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Biomaterials and Drug Delivery: Past, Present, and Future

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This special issue edited by Eric Simanek covers papers from the Symposium on Biomedical Polymers for Drug Delivery held in Salt Lake City, Utah, on March 26–27, 2010. I am very pleased that I was asked to write this perspective, since the symposium was organized by my former students to celebrate my 70th birthday. It provides me the opportunity to thank the organizers of the symposium, Drs. H. Ghandehari, Z. Gu, D. Putnam, K. Ulbrich, and C. Wang, for their considerate efforts, and the symposium sponsors, D. Putnam, Y. Kasuya, P.-Y. Yeh, C. Wang, H.-R. H. Lin, Watson Pharmaceuticals, National Engineering Research Center for Biomaterials at Sichuan University, TheraTarget, LTS, AgriAnalysis, Elsevier, USTAR, Department of Pharmaceutics and Pharmaceutical Chemistry, and Department of Bioengineering at the University of Utah, for their support. Thanks also to Eric Simanek for editing this issue, Kristina “Chi” Ong for coordinating the symposium, and numerous student volunteers for providing a pleasant and smooth environment for the exchange of ideas between more than 150 participants.

Three plenary lectures by Kazunori Kataoka, David Tirrell, and myself; nineteen invited presentations by (alphabetically) Karel Dušek, Jan Feijen, Hamid Ghandehari, Zhongwei Gu, Allan Hoffman, Alexander Kabanov, Sung Wan Kim, Thomas Kissel, Zheng-Rong Lu, Ram Mahato, Tamara Minko, Teruo Okano, David Putnam, Blanka Říhová, Abraham Rubinstein, Vladimir Torchilin, Karel Ulbrich, Chun Wang, and Dong Wang; and over 50 poster presentations reflected the state-of-the-art in the area of biomaterials and drug delivery.

The selection of plenary speakers reflected the two main areas of research in Kopeček’s laboratory: macromolecular therapeutics covered by Kataoka, and biomaterials by Tirrell. The research topics covered by invited lectures included recent designs of carriers (polymers, micelles, dendrimers, hydrogels, self-assembling polymeric materials) of anticancer drugs, genes, siRNA, drugs for the treatment of musculoskeletal diseases, and vaccines; macromolecular imaging agents; as well as studies on the mechanism of action of these compounds. Other lectures covered the design of new biomaterials by protein engineering, self-assembly, cell sheet engineering, and cartilage engineering.

The Symposium provided a great opportunity to meet former students and postdoctoral fellows and catch up on their professional and personal achievements. Many of these former lab members presented lectures, posters, and acted as session chairs. It was rewarding to witness their development from students to excellent scientists and colleagues.

The meeting also coincided with my 50 years of research. I started to work on my M.S. thesis in 1960 and joined the Ph.D. program in September 1961. So I shall try to provide a historical perspective that anchors my activities along a timeline representative of the interests of the community, as suggested by Eric Simanek. My graduate research focused on hydrogels (my Ph.D. advisor D. Lím invented hydrogels). After postdoctoral work at the National Research Council of Canada on membrane transport I returned to the Institute of Macromolecular Chemistry, Czechoslovak Academy of Sciences, in Prague and became an independent Laboratory Head in 1972.

My early biomedical polymer research program focused on the design and synthesis of biocompatible hydrophilic polymers. A systematic study of the relationship between the structure of cross-linked hydrophilic polymers and their biocompatibility was a basis for their translation into the clinic. One of the successful examples was the use of cross-linked poly(2-hydroxyethylmethacrylate) (HEMA)-based hydrogels in rhinoplasty, which produced long-term biocompatibility and excellent cosmetic results.¹

After translating hydrogels into the clinics, we focused our attention on water-soluble polymers.² First, the biocompatibility question was considered more challenging, and second, biocompatible soluble polymers could be used as drug carriers. Our focus was on *N*-substituted amides of (meth)acrylic acid; they represented a group of polymers whose properties could be easily manipulated by changing the substituent on the amide nitrogen. A new hydrophilic polymer, poly[*N*-(2-hydroxypropyl)methacrylamide] (poly-HPMA), was chosen as a candidate for a soluble polymeric drug carrier.³

