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Perspective

Polymers for Biomedical Applications: The Importance of Hydrophobicity in Directing Biological Interactions and Application Efficacy

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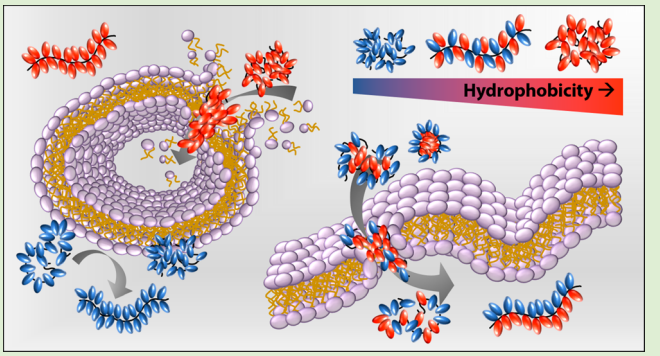
ABSTRACT: The past decades have seen significant research effort in the field of polymers for a range of biomedical applications, driven by the promising prospect of these materials for realizing next generation therapeutics in the clinic. In this regard, it is widely accepted that polymer properties such as chemistry, charge, and block composition, as well as properties of their self-assemblies including size, shape, surface chemistry, and biodegradation, all influence and direct their interactions with cells and biological membranes. In particular, polymer hydrophobicity is a property of interest, with growing evidence demonstrating the significant impact that hydrophobic interactions with lipid membranes and proteins can have on biomaterial application efficacy within the body. However, to date, this phenomenon has been relatively underexplored, and therefore there exists no clear universal understanding to direct polymer design. In this Perspective, we highlight important contributions to this field, focusing on seminal studies which investigate experimentally and theoretically how incorporation of hydrophobic moieties within polymer systems can influence their ultimate properties when used in biomedical applications. In this way, we aim to signify future directions in the design of highly performing polymers for biomedicine, making a case for the importance of standardized computational modeling to achieve widely applicable conclusions and facilitate future translational efforts.



INTRODUCTION

Polymers and polymeric self-assemblies are well-documented for their therapeutic potential in the clinical management of a range of diseases and medical needs. For instance, drug delivery systems, tissue engineering scaffolds, wound dressings, and polymer-coated biomaterials can improve treatment outcomes by enhancing cellular regeneration and drug delivery, safety, efficacy, and uptake into disease sites.[1−6] However, over the past decades as this field has developed, it has become apparent that the physical characteristics of the polymers employed, including chemistry, charges, composition, and biodegradation, as well as the properties of self-assembles such as size, shape, and surface chemistry, all play a major role in determining their behavior within biological environments.[7−9] In this regard, it is crucial to develop an understanding of how the physicochemical properties of polymeric materials direct these interactions in order to realize nanostructures that are capable of navigating the body, infecting and transforming cells, or detecting and repairing diseased cells. While many research efforts have been directed toward the understanding of relationships between polymer molecular weights, mechanical properties, self-assembly, solubility, and degradation behaviors, our overall understanding of how material chemistry and hydrophobicity direct interactions with tissues remains in its infancy. Promisingly, there are increasing reports to date highlighting the influence that the hydrophobicity (in conjunction with other factors such as chemistry and molecular weights) of drug delivery constructs and implants or scaffolds for tissue regeneration can exert in directing materials' interactions with tissues.[7,10−12] In this Perspective, we bring together literature with a specific focus on the property of polymer hydrophobicity within the fields of antimicrobial polymers, gene delivery, tissue engineering, and lipid membrane interactions. In particular, we not only focus on experimental investigations but also discuss the various structure−property relationship computational models that have been employed to validate experimental results. In this way, we aim to identify common concepts across these distinct

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fields in an effort to consolidate existing knowledge and shed light on future research directions.

