr	0000325
2ML4	2 ml	4 weeks	2.5 µl/hr	0000327