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Polymer Genomics: An Insight into Pharmacology and Toxicology of Nanomedicines ¹

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Abstract

Synthetic polymers and nanomaterials display selective phenotypic effects in cells and in the body that affect signal transduction mechanisms involved in inflammation, differentiation, proliferation, and apoptosis. When physically mixed or covalently conjugated with cytotoxic agents, bacterial DNA or antigens, polymers can drastically alter specific genetically controlled responses to these agents. These effects, in part, result from cooperative interactions of polymers and nanomaterials with plasma cell membranes and trafficking of polymers and nanomaterials to intracellular organelles. Cells and whole organism responses to these materials can be phenotype or genotype dependent. In selected cases, polymer agents can bypass limitations to biological responses imposed by the genotype, for example, phenotypic correction of immune response by polyelectrolytes. Overall, these effects are relatively benign as they do not result in cytotoxicity or major toxicities in the body. Collectively, however, these studies support the need for thoroughly assessing pharmacogenomic effects of polymer materials to maximize clinical outcomes and understand the pharmacological and toxicological effects of polymer formulations of biological agents, i.e. *polymer genomics*.

Keywords

artificial vaccines; DNA microarray; drug resistance; signal transduction; phenotype

1. Introduction

This work focuses on the pharmacogenomic effects of synthetic polymers and nanomaterials used in delivery of drugs, genes, and antigens. This new and relatively unexplored field is called “*polymer genomics*” [1]. Pharmacogenomics has emerged as an important field at the interface of pharmaceuticals and genetics, which studies how an individual’s genetic inheritance affects the body’s response to drugs [2]. It holds the promise that drugs might one day be tailor-made for individuals and adapted to each person’s own genetic makeup. Of equal importance are studies of cellular responses to drugs, particularly, in cancer chemotherapy and other areas where biological agents can select for genetic mutations that result in acquired resistance to these agents [3]. Such studies are employing methods of functional genomics, proteomics and bioinformatics to characterize cellular responses to drugs. Based on these foundations, polymer

¹In memory of Academician Victor Alexandrovich Kabanov (1934-2006).

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genomics investigates how polymers and nanomaterials alter cell and whole body responses to biological agents. Rapid development of nanotechnology has recently brought considerable attention to the problem of toxicity of nanomaterials [4]. Because of the enormous promise that nanotechnology holds for society, the concern about the safety of nanomaterials has also become very profound. Novel engineered nanomaterials have unique physicochemical properties including small size, shape, high surface area, surface activity and chemical composition. Due to these properties, these nanomaterials can exhibit toxic effects and may represent a considerable hazard. The toxicogenomic approach is now applied to nanotechnology to understand the origins of toxicity of nanomaterials and develop safe nanomaterials and processes.

Notably, the problem of toxicity of nanoscale materials is not new and was encountered by drug delivery researchers several decades ago in the context of toxicity of water-soluble synthetic polymers, liposomes, nanoparticles and other nanoscale objects [5–7]. The problem has been addressed in many cases by the development of biodegradable polymers, optimization of polymer chemical composition, surface coating of liposomes and nanoparticles and other approaches. Several, nanoscale drugs (“*nanomedicines*”), such as liposomal Doxorubicin, Doxil [8] or albumin-bound Paclitaxel, Abraxane [9] have been used in clinic. Others, such as Doxorubicin incorporated in polymer micelles, SP1049C [10,11] and NK911 [12] are in clinical trials. Clearly, some of the approaches developed in these earlier studies can and will be extended to improve safety of novel engineered nanomaterials, thus addressing major obstacles to their use in a human body. However, even safe polymers and nanomaterials developed for human use may affect the responses to biological agents in the body and these interactions are currently not well understood.

The relevance and urgency of such consideration in pharmaceuticals has become most obvious because of the tremendous growth of work using polymer-based drug and gene delivery systems. The central paradigm of polymer-based drug delivery systems considers polymers used in these formulations as *biologically inert* excipients that protect biological agents from degradation, prolong their exposure to tissues, and enhance transport of biological agents into cells. However, such a view is undergoing major revision due to growing evidence that selected synthetic polymers, when combined with the biological agents (low molecular mass drugs, DNA or antigens), can alter genetically controlled cellular responses to these agents [1,13–15]. This paper provides an overview of such studies. First, we consider polymers and nanomaterials that are currently used or developed for delivery of biologically active agents, and highlight those materials that are safe for human use. Second, we discuss biological membranes as a pivotal biointerface for interactions of polymers and nanomaterials. Third, we discuss toxicogenomics of nanomaterials. Fourth, we analyze the effects of synthetic polyelectrolytes on immune response and studies developing fully synthetic vaccines on the basis of polyelectrolytes. Fifth, we demonstrate that polymers can change gene expression profiles and improve gene therapy by activating transcription through affects on signal transduction pathways. Sixth, we discuss effects involving alterations of apoptotic signal transduction pathways in cancer cells by water soluble polymers mixed or covalently conjugated with antineoplastic drugs. Seventh, we describe effects of water soluble amphiphilic block copolymers in multidrug resistant (MDR) cancers and demonstrate that these effects are selective for MDR phenotype. Finally, we present evidence that amphiphilic block copolymers can drastically alter gene expression profiles and prevent development of drug resistance during biological selection of cancer cells with anti-cancer agents. Based on these studies, we formulate the polymer genomics hypothesis and discuss the significance and future directions of polymer genomics research.

2. Polymers and Nanomaterials

Polymer-based drugs (“polymer therapeutics”) emerged from the laboratory bench in the 90’s as a promising therapeutic strategy for the treatment of certain devastating human diseases [16,17]. A number of polymer therapeutics are presently on the market or undergoing clinical evaluation to treat cancer, rheumatoid arthritis, HIV/AIDS, hepatitis C, multiple sclerosis and other diseases. Most of them represent small drug molecules or therapeutic proteins that are chemically linked to water-soluble polymers, such as poly(ethylene glycol) (PEG), to increase drug solubility, drug stability or enable targeting of drugs to tumors. Separately, PEG-coated liposomes carrying chemotherapeutic drugs have been approved for clinical use [8,18]. They served as first examples of nanostructured biomaterials that found practical application in medicine, collectively called “*nanomedicines*”. Another example of a nanomaterial-based drug is albumin-bound Paclitaxel, Abraxane [9]. In addition, nanoparticles made of poly (D,L,lactide-*co*-glycolide) (PLGA) and other biodegradable materials have been studied for delivery of drugs, genes and vaccines [19]. More recently, a new generation of nanomedicines has emerged, which uses novel self-assembled materials and devices of nanoscale size for delivery of drugs and genes [20–24]. Examples are block copolymer micelles [25], DNA/polycation complexes (“polyplexes”) [26], block ionomer complexes [27,28] and nanogels [29,30]. Of these materials, polymer micelles have been evaluated in human trials for the delivery of anti-cancer agents [11,31]. The promising results of these trials suggest that these nanomaterial types are likely to find practical use in medicine. Further advances in polymer chemistry resulted in the appearance of polymer nanoparticles with unique spatial orientation of molecular groups, including dendrimers [32], and cross-linked polymer micelles [33,34]. In parallel, advances in nanomanufacturing and nanofabrication led to the development of carbon nanotubes [35], and nanofibers [36,37]. In terms of delivery of bioactive molecules, these materials are at relatively early development stages. However, their unique structural and mechanical properties hold great promise for drug delivery, bioimaging and tissue engineering research [38,39].