ANTIMICROBIAL PEPTIDE POLYMER MIMICS

The importance of polymer hydrophobicity has long been appreciated and exploited in the field of antimicrobial polymers, evolving from early studies exploring the potential of host-defense peptides as replacements for conventional small molecule antibiotics.[13,14] The balance between cationic groups and regularly distributed hydrophobic side chains on different sides of these facially amphiphilic helical structures was recognized to disrupt bacterial cell membranes, causing membrane leakage and eventually cell death.[16−18] Subsequent studies aimed to improve and enhance the selectivity of this toxicity toward bacteria using synthetic polymer mimics, by manipulating placement and chemistries of hydrophobic groups and overall amphiphilic balance, for example, through copolymer quarternization with hydrophobic groups or through random and block copolymer syntheses.[19−21] An early study by Kuroda et al.[15] systematically investigated the effect of the nature of hydrophobic groups, polymer composition, and length for a series of antimicrobial block polymers (Figure 1). Their results suggested that antimicrobial activity depended critically on the nature and content of hydrophobic groups, with little dependence on overall polymer length. Further theoretical investigations aimed to determine the mechanism behind the hemolytic activity of the copolymers toward red blood cells

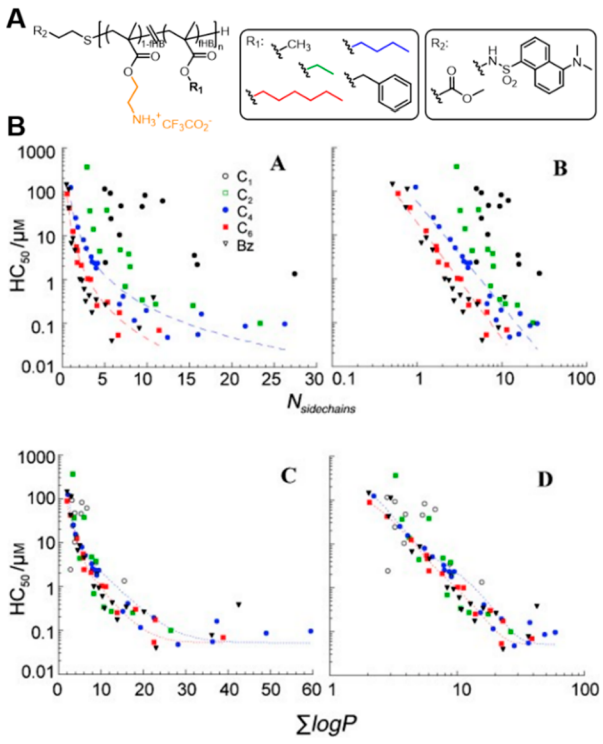

Figure 1. Top: Synthesis of amphiphilic polymethacrylate derivatives. Bottom: The HC_{50} of copolymers as a function of the number of side chains ($N_{side\ chains}$) (A and B) or $\sum \log P$ (C and D). $N_{side\ chains}$ is defined as $f_{HB} \times DP$. The data for polymers having butyl and hexyl groups were fitted to the equation $HC_{50} = C_3(N_{side\ chains})$ in which C_3 and C_4 are constant. The HC_{50} data for C_4- and C_6-polymer series in panels C and D were fit to eq 10. For the calculation of log*P* using eq 7, the number of carbon atoms in benzyl groups was set as $n = 7$. Adapted with permission from ref 15. Copyright 2009 Wiley-VCH.

(HC_{50}, the concentration that kills 50% red blood cells), using log*P* of the hydrophobic groups to estimate partitioning of alkyl side chains into hydrophobic regions of lipid bilayers. They concluded that the hemolytic activity of the copolymers could be described by the simple parameter of $\sum \log P$ (the summation of log*P* of alkyl side chains in a polymer, defined as $\sum \log P = \log P(n) \times N_{side\ chains}$; $\log P(n)$ is the relative partition coefficient for alkyl side chains) and was irrelevant to the identity of the alkyl groups, giving precedence that the hemolytic activity of polymers depends on their total hydrophobicity rather than the chemical nature of the side chain groups. Although cationic segments are crucial for antimicrobial activity, the hydrophobicity within the copolymers is the driving factor for lipid membrane binding and pore formation which ultimately causes cell death through a membrane-disruption mechanism. This copolymer design of hydrophobic and cationic blocks remains the predominant strategy in the field of antimicrobial polymers.[15,22−27]