What was achieved in the studies of HPMA copolymer-drug conjugates?⁴ Methods for attachment of drugs to the polymer backbone were developed; spacers stable in the bloodstream but susceptible to enzymatically catalyzed hydrolysis in the lysosomal compartment were identified; targeting of HPMA copolymer-anticancer drug conjugates to tumors using various biorecognition moieties: antibodies, antibody fragments, saccharides, and epitope-binding peptides was achieved; principles of biorecognition, internalization, and subcellular trafficking were recognized; activity of HPMA copolymer-drug conjugates was established in several cancer models; advantages of combination therapy using polymer bound drugs were demonstrated; and biocompatibility of the conjugates was determined. Finally, an HPMA copolymer-doxorubicin (DOX) was the first of several conjugates to enter clinical trials in the 1990s. These trials have proven the concept of macromolecular therapeutics, demonstrated the biocompatibility of the HPMA copolymer carrier and of the conjugates and confirmed that binding drugs to water-soluble polymers results in numerous advantages when compared to low molecular weight drugs. The advantages of polymer-bound drugs (when compared to low-molecular weight drugs) are (a) active uptake by fluid-phase pinocytosis (nontargeted polymer-bound drug) or receptor-mediated endocytosis (targeted polymer-bound drug), (b) increased *passive* accumulation of the drug at the tumor site by the enhanced permeability and retention (EPR) effect, (c) increased *active* accumulation of the drug at the tumor site by targeting, (d) long-lasting circulation in the bloodstream, (e) decreased nonspecific toxicity of the conjugated drugs, (f) potential to overcome multidrug resistance, (g) decreased immunogenicity of the targeting moiety, (h) immunoprotecting and immunomobilizing activities, and (i) modulation of the cell signaling and apoptotic pathways.⁴

However, the translation of laboratory research into the clinics has been slow. To enhance the development and translation, new approaches are needed. Research areas to be pursued are

- design of conjugates for the treatment of noncancerous diseases
- further studies on combination therapy
- new targeting strategies
- relationship between detailed structure of the conjugates and their properties
- mechanism of action
- mechanism of internalization and subcellular trafficking
- subcellular targeting

- design of backbone degradable, long-circulating polymer carriers

Progress has been made in all these areas, so the future is bright, and the translational potential of HPMA copolymer-drug conjugates is high. Recent work in these areas is described below.

HPMA copolymer conjugates with the well-established bone anabolic agent (prostaglandin E_1 ; PGE_1) are being developed for the *treatment of osteoporosis and other musculoskeletal diseases*.⁵ The biorecognition of the conjugates by the skeleton is mediated by an octapeptide of D-aspartic acid (D-Asp₈) or a bisphosphonate, alendronate (ALN).

Combination therapy using polymer-bound therapeutics has been studied vigorously.⁴ It was shown that combination therapy with HPMA copolymer-anticancer drugs (DOX and photosensitizer chlorin e_6) showed tumor cures that could not be obtained with either chemotherapy or photodynamic therapy alone.⁶ Moreover, most combinations of polymeric anticancer drugs produced synergistic effects. Recently, a new therapeutic strategy for bone neoplasms using combined targeted polymer-bound angiogenesis inhibitors (two per macromolecule: ALN and antiangiogenic TNP-470) was developed. The bispecific HPMA copolymer-ALN-TNP-470 is the first antiangiogenic conjugate that targets both the tumor epithelial and endothelial compartments, warranting its use on osteosarcomas and bone metastases.⁷

New targeting strategies involve identification of targeting peptides using combinatorial approaches: phage display and the chemical combinatorial technique, one-bead one-compound (OBOC) method. The peptides identified by these combinatorial methods frequently have a binding constant of the order of 10^{-6} M. There are two ways to further enhance binding: (a) due to the multivalency effect, conjugates containing several peptides per macromolecule have a higher avidity than those with one targeting moiety; (b) modifying the structure of the peptide selected from a random library by a highly focused secondary library may increase the binding constant by up to 3 orders of magnitude.

Relationship between Detailed Structure of the Conjugates and Their Properties

This seems to be a clear task; however, not enough attention is being devoted to analyze the interplay of individual factors in multivalent conjugates on the final properties. For example, an increase in the number of hydrophobic targeting peptides per macromolecule leads to enhanced avidity of the conjugate and better targetability. However, during internalization, the conformational changes of the macromolecule may lead to the association of side chains terminated in drug with concomitant decrease in the drug release rate. Combination of characterization techniques that include analysis of the conformation of the polymer conjugate by, e.g., FRET (fluorescence resonance energy transfer) needs to be undertaken to optimize the structure of the conjugate.⁸

Mechanism of Action

The hypothesis that free and polymer-bound drugs activate different signaling pathways is based on their internalization mechanisms. Free drug may initiate signaling pathways by interaction with membrane-bound proteins. In contrast, a polymer-bound drug may be hidden in the hydrophilic corona of the random polymer coil and prevented from interaction with membrane proteins during internalization. The drug in a conjugate may interact with proteins and DNA only after being released from the carrier in the lysosomes and translocated into the cytoplasm. Examples are in the literature on the differences in the

mechanism of action of free and HPMA polymer-bound drugs, including doxorubicin and geldanamycin.⁹

Mechanism of Internalization and Subcellular Trafficking

Macromolecular therapeutics are internalized by endocytosis, ultimately locating to the lysosomes. Numerous conjugates were synthesized and evaluated based on this biological rationale. Recently, however, research has been focusing on the identification of different routes of cell entry¹⁰ with the aim to deliver drugs into subcellular compartments different from lysosomes. This direction was mainly driven by attempts to deliver genes or oligonucleotides, i.e., compounds, which may degrade in the lysosomes, but there are other rationales: (a) the activity of many drugs depends on their subcellular location; and (b) the mechanism of action of polymer-bound drugs may be different from that of the free drug. Consequently, manipulation of the subcellular fate of macromolecular therapeutics may result in more effective conjugates.