Compared to the first generation polymer therapeutics, the new generation nanomedicines are more advanced (Figure 1). They entrap small drugs or biopharmaceutical agents, such as therapeutic proteins and DNA, and can be designed to release these agents at the target site. Many such materials contain a hydrophilic polymer brush at the surface, such as sterically stabilized liposomes [8], or have core-shell architecture, such as polymer micelles [40]. The hydrophilic polymers at the surface of these nanoparticles form a protective barrier preventing interaction of the nanoparticle-encapsulated material with the non-targeted cells. At the same time, the nanoparticles can accumulate in the target tissues or cells through a passive targeting (“enhanced permeability and retention effect”) [41] or attachment of specific targeting molecules (e.g. antibodies) to the nanoparticle surface. As a result of interaction of the nanomaterials within cells, their internal constituents are released and become exposed to the cell systems. Furthermore, the degradation of nanoparticles is needed to ensure the removal of the polymer material from the body through renal clearance. Hence, at different stages of the delivery process, various nanomaterial constituents, including those initially hidden within the nanoparticle, can become exposed to the body. The resulting material biointerface has a complex spatial and dynamic character and is a function of pharmacokinetics and biodistribution of the nanomaterial as well as the physical and chemical stability of the nanomaterial in the body.

At the present time, only a relatively small number of polymer materials have been administered in the human body (Figure 2). Of those materials, only an even smaller subset, including examples of amphiphilic, hydrophobic and hydrophilic molecules, has been clinically validated as safe for systemic administration in the body. Each of the validated

materials represents a *tremendous advancement* in therapy from both clinical and commercial viewpoints.

Examples of approved water-soluble or amphiphilic neutral polymers include PEG [42], poly(vinylpyrrolidone) (PVP) and its copolymers [43], copolymers of N-(2-hydroxypropyl) methacrylamide (PHPMA) [44], and poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO-PPO-PEO)² block copolymers (poloxamers or Pluronic®) [45]. These polymers have been utilized in a broad range of applications including, but not limited to, the preparation of soluble polymer-drug conjugates (PHPMA), the surface modification of proteins, liposomes and nanoparticles (PEG, PHPMA, Pluronic®), the preparation of micellar drug formulations (Pluronic®), as well as the employment of these polymers as biological response modifying agents (PVP, Pluronic®).

Examples of clinically validated water-insoluble, biodegradable polymers include PLGA [46], poly(ortho esters) (POE) [47], and polyisohexylcyanoacrylate (PIHCA) [48]. These polymers have been used for the preparation of nanoparticles, biodegradable implants and viscous injectable materials. In addition, one amphiphilic material, poly(styrene-co-maleic acid) copolymer conjugated with neocarzinostatin (SMANCS) dissolved in lipid contrast medium (Lipiodol) has proven efficacious in several clinical trials for the treatment of cancer [49,50].

Charged polymers are also part of the inventory of successful polymer therapeutics and nanomedicines. For example, high molecular mass polyanions, such as polyacrylic resins and copolymers of acrylic acid, have been utilized in various oral dosage forms. There is less clinical data, however, regarding their use in systemic drug delivery formulations [51]. The few well-known examples of systemically administered anionic polymers include heparin and its synthetic analogs, e.g. highly sulfated glycosaminoglycans [52]. However these are biologically active compounds that are used as drugs rather than components of drug delivery formulations. One example of a synthetic polyanion administered in the body is poly(dicarboxylatophenoxyphosphazene) (PCPP) that was evaluated in clinical trials as an immunoadjuvant in a number of preventive vaccines, such as influenza [53,54]. This is a biodegradable polymer, which slowly degrades to produce low molecular weight compounds, ammonium phosphate and hydroxybenzoic acid. This polymer is interesting not only because of its unique immunoadjuvant properties, but also because it can be used as a polyanionic component of constructs for drug delivery and drug release in the body [55,56].

In this context, it is important to point out that polycations, as a rule, are substantially more toxic than water-soluble nonionic polymers and polyanions. Therefore, the development of cationic polymer therapeutics, such as is currently under way for gene delivery applications, obviously represents a substantial challenge to the field. Nonetheless some polycation-based therapeutics have indeed been developed and tested in clinical applications. For instance, chitosan, has been incorporated in a number of oral, topical and injectable drug therapeutics and vaccines [57,58]. Another example of a successful polycation-based polymer therapeutic is “Polyoxidonium”, a partially N-alkylated, partially N-oxidized copolymer of poly(1,4-ethylenepiperaside) [59]. Polyoxidonium is biodegradable polymer that serves as an immunomodulating component of artificial polymer-antigen conjugated vaccines discussed below. The most notable of which is an anti-influenza vaccine “Grippol”, which has been approved by Russian regulatory authorities for human use [60].

Overall, numerous polymeric materials have been introduced in the body that display little if any side effects. These materials are generally regarded as safe. One should expect that several

²Poly(ethylene oxide) or PEO is the same as poly(ethylene glycol) or PEG.

new and novel materials for a diverse array of therapeutic applications will rapidly appear. The key question in the context of the current paper is how do these materials interact with biointerfaces that they encounter in the body?

3. Polymers and Biomembranes: A Pivotal Biointerface

There are, of course, multiple molecular and cellular targets for interactions of polymer therapeutics and nanomaterials in the body. Of those targets, we would like to focus primarily on biological membranes as a very important example of functional biological systems that can be affected by synthetic polymers and nanoparticles administered in the body. The plasma membrane is a functional barrier between the cell and its external milieu. It controls intracellular trafficking and is implicated in coordinating cellular signaling, intercellular communication and immune response [61]. Characteristic of the cell plasma membranes are different endocytosis processes, cellular membrane curvature formation, membrane transport proteins and signal transduction. Endocytosis can occur through various cellular pathways [61]. The best studied pathway depends on cytosolic proteins like clathrin. Clathrin initiates and stabilizes membrane curvature formation. The adaptor proteins bind to clathrin pits and augment the inward pull of the membrane towards the cytoplasm leading to vesicle formation [62]. Formation of membrane curvature is also thought to require changes in lipid composition, presence of integral membrane cytoskeletal proteins and microtubule activity, scaffolding through peripheral membrane proteins and active helix insertion of proteins into membranes.

Another endocytosis pathway is caveolae-mediated trafficking, which regulates cellular signaling [63]. Caveolae, are sphingolipid and cholesterol rich flask-shaped invaginations in the membrane. These invaginations are induced through binding of a dimeric protein, caveolin to cholesterol. Caveolae regulate cellular uptake and homeostasis of cholesterol, as well as activity of various ion channels. In addition, caveolae cluster multiple receptors, such as Insulin, EGF, CCK, Endothelin, Tyrosine Kinases, etc., which enables signal integration. Furthermore, caveolae hosts multiple components of the cell molecular machinery required for efficient signal transduction [64]. These components include, signal transducers (e.g., PKC, MAPK, eNOS and calmodulin), transporters (e.g. IP₃ receptor and Porin) as well as structural molecules (e.g., actin and myosin).

Caveolae and lipid rafts are biochemically distinct but share some similarities with each other [65,66]. Lipid rafts form spontaneously, through self-association of highly saturated lipid acyl chains in the presence of cholesterol. They appear to be very small and flat membrane microdomains devoid of clathrin. Apart from caveolae, lipid rafts compartmentalize various membrane transporters for instance P-glycoprotein (Pgp) implicated in multidrug resistance (MDR) in cancer [67]. In addition rafts assemble signaling receptors which undergo activation, e.g. nicotinic acetylcholine receptor-mediated neurotransmission, T-cell activation, HIV internalization, β -amyloid processing and cell death signaling [68–70]. Disturbing the function and/or assembly of lipid rafts, caveolae and other domains in biomembranes can profoundly affect the signal transduction and the homeostasis of the cells.

