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Editorial

The Silent (R)evolution of Polymeric Nucleic Acid Therapeutics

Ernst Wagner[1,2]

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The field of polymeric gene delivery systems (1–3) started 1962 and 1965 with the delivery of infectious poliovirus RNA into cultured cells using cationic proteins or diethylaminoethyl-dextran as carriers (4,5). It developed slowly but steadily in the shadow of more advanced technologies like therapeutic antibody and polymer conjugates (6). Compared with the latter technologies, polymeric nucleic-acid therapeutics have been more challenging and complex in every sense: both the carrier and the therapeutic payload are macromolecules, forming non-stochiometric complexes termed 'polyplexes' (7), initially complicating standardized pharmaceutical production processes. Milestones in the development of polyplexes include in 1988 the development of the first receptor-mediated polyplexes for hepatocytes targeting *in vivo* in rodents (8), the improved performance by incorporation of endosomolytic components, leading subsequently also to the first polymer-based *ex vivo* gene therapy trial under cGMP conditions in 1994 (9), the discovery of polymers such as polyamidoamine dendrimers or polyethylenimine (PEI) with inherent endosome-escape properties (10,11), the incorporation of shielding polymers such as PEG to improve *in vivo* compatibility (12–14), the use of PEGylated polymers in the first *in vivo* polymeric gene therapy trials in airway epithelium (15) or ovarian cancer (16). Additional conceptual break-throughs have been efforts towards biodegradable polymeric carriers (17–19), further developments were fueled also by the syntheses and screening of larger polymer libraries (20,21).

A silent revolution initiated with the discovery of RNA interference, micro RNAs and related mechanisms where gene expression can be modulated at the RNA level. The polymeric carrier field, while still struggling in the gene transfer area with technical problems such as delivery into the cell nucleus and persistence of gene expression, immediately was able to develop first solutions for siRNA delivery (22–24). The synthetic nature of siRNA opens a great opportunities for synthetic chemists. siRNAs can be stabilized by chemical modifications (25) and covalently conjugated with polymeric carrier at defined ratios (26). Covalent conjugates of antisense oligonucleotide analogues have been used to improve gene expression by either specific degradation of micro RNAs (27) or by triggering exon-skipping for partial repair of defective genes (28). In addition various other RNAs like aptamers (29) or poly inosine-cytosine (30) are being explored. The first clinical trials using transferrin-receptor targeted polymer siRNA formulations have been initiated in cancer patients in 2008 (31,32).

Articles (33–37) are examples highlighting the different opportunities and recent developments in the field of polymeric nucleic acid carriers. Injection of naked DNA presents an interesting option for vaccination and muscle transfection, without or with electroporation. This process is not enhanced but rather inhibited by polyplex formation, unless noncondensing copolymers are applied. Pome *et al.* (33) describe a novel noncondensing PEG-polytetrahydrofuran-PEG triblock copolymer which augments DNA based intramuscular gene transfer. The majority of polymeric approaches involve the formation of polyplexes. The cationic polymers required for the nanoparticle formation often cause dose-limiting toxic side effects. For example, the broadly used transfection reagent PEI (11) displays significant cytotoxicity dependant on size and type (branched or linear) of the polymer.

Bonnet *et al.* (34) report on the preclinical pharmacological properties of optimized GMP grade linear PEI which recently entered several clinical trials including phase II bladder cancer therapy and HIV immunotherapy. Systemic application of DNA and siRNA polyplexes triggered only a minor induction of proinflammatory cytokines. Notably, a transient interferon response was observed only with plasmid DNA but not siRNA or sticky siRNA containing polyplexes; the difference can be best explained by the presence of unmethylated CpG in the used plasmid.

Toxicity of cationic polymers can be reduced by introducing biodegradable linkages. Ideally, polymeric carriers should be stable during extracellular delivery in the blood circulation but degrade after cellular uptake. With the intention to create

[1] Pharmaceutical Biotechnology, Center for Drug Research, Ludwig-Maximilians-Universität (LMU) of Munich, Munich, Germany.
[2] To whom correspondence should be addressed. (e-mail: ernst.wagner@cup.uni-muenchen.de)

polymers with such a desired dynamics, Knorr *et al.* (35) designed PEG-oligoethylenimine block copolymers assembled via pH-sensitive ketal linkages. These polymers display high transfection activity at low cytotoxicity, they degrade and release PEG within few minutes at endosomal pH. For overcoming the many different delivery barriers, multidomain polymeric carriers have been designed for systemic delivery into solid tumors. Miyata *et al.* (36) developed polyplex micelles based on a new triblock copolymer containing PEG for enhanced biocompatibility, 1,2-diaminoethane groups containing poly(aspartamide) for efficient endosomal escape, and polylysine for condensation of DNA into 80 nm particles. These novel polyplexes have been successfully applied for systemic delivery into pancreatic tumors.

