Gene Therapy Needs Robust Synthetic Nonviral Platform Technologies

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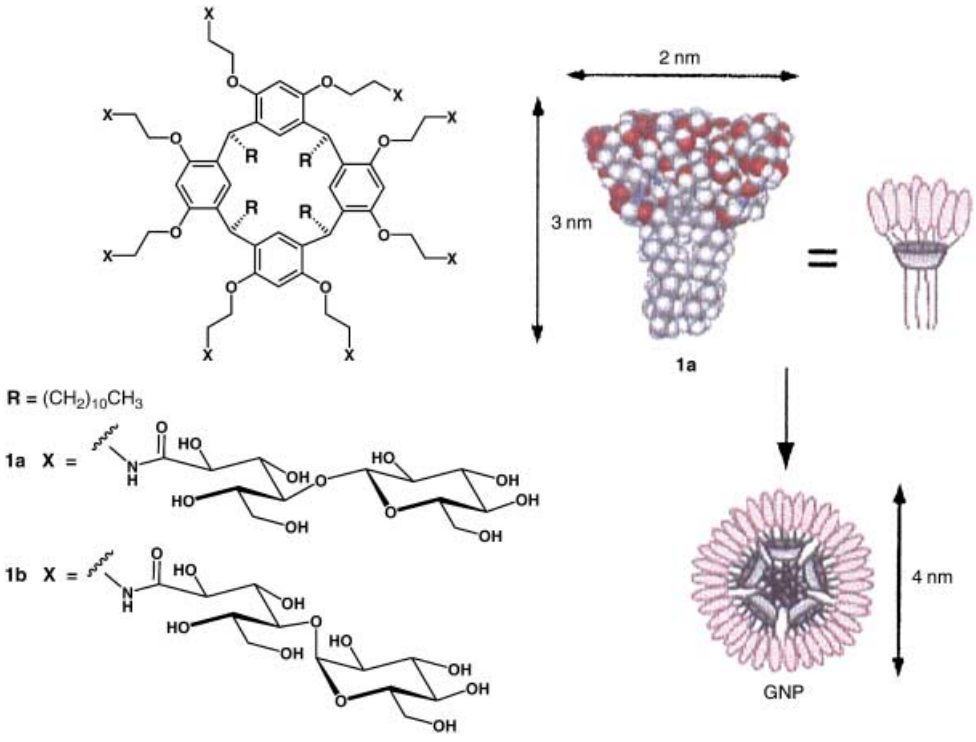
Gene therapy may be described as the use of genes as medicines to treat disease, or more precisely as the delivery of nucleic acids by means of a vector to patients for some therapeutic purpose. Gene therapy has enormous promise but clinical trials have failed to deliver.[1] The primary reason is the inadequacy of the vectors used. Researchers have driven for clinical applications long before vector technologies have been adequately developed or understood. Predictably, there has been a significant decline in scientific and public perceptions of gene therapy. This trend is unhelpful but can be reversed by a period of patient, logical, technical and scientific development of new vector systems, prior to any major second round of clinical trial activity.[1]

But which type of new vector system would be most appropriate to develop: viral or nonviral, synthetic or physical? Opinions may vary, but a balanced assessment suggests that synthetic nonviral vectors should be the main vector systems of choice for routine gene therapy in the future. Synthetic nonviral vector systems have many potential advantages compared with viral systems, including significantly lower toxicity/immunogenicity and potential for oncogenicity, size-independent delivery of nucleic acids (from oligonucleotides to artificial chromosomes), simpler quality control, and substantially easier pharmaceutical and regulatory requirements. Increasing public alarm, particularly with the toxic side effects of virus use, is also strengthening these significant advantages. So what is holding us back? Quite simply, synthetic nonviral vector systems do not appear to be stable enough in biological fluids, neither are they sufficiently reproducible or efficient enough at mediating transfection (i.e., gene delivery and expression).[2] There is a clear need for technical breakthroughs.

The recent paper by Aoyama et al.[3] may represent one such breakthrough. While many synthetic nonviral vector systems have relied upon the central use of cationic lipids or cationic polymers, Aoyama et al. describe a novel class of glycoconjugate amphiphiles (glycocluster amphiphiles) (Scheme 1). These form micelle-like pentameric aggregates in solution known as glycocluster nanoparticles (GNPs), whose size, shape and dimensions resemble those of a viral capsid protein. Glycocluster **1a** was studied in detail and shown to complex and condense plasmid DNA completely at a host/base or host/phosphate ratio (**1a**/P) = 0.5 to form glycoviral particles (approx. 50 nm) with low negative (< −10 mV) to zero ζ potentials (depending upon the exact **1a**/P ratio) that appear to contain only a single plasmid DNA molecule per particle (Figure 1). Remarkably, while these glycoviral particles contain no cationic charges, they are still able to mediate the transfection of HeLa, CHO, Huh-7, HepG2 or COS cells in vitro (serum-free or 10%-serum conditions) giving