It is well known that naturally occurring polypeptides can affect membrane transport and signaling processes [62,71,72]. It is also likely that ionic and amphiphilic synthetic polymers can interact with different membrane domains and alter membrane function(s). For instance, polyelectrolytes, such as polyanions and polycations can interact with positively and negatively charged groups of the membrane proteins, resulting in the formation of protein clusters within the membrane [73]. Polycations can also bind with negatively charged lipids resulting in neutralization of the lipid charges and formation of lipid-polymer domains due to lateral segregation of the lipids within the membranes [74,75]. These interactions are strongly dependent on the polyion charge density as well as the hydrophobicity of the backbone and

side groups of the polyions. It also critically depends on the membrane lipid composition and membrane phase state, i.e. on whether the membrane is “solid” (a rigid gel with little chain mobility) or “liquid” (a liquid crystal with considerable chain disorder). Using liposomes as models of biomembranes it was shown that polycations (e.g., poly(N-ethyl-4-vinylpyridinium) salts (PEVP)) adsorption can induce flip-flop of negatively charged lipids (cardiolipin, phosphatidylserine, and phosphatidic acid) from the inner to outer leaflet of the liquid liposomal membrane, but not in solid membranes [76,77]. Notably, these transitions are accompanied by the transfer of the equivalent amount of the neutral lipid molecules in the opposite direction from the outer to inner membrane leaflet, which enables the liposome membrane to keep its bilayer structure intact. The ability of polycations to induce the flip-flop was abolished when the charge density was decreased or the degree of polymerization (DP) was decreased [78]. The mechanisms of the flip-flop remain unknown but it is noteworthy that selected polycations, such as polyethyleneimine (PEI) were shown to form holes in lipid membranes [79]. These holes may be the “gates”, through which the transfer of the lipid molecules across the membranes can take place.

In contrast to hydrophilic polyions that bind to the external surface of the lipid membranes, polymers containing hydrophobic repeating units can insert within the hydrophobic interior of the membranes. For example, a copolymer of poly(2-propylacrylic acid) (PPAA), displays pH-dependent hemolytic properties and disrupts lipid membranes after a decrease of pH from 7.4 to 5.0 [80,81]. The decrease of pH results in the protonation of the carboxylic groups of PPAA and increase in the overall hydrophobicity of the polymer chain, which inserts in the membrane interior. Such pH-responsive polymers mimic the membrane disruptive properties of fusogenic peptides present in some viruses and toxins, which enable endosomal escape of the virus and toxin material into a cell [82,83]. Amphiphilic polymer surfactants, such as Pluronic[®], bind with the membranes, which results in incorporation of the hydrophobic PPO blocks into the lipid interior, while the hydrophilic PEO blocks remain exposed to the external solution [84]. Several studies have demonstrated that this can result in fluidization of the membranes in the cells [85,86]. Furthermore, select Pluronic[®] copolymers were also shown to significantly accelerate the interleaflet exchange of the lipids [87]. In this case, however, the lipid flip-flop was not associated with the change of the charge asymmetry of the membrane and was a passive process (in contrast to the polycation binding with negatively charged lipids). Both the membrane fluidization [88] and the rates of the flip-flop [89] increase as the length of the hydrophobic PPO block increases and/or the length of the hydrophilic PEO block decreases. Furthermore, the ability of the copolymer to induce flip-flop depended on the lipid composition of the membrane [90]. In particular, cholesterol and some other lipids that increased membrane microviscosity decreased responsiveness of the flip-flop to Pluronic[®].

An important concept to be considered in the context of this work relate to *cooperativity* of molecular interactions characteristic of polymer systems. Usually, binding between polymer species is highly cooperative due to a multiple point attachment of these species to each other. As an example, consider a polyion (A) that binds to an oppositely charged polyion (B) forming an “*interpolyelectrolyte complex*” through a system of cooperative electrostatic bonds [91]. The driving force for the complex formation is an entropic gain due to a release of the small counterions initially condensed with the reacting polyion chains. Although each charged repeating unit of a polyion (A) is only weakly bound to the oppositely charged unit of its polyion counterpart (B), the binding of the polyion chains is very strong and practically “irreversible” due to a significant number of interacting units within the complex. However, interpolyelectrolyte complexes in solution can participate in cooperative reactions of polyion interchange [92]. Namely, a free polyion chain (C) can replace a polyion chain (A) of the same charge sign (Figure 3, Scheme 1). If polyions (A) and (C) have different chemical structure the polyion substitution can be highly selective, and result in complete replacement of one chain with another. Furthermore, the rate and equilibrium of these reactions are strongly

dependent on the chemical structure and length of the reacting polyions as well as the concentration and chemical nature of small counterions [93]. Sharp cooperative transitions from one type of complex to another can occur in such systems upon variation of the environmental parameters.

Moreover, polyions bound to oppositely charged latex particles or liposomes can migrate from one particle to another and even “recognize” particles containing specific targeted molecules (Figure 3, Scheme 2) [94]. Based on these model studies, it was suggested that polyions adsorbed at the cell surfaces can recognize and transfer to selected membranes and/or cell types in the body [95]. In addition, polyions bound to a particular cell type can possibly compartmentalize within certain areas of the membranes, which have greater “affinity” for the polyion binding than other areas. Therefore, while some membrane functional systems or cell types may be affected by a polymer, others may remain unchanged resulting in apparent selectivity of the polymer pharmacological effect(s) in a cell. Similar considerations can be extended to polymers bearing nonionic functional groups, as well as polymers containing hydrophobic backbone or hydrophobic side chains, although molecular bases of their interactions with biomembranes need to be better defined. For instance, upon binding with the membranes of live cells (MDCK), Pluronic® P85 co-localizes with cholera toxin B, a marker for caveolae (G. Sahay, in preparation). Furthermore, this copolymer abolished cellular uptake of the cholera toxin B thus, perhaps, inhibiting caveolae-mediated endocytosis. At the same time, it had no effect (or even accelerated) the uptake of transferrin, internalized via a clathrin-mediated pathway. This reinforces a possibility of compartmentalisation of synthetic polymers within different membrane domains as well as a differential effect of polymers on functional systems in the membranes. Further examples, presenting rather selective effects of Pluronic® on drug efflux systems will be considered below.