Another exciting application of polymers for nucleic acid therapy presents the development of gene-activated matrices (GAM), i.e. biomaterial scaffolds comprising gene vectors for tissue regeneration or engineering. This field displays a tremendous potential, based on a rapidly growing knowledge of the cell biology of stem cells and differentiation processes. Exogenous DNA or siRNA turning on or off gene expression may induce cell differentiation or reprogram cells. Schillinger *et al.* (37) report on the generation of a gene vector-doted fibrin glue as a versatile injectable implant. The applied GAMs were based on DNA polyplexes protected by PEG copolymers and co-lyophilized with fibrinogen.

In perspective, a vast variety of therapeutic concepts have demonstrated preclinical efficacy of polymeric nucleic acid carriers at acceptable biocompatibility. Several recent human gene therapy trials have been initiated or been completed, demonstrating safety and also first hints for efficacy. For broad clinical use, polymeric carriers and their nucleic acid formulations have to retain chemical structures accessible for chemical synthesis, but should be more effective and more precise. Improved polymer chemistry including solid-phase, dendron and dendrimer technology will provide such polymers. Along these efforts, programmed, i.e. dynamic, bioresponsive polymers which can adapt to the different delivery barriers, respond to external physical stimuli, and/or carry inherent own therapeutic activity in addition to the nucleic acid part, will contribute to the next steps in the evolution of polymer based nucleic-acid therapeutics.

APPENDIX

Interview Questions for Dr. Ernst Wagner

1. What do you think holds the key to your success as a pharmaceutical scientist?

   To learn about biological mechanisms and try to either mimic or inhibit them by chemical means. The molecular program of life—our own material basis—has created amazing solutions for structures and dynamic processes, providing great inspirations for material sciences and developing therapies.
2. What do you consider to be your key research accomplishments?

   The development of synthetic virus-like gene delivery systems, by abstracting the highly effective delivery functions from viruses and try to restore them in new synthetic structures. The repertoire of synthetic chemical building blocks is far more diverse in the space of physicochemical properties than natural building blocks (like peptides, lipids, carbohydrates). This diversity makes it possible to design chemical analogues to viral protein functions with much simpler and more accessible structures (for example, simple polycations for nucleic acid condensation, PEG for nanoparticles shielding, hydrophobic structures for membrane destabilization).
3. What was the turning point in your career?

   At the age of 28, when I became group leader at the Research Institute of Molecular Pathology in Vienna, diving as chemist into a completely different research environment of world-class disease-focused molecular biology.
4. Who are the individuals who most influenced your research career?

   Prof. Christian Noe taught me that not collecting data like a sponge, but understanding the connections and putting them together in a rational model is what is important, as basis for a hypothesis-driven research. Prof. Albert Eschenmoser showed me how to motivate and build a team, to guide with farsighted vision and a clear program, at the same time providing each team member with freedom to operate and fostering the individual creativity which then fed back into the team's success. Prof. Max Birnstiel as a pioneer in novel research areas showed me that moving from solid established grounds into unexplored new directions may be like jumping into cold water but also most exciting and successful. The inspiring creative approaches of my colleague Dr. Matt Cotten in accessing science showed me that joy and fun in good rigorous science itself is much more important than being regarded as successful scientist by your peers.
5. Pharmaceutical scientists are faced with the dilemma of having to publish in biomedical or basic science journals. Does it mean cutting edge science will not likely be featured in the Pharmaceutical Research?

   Not, if Pharmaceutical Research makes clear that pharmaceutical sciences are more than just a collection of different disciplines, approaching pharmacy from different angles. If it highlights it as forum for unique cutting edge science that advances the performance of the drug development process, introduces novel drug concepts and improved technology for drug discovery, formulations and accelerated evaluation of clinical safety and efficacy.
6. Where is the field of Polymeric Carriers for Nucleic Acid Delivery going? How do the articles in the theme section fill the gap?