Scheme 1. Structures of glycocluster amphiphiles and schematic drawing to show formation of a pentameric glycocluster nanoparticle (GNP) from glycocluster amphiphiles.[3]

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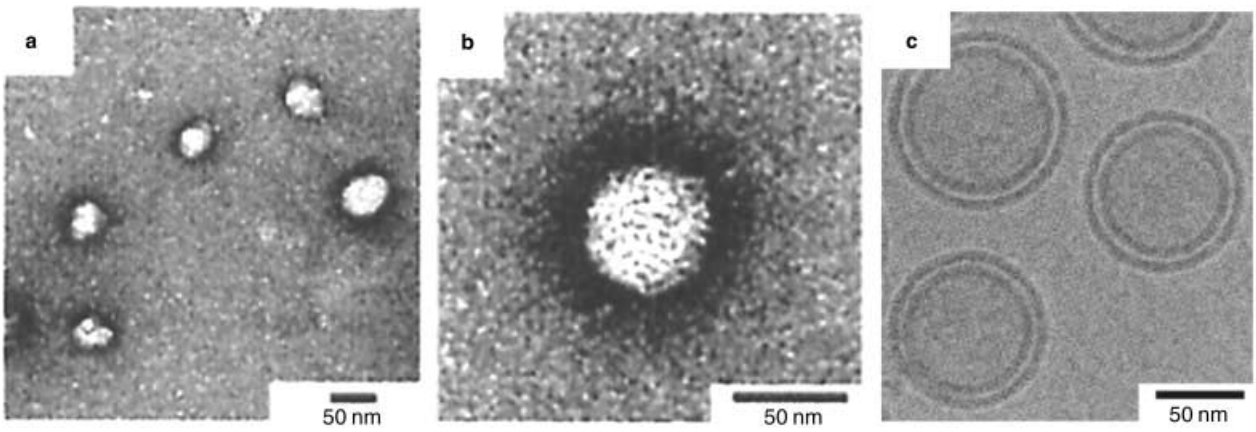

Figure 1. Electron microscopy of particles. a) Transmission electron microscopic (TEM) images of glycoviral particles formed in water from **1 a** and plasmid DNA in a **1 a**/P ratio of 0.7, with uranyl acetate as a negative stainer.[3] b) Enlargment from (a). c) Cryoelectron microscopy image of liposome/mu/DNA (LMD) particles for comparison.[7]

acceptable levels of gene expression, competitive with cationic liposome-mediated gene delivery in vitro.

Recent research, our own included, has amply demonstrated that attempts at systematic improvements of synthetic nonviral vector systems are destined to be fruitless unless the most fundamental problems associated with achieving reproducible and scalable formulations, resistance to aggregation, long term storage and properly reproducible transfection outcomes are convincingly solved prior to future attempts at systematic improvements.[2] By definition, any true synthetic nonviral vector platform technology must embody solutions to all of these fundamental problems. Such size-defined, self-assembly virus-like nanoparticles have been much sought after in the field of synthetic nonviral vector systems, not least because they can lay claim to presenting solutions to the fundamental problems above. Good recent examples are the stabilised plasmid-lipid particle (SPLP),[4, 5] and liposome/mu/DNA (LMD) systems (Figure 1),[6, 7] which comprise at least some cationic lipids. Both are primary vehicles that have been constructed from primary toolkits of well-defined chemical components. Both have been shown to mediate transfection in high serum conditions and even in vivo. Both can now be developed in a sequential and logical fashion for expanded use in vivo by means of modular adaptations/modifications by using secondary toolkits of chemical components, designed to enhance the transfection process and overcome specific barriers to transfection.[8]

The glycoviral particles of Aoyama et al. could well be another valuable synthetic nonviral vector platform technology, valuable especially for being of neutral charge and comprising novel classes of defined chemical components. Evidently, Aoyama et al. will need to provide further evidence to demonstrate that glycoviral particles may be formulated in a reproducible and scalable manner, be amenable to long-term storage and give properly reproducible transfection outcomes. Moreover, assuming that this information will be provided, then Aoyama et al. will also need to demonstrate more than a rudimentary biological efficacy for their particles. Transfections in the presence of high levels of serum in vitro and transfections in vivo will surely be the acid test for these exciting new particles. Nevertheless, the novelty and potential of this research should not be underestimated, and Aoyama et al. are to be congratulated for breaking potentially impressive new ground in the quest for the ideal, clinically viable, synthetic nonviral vector system for gene therapy.

Keywords: DNA · gene expression · gene therapy · nucleic acids · platform technologies

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