## Drug delivery

### Maximizing efficiency of nonviral gene delivery vehicles

The possibilities for gene therapy in treating disease are many and fascinating. The first gene delivery vehicles consisted of modified viral vectors. Viruses have developed mechanisms to deliver genetic material to mammalian cells, mechanisms that have been optimized over time through evolution. However, during that same evolutionary time frame, mammalian hosts have developed immune defense mechanisms to combat the viruses. This can create dose- and/or therapy-limiting complications when the immune defenses effectively eliminate the viral vector. In addition, in early studies, even though the viral vectors were designed to be replication incompetent, they were found to replicate anyway, which led to the destruction of transfected host cells.

Because of these complications with using viral vectors as gene-delivery vehicles, recent research focus has been on the use of non-viral, polymeric gene-delivery vehicles. The advantage of these vehicles lies not only in getting away from the safety problems that are inherent in viral vehicles but also in the wide range of polymers available. Through the use of different polymers and other components, the complex stoichiometry, surface charge density and hydrophobicity can all be manipulated. All of these factors have a role in the interactions of the vehicle with the cellular lipid components. However, the number of possibilities presents challenges when developing a formulation, especially because *in vitro* measurements do not always correlate well to actual *in vivo* results. To date, relatively little research has been pursued to systematically survey the large number of polymers that are available for use in this new area of gene-delivery vehicles.

Prokop and co-workers recently published a study in which they surveyed a large number of polymeric systems for their efficiency in *in vivo* gene transfer [1]. They surveyed ~40 polymers in various combinations. Favored formulations featured weakly cationic polymers, resulting in formulations with moderate negative charge on the particle periphery. Surprisingly, this observation contrasted with the *in vitro* application of plasmid gene-delivery, where positively charged micelles are necessary. Considering the number of formulations studied, no attempt will be made here to summarize the findings: however, some of the highlights are summarized below.

The various plasmid–polymer formulations were delivered by a gene gun to the subcutaneous tissue of Sprague–Dawley rats. The plasmid consisted of a modified commercially available cytomegalovirus promoter in which luciferase was cloned in as a reporter gene. Soluble plasmid in vehicle was used as a control. Forty-eight hours after application, animals were euthanized and the skin harvested using a biopsy punch. Transfection activity was then measured by a commercial luciferase assay.

Among the polymers tested, many Tetronic® (BASF, Mount Olive, NJ, USA) polymers were found to be effective as gene delivery vehicles. Tetronic surfactants are tetrafunctional block copolymers derived from propylene oxide and ethylene oxide and ethylenediamine polymerization. Only water-soluble polymers were usable as gene delivery vehicles. The proportion of polyethylene (a hydrophilic block) to polypropylene (a hydrophobic block) varies for different classes of these polymers enabling some control over the average hydrophobicity. The tertiary amine moiety provides slightly cationic properties. Among these polymers, a moderately hydrophobic polymer, designated T704, yielded the best activity. Other polymers in this series also exhibited good transfection. These results are believed to be a result of a combined effect of polymer charge and hydrophobicity, both under a certain control by polymer selection. The authors suggest that directed synthesis of polymers could be a possible way of improving characteristics for gene transfection.

Other polymers tested included polycations with a much higher net charge than the Tetronic series and groups of weakly or noncharged polymers. Other polycations exhibited some transfection ability, whereas the weakly or noncharged polymers exhibited a reduced expression when compared with soluble control plasmid. Previous cell-culture studies had indicated that polymers with a net positive charge were optimal for transfection. Unfortunately, there appears to be no systematic correlation between polymer selection for *in vitro* transfection efficiency and *in vivo* gene expression. More systematic studies like this one would be helpful to those in the field of gene therapy who are attempting to optimize polymeric gene delivery vehicles.

**1** Prokop, A. *et al.* (2002) Maximizing the *in vivo* efficiency of gene transfer by means of nonviral polymeric gene delivery vehicles. *J. Pharm. Sci.* 91, 67–76

***John Weidner***
*Scientist, Parallel Synthesis*
*Medicinal Chemistry*
*Emisphere Technologies*
*765 Old Saw Mill River Rd*
*Tarrytown, NY 10591, USA*
*tel: +1 914 785 4792*
*fax: +1 914 593 8250*
*e-mail: Jweidner@emisphere.com*



 1359-6446/02/$ – see front matter  PII: S1359-6446(02)02249-3