4. Toxicogenomics of Nanomaterials

It is increasingly evident that polymers and nanomaterials may affect cell signaling through interactions with cell plasma membranes. They can also be transported within a cell via endocytosis and interfere with normal cell function by interacting with intracellular molecular targets. Compared to relatively simple polymer systems considered in the previous sections, the nanoparticles may have more complex structure, and display multiple functional groups at the surface, which can be hydrophilic, lipophilic, charged or even chemically reactive. Nanoparticles are small and have tremendous surface area and thus can exhibit profound affect on cell function. The interaction of the nanoparticles with the lipid membranes and the transport of nanoparticles into cells are very poorly understood. However, there is an increasing body of evidence that the surface properties of nanoparticles can lead to considerable toxicity. The possible toxic effects of nanomaterials are reviewed in detail in [4]. Toxic responses to nanomaterials can be very profound and are believed to involve oxidative stress in response to generation of reactive oxygen species in the membranes (ROS). For example, water soluble fullerenes (C60) induce formation of the superoxide radical (O_2^-), lipid peroxidation and cytotoxicity in cells. Carbon nanotubes added to keratinocytes induce ROS generation, lipid peroxidation, oxidative stress, mitochondrial dysfunction, and changes in cell morphology [96]. At the lowest level of oxidative stress, cells are responding by the induction of antioxidant and detoxification enzymes controlled by the Nrf-2 signaling pathway. At higher levels of oxidative stress, inflammation is initiated through the activation of pro-inflammatory signaling cascades, for example, mitogen-activated protein kinase (MAPK) and nuclear factor κ B (NF- κ B) cascades. Finally, at the highest level, mitochondrial perturbation disrupting electron transduction and the release of proapoptotic factors leads to a programmed cell death. As a result many nanomaterials may represent considerable hazard, and may be toxic to humans and animals when inhaled or injected. Of course, these materials are not considered for human use in their current form. If ever used as components of nanomedicines, such materials would

have to be substantially modified. For example, their surface would need to be modified to acquire antioxidant properties [97] or covered with PEG to prevent hydrophobic binding to membranes.

Surprisingly, more conventional materials that are used in biomedical and pharmaceutical research may also exhibit considerable toxicogenomic effects. A study of a DNA delivery systems comprised of common transfection agents, Lipofectin and Oligofectamine, revealed marked changes in the expression of several genes in epithelial cells [98]. Particularly, Lipofectin formulation initiated upregulation of the replication protein a1 (*rpa1*), whereas Oligofectamine formulation induced the overexpression of genes involved in apoptosis, such as bcl2-related protein a1 (*bcl2a1*), caspase 8 isoform c (*cas8*), heat shock protein 70 (*hsp70*), and heat shock 60KDa protein 1 chaperonin (*hspd1*). Further, Lipofectin triggered suppression of endothelin receptor type b isoform 2 (*ednrb*), ribosomal protein 16 (*rp16*), and endothelin receptor type b, isoform 1 (*ednrb*). At the same time, Oligofectamine inhibited the expression of the genes annexin a2 (*anxa2*), retinoid x receptor alpha (*rxra*), and s100 calcium-binding protein a8 (*s100a8*). Overall, the downstream functional consequences of the transfection-induced gene expression led to an increased tendency of cells toward early apoptosis.

In another transfection study, it was shown that a polycation, PEI, formulated with plasmid DNA and administered to mouse lungs activated p38 pathway involved in endocytosis, phagocytosis and hydrogen peroxide production [99]. The considerable toxicity of such PEI polyplex formulations observed *in vitro* and *in vivo* was therefore linked to a general stress reaction, inflammatory responses, cell cycle regulation and DNA damage repair.

Clearly, analysis of toxicogenomic responses to new polymers and nanomaterials along with conventional assessment of their toxicity in animal models is now becoming essential for development of safe pharmaceutical formulations with wide therapeutic index and low toxicity profiles [1,13–15]. It is widely accepted that new polymer materials and nanomedicines should undergo a rigorous evaluation for possible toxicogenomic responses. However, what if a polymer is already used in humans and generally regarded as safe? Can such material alone or in combination with biologically active molecules alter cell signaling and biological responses in cells and organisms? To highlight possible implications of polymer genomics for nanomedicine development, we will consider effects of several known polymeric excipients that are used in clinics or in human trials, specifically, Polyoxidonium, PHPMA, PEG and Pluronic®.

5. A Synthetic Polyelectrolyte-Based Vaccine

The studies discussed in this section were conducted by polymer chemists led by V.A. Kabanov and immunologists led by R.V. Petrov and R.M. Khaitov over thirty years ago [100–103]. Their initial studies discovered that linear synthetic polyelectrolytes of diverse chemical structure, when introduced into organisms, markedly intensified bone marrow formation and subsequent migration and settling of stem cells, precursors of immune cells, including macrophages, T- and B-lymphocytes. The examples included a polyanion, PAA and a polycation, poly-4-vinylpyridine, as well as other polyelectrolytes. These synthetic polyelectrolytes had no structural relationship to biopolymers, and hence, they were not antigenic. However, when introduced in mixtures with proteins, natural microbial polysaccharides, or other typical antigens, they acted as immunostimulants enhancing the immune response and increasing antibody production by several times.

Thereafter, it was shown that very small amounts of these polyelectrolytes added to a suspension of isolated B-lymphocytes activated DNA synthesis, induced the cell division, and, in the presence of antigens, triggered antigen-dependent differentiation of cells. Thus, the

polyelectrolytes initiated an immune response in B-lymphocyte cell suspension, without assistance of T-lymphocytes, antigen presenting cells (APC), or other cells of the immune system. Such a mechanism is unique and not encountered in nature. Furthermore, the polyelectrolytes triggered activation of B-lymphocytes *in vivo* in mice devoid of T-cells, which did not respond to the ordinary antigens. However, when the same antigens were introduced in the mixture with the polyelectrolytes, the mice exhibited an equally strong immune response as immunocompetent animals did. Thus, synthetic polyelectrolytes can activate an immune response that bypasses the control programmed by the immune response genes (Ir-genes) of the main histocompatibility complex. As a result, either well or poorly genetically protected species reacted practically equally strong, which provides a unique example of *phenotypic correction* of Ir-gene control of immune response [104]. This phenomenon is based on the general property of linear polyelectrolytes to bind with cellular membranes and aggregate membrane proteins [73]. It was shown that polyelectrolytes induce potassium vs. sodium and calcium ions exchange that activated (K^+ , Na^+)- and Ca^{2+} -ATPases, and served as a signal for activation of division and differentiation of the B-lymphocytes [105].

This group developed a non-toxic biodegradable polycation, “Polyoxidonium” (Figure 2), a water soluble ternary copolymer of 1,4-ethylenepiperazine, 1,4-ethylenepiperazine-N-oxide, and (N-carboxymethylene)-1,4-ethylenepiperazinium bromide permitted in Russia for administration as an immunosimulant to humans [73]. The potency of Polyoxidonium and other polyelectrolytes as immunostimulators was greatly increased when they were covalently conjugated to antigens. It was found that such conjugates enhanced specific immune response to antigens by orders of magnitude. Furthermore, copolymer of acrylic acid (AA) with *N*-vinylpyrrolidone (VP) was shown to significantly increase immune response to the conjugated low-molecular mass trinitrophenyl (TNP) group, which is not even immunogenic [106]. The repeated administration of the conjugate into mice was accompanied by a strong production of TNP-specific antibody-forming cells in the animal’s spleen. A similar effect was observed when bovine serum albumin was attached to the synthetic polyelectrolyte [107]. Discovery of a biological effect through polyelectrolyte conjugation with antigens has opened a way to a novel generation of synthetic vaccines [73]. In particular, individual bacterial or viral antigens, which are not sufficiently active alone, induced specific immune responses when chemically conjugated to Polyoxidonium [108,109]. The immunization of animals with such conjugates elicited protection against lethal doses of the corresponding bacteria or viruses. These studies led to the development of the polymer-subunit human vaccines. One such vaccine, “Grippol”, was obtained by conjugation of Polyoxidonium with protein subunits of influenza viruses, hemagglutinin, and neuraminidase [110,111]. About 50 million recipients were vaccinated with Grippol during the last seven years, and extensive statistical data indicates the high efficiency and innocuous character of the obtained vaccine [60].