As the polymer chemistry field has developed, the strategies toward synthesizing antimicrobial polymers have diversified, leading to new insights into how polymer hydrophobicity influences activity.[26,28−32] In contrast to traditional radical polymerizations which form linear copolymers with pendant hydrophobic and cationic moieties, polymers synthesized through ring-opening polymerization (ROP) or ring-opening metathesis polymerization (ROMP) form polymers with inherent highly hydrophobic backbones. In particular, ROMP polymers have been identified to have great potential as highly potent and selective biomimetic antimicrobial agents, as facially amphiphilic polymers can be readily synthesized using norbornene monomers with pendant cationic groups.[26,31,33,34] In this case, facial amphiphilicity is introduced at the monomer level as each repeat unit carries both a charged and a nonpolar group, which after polymerization result in the two different groups positioned on opposite sides of the backbone. In a comparison between facially amphiphilic and traditional segregated copolymers, the former were demonstrated to be more selective toward bacteria over normal cells, highlighting that the balance of hydrophobic/hydrophilic spatial location at the local monomer level is more critical than global amphiphilicity or overall charge density.[32,35] Tew and co-workers proposed that the reasoning for this difference in activity is due to differing polymer−lipid membrane interactions (Figure 2A). For segregated statistical copolymers without a perfectly alternating hydrophobic/hydrophilic structure, lipid membrane disruption can be negatively impacted due to inefficient close contact. Instead, the facially amphiphilic polymers have a homogeneous distribution of cationic and hydrophobic moieties, leading to improved contact between polymer and membrane. The authors subsequently investigated different facially amphiphilic polymers that showed broad spectrum antimicrobial activity and selectivity, whereby rigid backbone conformations (thus improved facial amphiphilicity) enhanced antimicrobial activity.[36−38] As a final comparison, the role of amphiphilicity and/or disrupted hydrophobicity (i.e., the hydrophobic region is separated by at least one polar moiety) on the antimicrobial activity of copolymers was investigated, where it was found that topologically homogeneous amphiphilicity was crucial for antimicrobial activity (Figure 2B).[39]

Facially amphiphilic polymers had broad spectrum activity against both Gram-positive *S. aureus* and Gram-negative *E. coli*, whereas the insertion of a disruptive amide linker between the backbone and hydrophobic side chain led to a complete loss of