Subcellular Targeting

Approaches that seem to be effective are nuclear delivery of drugs mediated by steroid hormone receptors that shuttle between the cytoplasm and the nucleus⁴ and mitochondrial targeting mediated by delocalized hydrophobic cations.¹¹

Design of Backbone Degradable, Long-Circulating Polymer Carriers

High-molecular weight (long-circulating) polymer conjugates accumulate efficiently in tumor tissue due to the EPR effect. The higher the molecular weight of the conjugate, the higher the accumulation in the tumor tissue with concomitant increase in therapeutic efficacy.¹² However, the renal threshold limits the molecular weight of the first generation of polymeric carriers to below 40 kDa; this lowers the retention time of the conjugate in the circulation with concomitant decrease in pharmaceutical efficiency. Higher molecular weight drug carriers with a nondegradable backbone deposit and accumulate in various organs, impairing biocompatibility. To this end we designed new second-generation anticancer nanomedicines based on high molecular weight HPMA copolymer-drug carriers containing enzymatically degradable bonds in the main chain (polymer backbone). These multisegment block copolymers are synthesized by RAFT polymerization followed by click (alkyne-azide and/or thiol-ene) reactions.¹³

In parallel we studied *smart biomaterials*, namely, hydrogels containing azoaromatic cross-links for colon-specific delivery;¹⁴ hydrogels translating nanoscale conformational change into macroscale motion;¹⁵ and hydrogels self-assembled from genetically engineered triblock and diblock copolymers,¹⁶ from synthetic polymers and genetically engineered peptide motifs,¹⁷ and from HPMA copolymers containing grafts forming α -helices¹⁸ or β -sheets.¹⁹

Hydrogels containing azoaromatic cross-links are susceptible to degradation in the reductive environment of the colon. They have the potential for colon-specific delivery of peptides, proteins and other compounds sensitive to the low pH in stomach and/or enzymes in the small intestine.¹⁴

Hydrogels Translating Nanoscale Conformational Change into Macroscale Motion

A new design of enzyme-based hybrid hydrogels that employs substrate-enzyme (adenylate kinase-ATP) recognition to induce a conformational change with concomitant decrease of

the hydrogel volume was recently described.¹⁵ This provides a new paradigm for hybrid hydrogel design, where biorecognition induced nanoscale conformational changes translate into macroscale mechanical motion.

Hydrogels Self-Assembled from Block and Graft Copolymers

The coiled-coil, a supercoil formed by two or more strands of α -helices, reveals the potential for the design of well-organized assemblies. Self-assembly of macromolecules mediated by protein domains demonstrated that it is possible to *impose properties of a well-defined coiled-coil peptide motif onto a hybrid hydrogel* containing synthetic polymer primary chains.¹⁷

For example, two distinct, oppositely charged, penta-heptad peptides (CCE and CCK) were designed to create a dimerization motif.¹⁸ Their structure was designed with the goal of achieving an *antiparallel heterodimeric conformation*. A mixture of graft copolymers, CCE-P and CCK-P (P is the HPMA copolymer backbone), spontaneously self-assembled into hybrid hydrogels with a high degree of biorecognition.¹⁸

Drug-free macromolecular therapeutics presents a new paradigm in drug delivery.²⁰ It was hypothesized that the unique biorecognition of CCK and CCE peptide motifs could be expanded past biomaterials design, be applied to a living system and mediate a biological process. This would provide a bridge between the designs of biomaterials and macromolecular therapeutics, the two main interests in our laboratory. Indeed, apoptosis in CD20 positive B cells could be induced by cross-linking of CD20 receptors at the cell surface mediated by antiparallel coiled-coil heterodimer formation.²⁰

Approximately 50 years ago, Lím invented hydrogels²¹ and Jatzkewitz synthesized the first polymer-drug conjugate.²² Since then, the developments in the design of new macromolecular drug delivery systems and smart biomaterials have been amazing. A stage has been reached where it is feasible to mimic the systems developed during evolution, and it commenced to a level where new systems not present in Nature can be designed and evaluated. Such designs are/ will be based on the exact knowledge of the relationship between the structure of macromolecules and their properties. There are still numerous gaps in our knowledge to be filled, but a solid base was created that may act as a springboard for future expansion.

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