Overall these studies suggest that synthetic polyions combined with specific antigens can enhance immune response and can be used successfully for developing a synthetic vaccine. At the time, when these studies were conducted, the use of genomics in immunology was in its infancy and little was known about the cell signaling processes underlying the immune response. Yet, this fascinating work by Russian scientists was, perhaps, the first example of a systematic study demonstrating significance of the ability of synthetic polymers to alter the biological responses and, in our opinion, serves as a precursor to polymer genomics.

6. Activation of Gene Expression by Polymers: A New Gene Delivery Paradigm

This section discusses the effects of synthetic polymers on the delivery and expression of DNA. Non-viral vectors based on polycations have been developed to enhance pDNA delivery [26, 112]. These agents act by 1) binding with the pDNA, 2) condensing the pDNA, 3) protecting

the DNA from nuclease degradation, and 4) enhancing transport of the pDNA into the target cell. Numerous polycations of different structure including linear, branched, dendrimer, block and graft copolymer have been evaluated as carriers for DNA delivery. Considerable effort has been devoted to improving the efficacy of the delivery of the pDNA into the cell and particularly to the nucleus [113]. Much less attention has been in elucidating how the polymers *per se* can affect the expression of genes that are already present or delivered into the cells. This situation is likely to change in the nearest future. For example, a recent study demonstrated that Pluronic® block copolymers enhanced expression of reporter genes under the control of CMV promoter and NF- κ B response element in stably and transiently transfected mouse fibroblasts and myoblasts *in vitro* [1,114,115]. These block copolymers acted as biological response modifying agents by up-regulating the transcription of genes through the activation of selected signaling pathways, in particular, NF- κ B [115]. Furthermore, Pluronic® P85 promoted transport of the pDNA to the nucleus in cells transiently transfected with DNA/PEI polyplex (Z. Yang, in preparation). Pitard et al. have shown that co-injection of Pluronic® L64 with pDNA in the cytoplasm of cardiomyocytes increased the percentage of the cells expressing the reporter gene, while co-injection in nucleus did not [116]. This also suggests that Pluronic® enhanced pDNA nuclear transport. In this respect it is important to note that NF- κ B can bind pDNA in the cytoplasm and transport it to the nucleus through the nuclear import machinery [117,118]. Furthermore, the nuclear localization sequence (NLS) of NF- κ B can import pDNA across plasma membrane in the cell cytoplasm [119,120]. Thus the early stages of gene transfer may be also related to the activation of NF- κ B, which is a rapid response transcription factor. It was shown that Pluronic® rapidly activated NF- κ B in the cells, which can promote the pDNA nuclear import as well as enhance transcription of genes under the control of the NF- κ B response elements (Figure 4) [115]. Alterations in hsp68 expression further suggest that Pluronic® affect stress-related pathways or there is a cross-talk between the stress and other pathways activated by the copolymer. However, these effects were not associated with increased cytotoxicity or decreased cell viability [115].

Pluronic® has also shown substantial promise by increasing gene expression of plasmid DNA in skeletal and cardiac muscle, as well as in solid tumors [116,121–126]. Notably, some of these studies used a mixture of Pluronic® L61 and F127 (called SP1017) [121], which has ca. 1000-fold safety margin upon tissue injections and shown to be safe after intravenous (i.v.) administration in humans in combination with an anticancer drug [10,11]. This opens a real possibility to use Pluronic® clinically to enhance gene delivery in the muscle. Injection of plasmid DNA alone (“naked” DNA) into skeletal muscle results in direct gene transfer into myocytes and sustained gene expression *in vivo* [127,128]. Gene therapies involving intramuscular (*i.m.*) injection of naked DNA were evaluated for the treatment of muscular dystrophies (MDs), local or systemic secretion of therapeutic proteins, and elicitation of immune responses against specific antigens of infectious diseases or cancer [129]. The use of naked DNA can avoid some inherent problems of recombinant viral vectors, including immune and toxic reactions as well as viral recombination [130]. However, the potential of this technique for gene therapy is limited, due to poor transduction efficiency and high variability of gene expression observed with naked DNA. Selected non-ionic polymers, such as PVP, were shown to improve gene expression of naked DNA in the muscle [131,132]. The levels of gene expression in the muscle observed with Pluronic®-formulated DNA were much greater than those reported for PVP-DNA formulations [121] and comparable with the expression achieved by electroporation [116]. Furthermore, Pluronic®-based formulation of a plasmid DNA applied in combination with electrotransfer further increased gene expression in the muscle [126].

Contrary to polycations, Pluronic® does not bind and condense the plasmid DNA, and therefore, does not protect DNA from degradation or facilitate transport of the DNA into the cell [133]. However, compelling evidence suggests that Pluronic® effects involve

transcriptional activation of gene expression. This was reinforced by the discovery of the promoter selectivity of the Pluronic[®] effect in muscle [1,133–135]. In particular, Pluronic[®] activated expression of a reporter gene driven by the CMV promoter, NF- κ B and p53 response elements, while having much less or no effect on the gene driven by the SV40 promoter or AP-1 and CRE response elements [133]. Analysis of the transcription factor binding sites in the CMV and SV40 promoters suggest that while AP-1 and CRE sites are present in both promoters, the NF- κ B is unique for the CMV promoter, and p53 is not present in either of the promoters used. Thus, the effect of Pluronic[®] is consistent with the activation of gene expression by activating the NF- κ B and p53 signaling pathways. Interestingly, some natural amphiphilic agents, such as green tea polyphenol epigallocatechin-3-gallate, were shown to activate both NF- κ B and p53 in the muscle cells [136]. Importantly, AP-1, a pro-apoptotic factor that is also frequently regulated by the NF- κ B system, was not responsive, which indicates that Pluronic[®]-mediated effect on transcription is selective and not a result of a general non-specific activation of immune defense system such as NO-mediated burst.

NF- κ B and frequently p53 are involved in inflammatory events, suggesting that activity of Pluronic[®] in gene delivery is related to the inflammation signaling pathways. Remarkably, the effect of Pluronic[®] on gene expression in the muscle was exerted in immunocompetent mouse strains Balb/c and C57Bl/7, but was lost in the athymic nude mouse strain, which has a defective immune system [133]. Pluronic[®] copolymers have long been known as adjuvants increasing immune responses and inflammation *in vivo* [137–142]. These biological effects involve histamine release in the mediator containing cells, which in turn correlates with the ionophore properties of the block copolymers selective for monovalent cations [143,144]. Therefore, it is likely that the effects of Pluronic[®] on cell signaling and activation of gene expression are also related to its interactions with biomembranes.

Overall, Pluronic[®] block copolymers can induce expression of genes through mechanisms that differ from the delivery of the DNA into the cell. This raises the question as to whether up-regulation of expression of genes delivered into cells can also take place in the case of various other nonviral polymer-based gene delivery systems? Future studies should address this basic question as well as characterize the mechanisms of the effects of the amphiphilic block copolymers on gene expression, identify the structural determinants of the polymers responsible for such effects, and determine to what extent these effects depend on the type of genes or promoters that control the gene expression.