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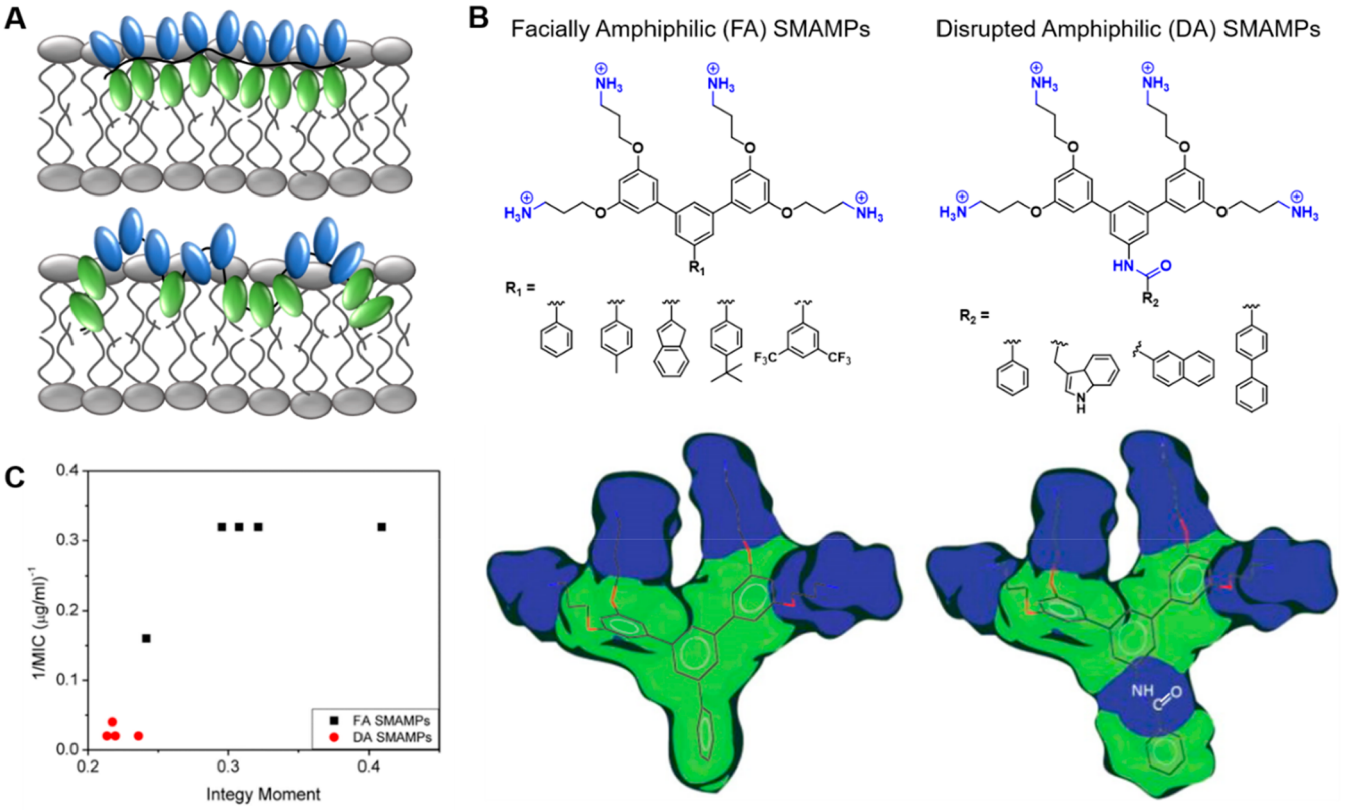

Figure 2. (A) Cartoon proposing that polymer interactions with the polar headgroup (gray circles) and the nonpolar lipid tails (squiggly lines) of a lipid membrane are significantly different for polymers from segregated monomers (top) versus that of polymers from facially amphiphilic (FA) monomers (bottom). (B) Design of aryl synthetic mimics of antimicrobial peptides (SMAMPs) with pendant aromatic groups as either an FA topology or disrupted amphiphilic (DA) topology. The hydrophilic regions are indicated in blue, and R1 and R2 represent the pendant aromatic groups. Below are the SMAMPs depicted as 3D molecular interaction fields, where blue and green represent the hydrophilic and hydrophobic regions, respectively. (C) Plot of the minimum inhibitory concentration (MIC) against *E. coli* vs the integy moment (IW) for the SMAMPs in the DA series (red dots) and the FA series (black squares). Adapted with permission from ref 39. Copyright 2013 American Chemical Society.

activity against *E. coli*. Gram-negative bacteria have a high resistance to antibiotics and represent a significant public health concern, and therefore, understanding the contributors to antimicrobial polymer efficacy against this class of bacteria is of great importance.[40] In order to further understand these results, the amphiphilicity of each polymer was quantified by analyzing 3D molecular interaction fields, focusing on the integy moment (IW), which quantifies the segregation of hydrophilic and hydrophobic regions within each polymer chain based on the distance from center of mass to the center of the hydrophilic region. Plotting antimicrobial activity against *E. coli* versus IW confirmed that the amide linker "disrupted" the amphiphilicity, giving lower IW values in corroboration with the observed lower toxicity against bacteria, even with the addition of bulky hydrophobic groups as the R_2 substituents (Figure 2C). Conversely, the facially amphiphilic polymers had the higher antimicrobial potency in addition to greater IW values, which suggests a possible trend between the two factors and a certain threshold value of amphiphilicity required in order to achieve activity against Gram-negative bacteria.