   The field has moved into a most interesting age. It started already 45 years ago, developing slowly in the shadow of the more prominent polymer therapeutics field. Clinical developments were limited by moderate efficiency, significant toxicity, and CMC issues in chemistry, manufacturing, quality control of polymers. Step by step solutions for the mentioned

problems were developed. About a decade after the first *ex vivo* gene therapy trial in 1994, recently several *in vivo* gene therapy trials have been initiated or been completed, demonstrating safety and first encouraging hints for efficacy. The advent of siRNA as possible therapeutic modality provides a further boost for the synthetic polymer field. The articles in the theme section illustrate the different opportunities in the field. For example, a novel noncondensing copolymer is described which augments naked DNA based intramuscular gene transfer. Preclinical pharmacological properties of GMP grade linear PEI in DNA and siRNA polyplexes are interesting as this broadly used transfection agent recently entered several clinical trials. PEG-oligoethylenimine block copolymers with ketal linkages designed to rapidly degrade in endosomes maintain high transfection activity at strongly reduced cytotoxicity. Polyplexes based on a new triblock copolymer have been successfully applied for systemic delivery into pancreatic tumors. Other interesting polymer applications are gene-activated matrices (GAM) for tissue regeneration. An injectable fibrin glue-based GAM based on copolymer protected polyplexes co-lyophilized with fibrinogen are also described in this theme section.

7. What are the challenges for Polymeric Carriers for Nucleic Acid Delivery and how can they be overcome?

   Preclinical studies have demonstrated efficacy at acceptable biocompatibility. For broad clinical use, polymeric carriers and their nucleic acid formulations have to remain simple, but should be more effective and more precise. Along these efforts, programmed, i.e. dynamic, bioresponsive polymers which can adapt to the different delivery barriers will enhance the therapeutic window. Improved polymer chemistry including solid-phase, dendron, and dendrimer technology will provide monodisperse polymers with uniform size and topology.

8. What is the key to developing successful collaborative relationships?

   Partners have to be complementary in delivering ideas, technologies or materials into the collaboration which otherwise could not be done by the individual groups alone.

9. What is your philosophy of educating graduate students?

   I rather act as coach than as supervisor. My philosophy is to nurture their own talent and interest in a high scientific standard of performing and describing their experiments. To raise their critical mind and their creativity, to let them find interesting questions and try to solve them. To let them develop the notion that so-called 'problems' might actually be the more interesting part of science where we may learn most, and confidence that many problems can be overcome by careful analysis and subsequent measures.

10. What are the challenges facing the pharmaceutical sciences?

    Redefining its identity and unique position in a rapidly changing field of bioscience, integrating rising opportunities such as novel molecular therapeutics, genomics/proteomics or individualized medicines.

11. What is the place for collaboration with industry in academia?

    Pharmaceutical industry has to be product-driven, with a strong focus on development and finally marketing better drugs for health care. Academia is research-driven, exploring into many directions without market restrictions. Out of serendipity, academia discovers novel mechanisms, technologies, or therapeutic modalities, which only with the expertise and the budget of pharmaceutical industry can be converted into medicines—this is the place and necessity for collaboration.

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Ernst Wagner is Professor of Pharmaceutical Biotechnology at the Center for Drug Research, Ludwig-Maximilians-Universität (LMU) of Munich. As member of the Center for Nanoscience and the cluster of excellence Nanosystems Initiative Munich he coordinates the area ‘Programmed drug delivery’. Dr. Wagner received a PhD in organic chemistry from the Technical University of Vienna. After a postdoctoral stay at the Federal School of Technology in Zurich, he became group leader in 1988 at the Institute of Molecular Pathology and the Vienna University Biocenter, Vienna, Austria, developing synthetic virus-like gene transfer systems. He was F.C. Donders Professor for Biopharmaceutical Sciences at Utrecht University in 1996, Director for Cancer Vaccines and Gene Therapy at Boehringer Ingelheim Austria (1992–2001) where he developed the very first polymer-based clinical gene therapy study in 1994, before joining the LMU in 2001. Dr. Wagner has authored 230 publications and more than 20 patents. He is Editor of Current Opinion in Molecular Therapeutics, Editor of Pharmaceutical Research, and Associate Editor of Molecular Therapy and Journal of Gene Medicine.