7. Alteration of Responses to Drugs by Polymers: A New Drug Delivery Paradigm

Although polymer-drug formulations are used primarily to improve the *delivery* of the drugs to their molecular targets, evidence began to mount that polymer excipients in these formulations may also affect the intracellular signal transduction pathways that underlie specific responses of the cells to the drugs. Examples include anti-cancer drugs that are chemically bound to polymer carriers, incorporated into polymer micelles, or simply co-administered with the polymers, such as Pluronic[®] [145–148]. For example, conjugation of Doxorubicin (Dox) with PHPMA results in the induction of additional (compared to the free drug) caspase-dependent apoptosis signaling pathways in human ovarian carcinoma cell lines, A2780 and A2780/AD [145]. Specifically, the polymer-drug conjugate induced apoptosis by up-regulating *Caspases* 3, 6, 7, 8, and 9, while the free drug caused induction of only *Caspases* 9, 3, and 7. Moreover, following treatment of cells with PHPMA-Dox conjugate, the *bcl-2* gene responsible for the cellular defense mechanism was down-regulated, resulting in the further stimulation of the pro-apoptotic route. Conversely, free Dox increased expression of *bcl-2* gene, which partially mitigated its cytotoxic activity.

Alterations in signal transduction were also demonstrated for another polymer-drug conjugate in A2780 cancer cell line [146]. It was shown that conjugation of Camptothecin with PEG considerably increased expression of pro-apoptotic genes encoding caspase activators, *SMAC* and *APAF1*, as well as *Caspases* 9 and 3, compared to the effects of the free drug. Polymer-conjugated drug also induced up-regulation of genes related to the cellular defense, *bcl-2* and *bcl-xl*. Nevertheless, induction of apoptosis with the polymer-conjugated drug was developed faster and to a greater extent than with the free drug, which resulted in the enhancement of the cell death pathways.

Pluronic® P85 formulated with Dox also enhanced the pro-apoptotic response in MDR human breast carcinoma cell line, MCF7/ADR [148]. Specifically, in the presence of the block copolymer the expression of proapoptotic genes, *BAX*, *P53*, *APAF1*, *Caspase 9* and *Caspase 3*, was up-regulated, while the antiapoptotic cellular defense, *BCL2*, was decreased compared to Dox alone. Therefore, in addition to enhanced drug delivery in the cells due to the inhibition of the drug efflux systems, the block copolymer also enhanced the cytotoxic effect of the delivered drug by altering the cell death pathways in the MDR cells.

DNA microarray has been a useful tool to elucidate complex and interrelated cellular responses to polymeric drug formulations [149]. It was applied to analyze the responses in non-small cell lung cancer PC-14 cells to Cisplatin (CDDP) incorporated in polymeric micelles with PEG-poly(glutamic acid) block copolymer [147]. Interestingly, the expression levels of genes related to cell cycle regulation, apoptosis, detoxification, and DNA repair were different in the cells treated with free CDDP and CDDP formulated with the micelles (CDDP/m). For example, incorporation of CDDP into polymer micelles resulted in down-regulation of the gene expression of integrins and matrix metalloproteases (MMPs) families, while free CDDP up-regulated these genes. This may reduce tumor invasion, metastasis, and angiogenesis, leading to additional therapeutic benefits of CDDP/m.

Overall, these studies suggest that various polymer components in drug delivery formulations can change the biochemical effects of the anti-cancer drugs in the cells by altering the normal function of signal transduction pathways. This can drastically change the modern drug delivery paradigm with its focus on improved formulation and delivery using existing polymeric excipients or novel nanomaterials. Due to possible effects on cell signaling, a polymer-drug combination should be considered as a whole like a new drug rather than a simple reformulation form. Of course, this approach has certain limitations, as in many cases the effects of current polymeric excipients on the cellular and body responses are negligible. However, the concept of pharmaceutical excipients as components of a formulation other than the active substance has recently undergone a substantial paradigm shift [150]. This is because many excipients that were considered “inert” actually display important and sometimes very useful activities, making them essential constituents of the formulation. As a result, those components are now sometimes termed “functional excipients”. Therefore, it is highly likely that we will face an increasing number of cases where synthetic polymers and nanomaterials used in formulations exhibit a significant effects on the cellular responses to the drugs. The following section considers one such example that has been, perhaps, most thoroughly studied as of today.

8. Phenotype-Selective Sensitization of MDR Tumors by Pluronic®

In contrast to genotype, which is defined as inherited genetic makeup of the body, the phenotype includes properties acquired as a result of environmental effects. In an evolutionary sense, biological selection acts on the phenotypes, because differential reproduction and survival selects for certain phenotypes. Thus, alterations in phenotype are the earliest, preceding the genotype changes, while still playing a critical role in the responses and survival of the cells. One example of phenotypic changes of high significance for chemotherapy of cancer and other

diseases is the development of MDR in response to chemotherapy. Tumors with MDR phenotype overexpress drug efflux transporters belonging to a superfamily of ATP binding cassette (ABC) proteins, such as Pgp (MDR1), multidrug resistance-associated proteins (MRP), and breast cancer resistance protein (BCRP) that pump drugs out of a cell and thus reduce their cytotoxicity [151,152]. Furthermore, the glutathione (GSH)/glutathione *S*-transferase (GST) detoxification system is frequently activated in MDR cells, further contributing to drug resistance [153,154]. Another impediment to treatment, which is present in MDR cells, involves the sequestration of drugs within cytoplasmic vesicles, followed by drug extrusion out of the cell [155]. Drug sequestration in MDR cells is achieved through the maintenance of abnormally elevated pH gradients across organelle membranes - by the activity of H⁺-ATPase, an ATP-dependent pump [156,157]. The combination of several mechanisms of drug resistance complicates chemotherapy and reinforces the need for developing novel drug formulations effective against drug resistant cancers.

The use of Pluronic[®] block copolymers in formulations is one approach to overcome MDR in drug resistant cancers [158,159]. Pluronic[®] molecules have been shown to inhibit Pgp and drastically enhance cytotoxicity of many anticancer drugs in MDR cells. The block copolymers exert a “double punch” effect on Pgp through 1) decreasing Pgp ATPase activity [160] and 2) intracellular depletion of ATP necessary for Pgp function (Figure 5) [86,161]. The synergy between these two effects results in a potent inhibition of Pgp efflux system, as well as some other ATP-dependent drug efflux transporters, such as MRP [160,162]. Furthermore, Pluronic[®] inhibits other energy-dependent drug resistance mechanisms, such as GSH/GST detoxification system and cytoplasmic drug sequestration in MDR cells [159,162]. Consequently, very potent chemosensitization of MDR cancer cells by Pluronic[®] copolymers is accomplished.

The sensitization effect of Pluronic[®] in MDR cells is due to the ability of block copolymer molecules to incorporate into cellular membranes and alter the membrane structure [88]. Two major targets of Pluronic[®] effects are the cell plasma membrane (where Pgp is localized) and the mitochondria membrane, where Pluronic affects normal functioning of the drug efflux transporters and respiratory chain, respectively. The potency of Pluronic[®] molecules in inhibition of Pgp ATPase is increased as the length of the hydrophobic PPO block is increased [88,163]. This correlation reflects the ability of the copolymers with larger hydrophobic chain to illicit more profound perturbations in the membranes. The most efficacious Pluronic[®] copolymers in terms of sensitization of MDR cells are those with an intermediate length of the PPO chain and relatively short PEO chain [88,163]. Such copolymers can translocate inside the cell and thus affect intracellular compartments, in particular, mitochondria, inducing the greatest ATP depletion effects [88]. To determine the stages of energy conservation affected by Pluronic we examined the rates of total cellular metabolism (glucose uptake), anaerobic metabolism (lactate secretion) and aerobic metabolism (oxygen consumption) in MDR and non-MDR cells (paper in preparation). These experiments suggest that Pluronic[®] inhibits total metabolism and decreases ATP intracellular levels in MDR cells by inhibiting respiration in mitochondria. In contrast, anaerobic metabolism was enhanced by Pluronic[®], especially in MDR cells. Therefore, respiration rather than glycolysis is a principal target for Pluronic[®] effects on energy metabolism.