Similarly, highly selective antimicrobial polymers synthesized through ROP have also been identified.[28,29,41] In this case, due to the cyclic ester and carbonate monomers employed, the ROP synthetic strategy inherently provides the final materials with the added advantage of backbone degradability, which is arguably a crucial factor for ultimate clinical success.[42] Yang and co-workers have been investigating these polymers using similar design criteria to those of the ROMP polymers—a hydrophobic polymer backbone with charged pendant groups (typically guanidinium moieties)—however, to extend this research, the authors have focused on the addition of further hydrophobic moieties within the monomer unit, either as a spacer from the backbone or conjugated to the cationic center, and the implication of these modifications on activity.[28,43] Consistent with the literature to date, increasing the length of an alkyl spacer before the cationic headgroup in the repeat unit corresponded to an increase in polymer antimicrobial efficacy. However, this was a result of enhanced hydrophobic interactions with cell membranes in general, leading to a decrease in selectivity of bacterial cells over red blood cells, expressed as the ratio between HC_{50} (polymer concentration that induces 50% hemolysis)/MIC. Striking the correct balance between the two components, in this case, an ethyl spacer group with a guanidinium headgroup, led to unprecedented selective antimicrobial activity but importantly only when in combination with the hydrophobic polycarbonate backbone.[28] The authors further analyzed these results in terms of polymer hydrophobicity, by experimentally modeling the log*P* values with the inclusion of a model surrogate for membrane-bound fatty acids within the octanol layer. The polymer featuring the ethyl spacer group was observed to partition within the octanol layer even at low concentrations of the fatty acid, thereby indicating transport through bacteria membranes, whereas the control polymer with no hydrophobic spacer remained within the water layer and thus

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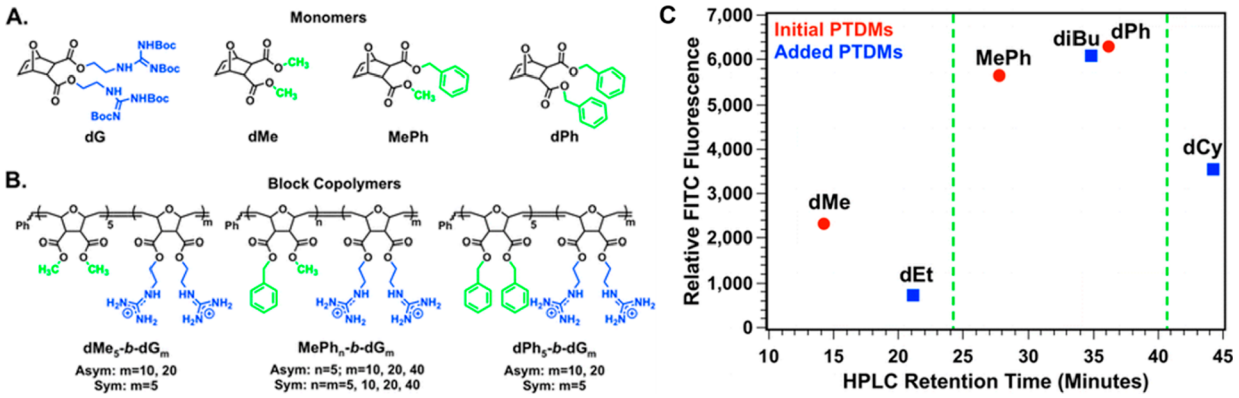

Figure 3. (A) Monomers and (B) block copolymers studied and (C) a plot of relative FITC fluorescence in Jurkat T cells as it relates to monomer high-performance liquid chromatography (HPLC) retention times. Green dashed lines indicate the hydrophobic window for optimal protein transduction domain mimic (PTDM) performance. Red data points represent hydrophobic monomers initially used. Blue data points represent hydrophobic monomers added after monomer hydrophobicity assessment by HPLC. Reproduced with permission from ref 44. Copyright 2016 American Chemical Society.

would not be expected to enter bacterial cells. Ultimately, this provides the precedent that both monomer design and overall polymer hydrophobicity are key factors to be considered when directing biological interactions with polymer materials.