The membrane-active properties of Pluronic[®] are rather nonspecific. Thus, it is quite remarkable that both the ATP depletion and sensitization of MDR cells correlate with the expression of the drug resistance proteins in these cells [164]. As the expression of Pgp was increases, ATP depleting potency of the block copolymer also increases. Furthermore, respiration in isolated mitochondria from MDR cells is inhibited by Pluronic[®] to a much greater extent than in mitochondria from non-MDR cells (in preparation). The selective suppression of energy production in MDR cells by Pluronic[®] was attributed to differences in the major

energy sources used by MDR and non-MDR cells. In MDR cells that utilize fatty acids as a source of energy [165], Pluronic® has a pronounced effect on respiration. In contrast, Pluronic® exerted only mild effect on glucose-based respiration of drug-sensitive cells. Thus, appearance of the MDR1 phenotype imposes high energy requirements in MDR cells and contributes to the elevated responsiveness of these cells to Pluronic®.

Such selectivity of Pluronic® with respect to cells displaying the MDR1 phenotype is unique. It serves as an example that the cell phenotype can determine the cellular responses to polymer-drug formulations. Based on these studies, MDR1 is a valuable pharmacogenomic marker for prediction of successful outcomes of chemotherapy with drug-Pluronic® formulations [164]. Indeed, of several dozen different cancer lines examined in our laboratory, cancers overexpressing Pgp were found to be the most responsive to the sensitization effect of Pluronic® (paper in preparation). This reinforces the use of Dox-Pluronic® formulation (SP1049C) in human trials for treating adenocarcinoma of the oesophagus and other cancers with high incidence of MDR [11]. Future studies will extend the pharmacogenomic approach to correlate gene expression profiles and chemotherapeutic responses to polymer drug formulations. This helps to identify genetic markers that can predict the greatest response to such formulations and maximize the clinical benefits. It is possible that similar studies could also be useful for improving therapeutic outcomes using other polymer therapeutics and nanomedicines, including drugs incorporated in liposomes, polymer micelles, and polymer-drug conjugates.

9. Alteration of Genomic Profiles in Drug-Selected Cancer Cells by Pluronic®

Cancer cells mutate in response to chemotherapeutic agents resulting in drastic changes in gene expression profiles and development of drug resistance. Currently, the principal question is whether polymer excipients can affect the outcomes of these changes and prevent (or redirect) the development of drug resistance. Recent studies demonstrated that Pluronic® P85 altered gene expression profiles in cancer cells selected with Dox [1,166,167]. Specifically, MCF7 cells cultured in the presence of Pluronic® were unable to stably grow at concentrations of Dox that exceeded 10 ng of Dox/mL in culture medium. In sharp contrast, MCF7 cells cultured in the absence of the block copolymer resulted in the selection and stable growth of cells that tolerated a 1000 times higher concentration of the drug. Detailed characterization of the isolated sublines demonstrated that those cells selected in the polymer-drug formulation did not show amplification of the MDR1 gene, resulting in their high sensitivity to the drug. Conversely, cells selected with Dox alone showed an elevated level in the expression of the MDR1 gene along with an increase in the expression level of the drug efflux transporter, Pgp, contributing to the high resistance of the cells to Dox. This result for the first time suggests that the formulation of the antineoplastic drug, Dox, with Pluronic®, also *prevents the development* of MDR in breast cancer cells [167]. Subsequently, a similar result was obtained for leukemia cancer selected with Dox-Pluronic® *in vitro* and *in vivo* (in preparation). In view of the fact that MDR1 is found at high levels or more frequently in recurrent or relapsed cancers as well as after the initial chemotherapy treatment [168], Pluronic® may be of substantial benefit in cancer chemotherapy.

Besides the amplification of the MDR1 gene, a number of other mechanisms of resistance are known to be triggered in cancer cells in response to exposure to an antineoplastic agent. These include altered expression and mutations of topoisomerase II and I [169]; activation of metabolic enzymes such as cellular retinoic binding protein, epoxide hydrolase, and thioredoxin [170,171]; and inhibition of apoptotic signal transduction pathways in the affected cells [170,172,173]. Given the high level of genomic instability and mutations in cancer cells, these mechanisms are often displayed in complex and multifactorial fashion, allowing the

cancer cell many escape routes to survive the chemotherapy. To address this possibility, microarray analysis of the global profile of genes expressed in the selected sublines was carried out [167]. An unexpected result of this study was that selection of the cells with the drug in the presence of Pluronic® led to profound alterations in the levels of genes including some genes that were not affected in the cells selected with the drug alone or with the block copolymer alone. Notably, in some cases Pluronic® appeared to enhance the effect of the drug on gene expression. Examples include genes related to drug resistance, such as the vacuolar proton pump that may promote degradation of the drugs in the lysosomes [174], and β -tubulin that may result in appearance of drug resistance to paclitaxel via altered microtubule assembly, drug binding, and dynamics [175]. In addition, increased expression of an estrogen-dependent factor gene TFF1 may result in enhanced cell proliferation and invasiveness [176]. Another group of genes reportedly upregulated in cells selected with Dox and Pluronic® is involved in signaling and regulation of apoptosis, such as programmed cell death 5 (*PDCD5*) and tumor necrosis factor receptors (*TNFSF10A*) [167].

Overall, these results reinforce the need to consider the pharmacogenomic effects of polymer excipients, including a potential for development of new resistance mechanisms, which are not found with low molecular mass drugs. It is remarkable that a formulation of a chemotherapeutic drug with a polymer excipient, which is not even covalently bound to this drug and which alone has little if any effect on gene expression, can drastically change the pharmacogenomic responses to the drug. This conclusion may be of general significance for other nanomedicines, such as polymer drug conjugates and drugs entrapped in polymer micelles, liposomes, and others.

10. Polymer Genomics Hypothesis and Future Studies

The studies described in this review suggest that synthetic polymers and nanomaterials can have rather selective *phenotypic effects* in cells and in the body by affecting cell signaling involved in inflammation, differentiation, proliferation and apoptosis. These effects result from interactions of polymers and nanomaterials with cell membranes and delivery of polymers and nanomaterials to intracellular organelles. The ability of the cells and organisms to respond to these effects can be dependent on *phenotype* (e.g., ATP depletion in MDR cells) or *genotype* (e.g., effects of Pluronic® on gene expression in the muscle in immunocompetent and nude mouse). In selected cases, polymer agents can bypass major limitations to biological response imposed by the phenotype or genotype (e.g. sensitization of MDR cells by Pluronic® or bypass of Ir-genes control of immune response by synthetic polyelectrolytes). For many polymer excipients these effects can be relatively benign, as they do not result in cytotoxicity or toxicities in the body. Indeed, the polymers described in this paper are generally safe and some of them, e.g. Polyoxidonium, PHPMA, PEG and Pluronic®, are used in humans without major side effects. Furthermore, exposure of cells to these polymers alone, such as Pluronic®, does not induce major genomic alterations, suggesting that the cells can withstand the phenotypic affects of the polymers without major changes in cell function and life cycle. However, when combined with antigens, bacterial DNA, or cytotoxic agents, either by mixing or covalent conjugation, these polymers can drastically alter genetically controlled responses to these agents. Thus the central hypothesis of polymer genomics is that “*weak phenotypic effects of polymer materials can be greatly amplified in the course of responses of a cell or organism to biologically active agents by changing the magnitude, and in selected cases, direction of these responses*” [15]. Several examples have shown that combinations of synthetic polymers and biological agents can be beneficial for health, such as enhancement of protective immune response after vaccination or prevention of development of MDR during chemotherapy. In other cases, these effects may result in unwanted consequences, which should be analyzed from the standpoint of toxicity, immune reactions, and development of new resistance mechanisms.