POLYMERS AND SELF-ASSEMBLIES FOR GENE DELIVERY

As a result of the general observation that adding hydrophobicity into antimicrobial polymer systems (either through direct incorporation of hydrophobic groups or through the use of bulky counterions) generally improves membrane interactions, cellular uptake, and delivery efficiencies, such investigations are now being translated into other drug delivery fields. For example, nucleic acid delivery is a promising therapeutic strategy for a variety of clinical conditions including cancers, heart disease, neurological disorders, and viral infections. However, the efficacy of these constructs is dependent upon the ability of the nucleic acid cargo to reach its site of action: the cell nucleus. This requires specific delivery vehicle criteria, most notably the ability for the construct to pass through biological membranes, akin to the behavior of antimicrobial polymers. Tew and co-workers assessed the efficiency of siRNA intracellular delivery using ROMP polymer protein mimics—referred to as protein transduction domain mimics (PTDMs)—comprised of both hydrophobic and charged pendant monomer groups. Specifically, the authors investigated how alterations in the relative hydrophobicity of the noncharged block (from methyl through to benzyl moieties), as well as the length of the charged block (from 5 to 40 monomer units), impacted the cellular internalization of the polymer complexes (Figure 3).[44] The PTDMs were complexed with fluorescein isothiocyanate (FITC) labeled siRNA (FITC-siRNA), enabling quantification of siRNA internalization through flow cytometry analysis. When the hydrophobic block length was held constant (n = 5), an increase in side chain hydrophobicity (dimethyl, methyl-phenyl, diphenyl, Figure 3A) was found to be an important factor in improving cellular internalization as determined by increased intracellular FITC fluorescence. To probe this further, HPLC retention times were analyzed to quantify the hydrophobicity of the monomers, whereby larger retention times reflected increased hydrophobicity. These values had a linear correlation with theoretical monomer log$P$ calculations, suggesting HPLC as a viable experimental model for assessing relative hydrophobicity. Using these models, the authors were able to selectively target new monomer chemistries based on their log$P$ values, expanding the monomer set to also include ethyl, isopropyl, and cyclohexane side chain moieties. Through siRNA internalization experiments, the authors could finally identify that the optimal hydrophobic window of log$P$ values was between 1.78 and 3.50 for optimum efficacy. They further observed that polymers that fell within this critical window featured both aromatic and alkyl side chains, indicating that overall hydrophobicity is of greater importance than specific monomer side chain structure.

Similarly, Yang and co-workers synthesized a series of poly(carbonate) homopolymers for gene delivery, varying the hydrophobic spacer between the backbone and the charged quaternary and tertiary amine moieties, and investigated the impact on transfection efficacy and hemolytic activity.[45] As described above, experimental log$P$ values were calculated in a water/octanol system using fluorescently labeled polymers, by quantifying the polymer concentration within each layer at equilibrium. As expected, there was a trend of increasing hydrophobicity with an increase in pendant alkyl chain length, with the aromatic side chain polymer falling in the middle of the range. When investigated *in vitro*, an alkyl spacer length of 6 carbons (log$P$ −1.5) was found to improve gene transfection with minimal toxicity toward mammalian cells at effective concentrations, while a further increase to 9 carbons (log$P$ −0.8) reduced gene expression concurrent with higher toxicity. Interestingly, despite the similar log$P$ value to the hexyl side chain polymer (log$P$ −1.6 and −1.6, respectively), the incorporation of an aromatic 4-methyl benzyl group improved cellular uptake and gene transfection but with higher hemolysis to red blood cells and cytotoxicity. This would suggest that for some polymer systems, the specific monomer chemistry does contribute to the optimum balance of cationic charge density and hydrophobicity. Undoubtedly, these findings further confirm that while polymer chemistry cannot be ignored, polymer hydrophobicity must be carefully considered during polymer design in order to enhance application efficacy while minimizing unwanted cytotoxic effects arising from hydrophobic interactions and perturbations of membrane phospholipid bilayers.