Future studies should determine the molecular mechanisms by which polymers and nanomaterials modify biological responses. Based on available work, such as effects of Pluronic® in MDR tumors, these mechanisms can be complex and include multiple components. Mechanistic studies should focus on identification of structural determinants of the polymers and nanomaterials that render them biologically active. One unifying property of polymers and nanomaterials is the ability to engage in cooperative interactions with biomacromolecules and sub-cellular components. Such interactions can result in profound changes in various cell processes, for example, changes in the membrane transport, signal transduction or energy conservation. From this standpoint, even polymeric species assembled from small molecules, such as liposomes or nanosuspensions of hydrophobic drugs, can also engage in cooperative interactions within the cells and thus modify activity of biological agents. This consideration becomes particularly significant because of increasing use of polymer therapeutics in humans and the development of novel nanomedicines for drug and gene delivery. In addition to being *sufficiently large*, and being capable of cooperative interactions with other macromolecular objects, polymers and nanomaterials are also *sufficiently small*, and can be transported into cells reaching different critical sub-cellular targets. Thus, nanoscale range of sizes is particularly important in view of biological response modifying activities of polymers and nanomaterials.

The mechanistic studies should also focus on identification and analysis of the signal transduction pathways affected by different types of polymers and nanomaterials. In this respect approaches using functional genomics, proteomics and bioinformatics can be of great help. It is possible that this analysis will allow relating different pathways affected by polymers and nanomaterials to specific molecular and supramolecular characteristics of these materials. Obtaining information about structure-functional properties of polymers and nanomaterials will further facilitate engineering of such materials tailored for different types of biological applications. It can also enable designing “responsive” materials, which can be delivered to specific sites in a body and within a cell to locally modulate signaling pathways using external forces, such as applied magnetic field, heat or ultrasound. Even though such a goal may currently appear futuristic, it is achievable with modern developments in site specific drug delivery and expanding possibilities for nanomaterial design, particularly in the area of magnetic and temperature-responsive nanoparticles.

Once substantial clinical information regarding various polymer therapeutics and nanomedicines becomes available, it will also be possible to relate the toxicological and immunological properties of such materials, as well as their efficacy in specific applications to their potential to modify biological response and pharmacogenomic effects. This will underscore the clinical significance of the polymer genomics approach to human health.

11. Conclusions

Studies described in this paper present a compelling case that synthetic polymers and nanomaterials can have significant effects on signal transduction mechanisms, gene expression, and effects of biological agents. The field of polymer genomics has been identified recently [1]. However, some of the ideas underlying this new field have been discussed before. It is illustrated by the following citation: “...*exciting prospects are linked to the studies of synthetic polyelectrolytes as novel types of physiologically active agents. Ability for cooperative interaction with charged cell membranes and other components of living systems, most likely, allows for extremely strong affects of ionizing macromolecules of different chemical composition on the basic functions of the living organisms, affects that are hard to predict now, and which can be directed towards the benefit of the organism as well as to its harm.*” (translated from [177]). It has become clear that the range of materials that can affect cell responses is much broader than just ionized macromolecules. These materials include

nonionic water soluble amphiphilic polymers, and perhaps a variety of nanoparticles of different composition and structure. Importantly, among these materials are safe polymer excipients, such as Pluronic® block copolymers and PEG currently listed in Pharmacopeias, PHPMA and others. The pharmaceutical industry refer to “GRAS” compounds (“*generally regarded as safe*”), among which some polymer excipients are listed. Based on the present consideration it is proposed that this approach should be used with certain caution since, at least some polymer excipients, particularly, in combination with biologically active agents cannot simply be considered inert. As a consequence, the combinations of a biological agent and excipient in a drug or gene delivery system may need to be considered a “new” entity rather than a re-formulation of the existing agent. In selected cases, the activities of polymer excipients may have beneficial effects, such as protective immune response during vaccination or overcoming drug resistance in chemotherapy. In some cases these activities may result in unexpected and unwanted side effects. In any case, the pharmacogenomic effects of polymers and nanomaterials in formulations with biological agents require thorough and systematic characterization to maximize clinical outcomes and understand the pharmacological and toxicological effects of these formulations, i.e. *polymer genomics*.

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Fig. 1.

Examples of self-assembled nanomaterials with core-shell structure: **(A)** PEGylated liposomes produced from mixture of PEGylated and non-PEGylated lipids [8]; **(B)** polymer micelles with hydrophobic core formed by amphiphilic block copolymer [21]; **(C)** polyplex obtained by reacting DNA with cationic block/graft copolymer in presence of Pluronic® [178]; **(D)** polymer micelles with ionic core synthesized by condensing double hydrophilic block copolymer containing ionic block (poly(methacrylic acid) (PMA)) by Ca^{2+} , cross-linking of the ionic blocks in the core of the formed micelles and removal of the condensing agent [33].

Water-soluble or amphiphilic neutral polymers

Water-insoluble polymers

Anionic polymers

Cationic polymers

Fig. 2.
Chemical structures of some polymers administered in the body.

Scheme 1

Scheme 2

Fig. 3.

Scheme 1: A cooperative polyelectrolyte interchange reaction: At the first stage the free polyion (C) diffuses to the interpolyelectrolyte complex (AB); the chains reside together in the intermediate tertiary complex, while the oppositely charged polyion (B) migrates from polyion (A) to polyion (C); at the last stage the new interpolyelectrolyte complex (AC) is formed [91].

Scheme 2: Migration of polyions adsorbed at the oppositely charged particles: Polyelectrolyte adsorbed at the surface of untargeted particles (U) cannot dissociate into a solution; instead the particles migrate to each other forming an intermediate complex; the adsorbed polyion migrates to the surface of the targeted particles (T) where it is anchored through the ligand-receptor binding [95]. This mechanism was proposed for the recognition of specific immune cells by polyelectrolyte-antigen conjugates used as artificial vaccines as discussed in Section 5.

Fig. 4. Pluronic® block copolymers activate Nf-κB signaling in cells: (A) Pluronic® affects NF-κB signaling presumably by interacting with cell plasma membrane; (B) this leads to rapid phosphorylation of I-κB accompanied by the release of the Nf-κB [115]; (C) the released Nf-κB translocates to the nucleus and activates transcription of the corresponding genes.

Fig. 5.

Sensitization of MDR tumors by Pluronic® block copolymer: (A) Pluronic® binds with the cell membrane and inhibits Pgp ATPase; (B) Pluronic® also translocates inside cells and inhibits respiration in the mitochondria resulting in ATP depletion; (C) Pgp efflux pump shuts down; (D) the drug enters the cell and induces cytotoxic effect; (E) in the presence of Pluronic® the pro-apoptotic signaling in response to the drug is enhanced.