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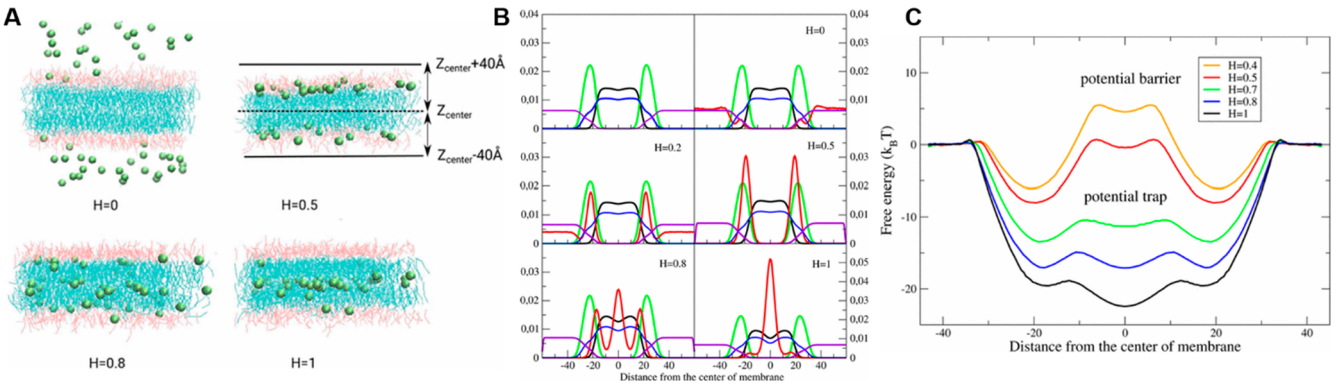

Figure 4. (A) Lipid membranes interacting with 50 NPs, at different values of hydrophobicity (H = 0, 0.5, 0.8, 1). Solvent beads are not shown to improve visibility. The solid lines displayed for H = 0.5 indicate the thresholds for calculating translocation events. (B) Density profiles of the various components of the lipid membrane, as well as the NPs at different degrees of hydrophobicity (H = 0, 0.2, 0.5, 0.8, 1). Tail groups (black), head groups (green), lipid molecules (blue), NPs (red), and solvent beads (purple) are presented as functions of distance from the center plane of the membrane, z. (C) Free energy profiles as a function of the distance from the membrane center, in the presence of 50 NPs, and at different hydrophobicities, H.

INTERACTIONS WITH LIPID MEMBRANES

As highlighted in the above examples, hydrophobic regions within polymeric materials show strong interactions with hydrophobic lipid membranes, and this is attributed to their ultimate application efficacy. However, beyond considerations of red blood cell lysis, these interactions are often assumed to have no further consequences. In actuality, within the drug delivery field there are a number of studies which highlight the resultant modulation of membrane dynamics, interactions with membrane rafts, and membrane lysis that can occur following exposure to polymers within biological systems.[46−49] Given the ubiquitous nature of lipid membranes within biological systems, it is crucial to understand how interactions with hydrophobic polymers occur at the molecular level, as any alterations in membrane structural properties and elasticity can affect important downstream cellular processes and overall cell function. The plasma membrane generally segregates into a series of dynamic and ordered nanoscale membrane domains, due to differential interactions between lipids and proteins, and nanoparticles can selectively segregate within different domains on account of polymer hydrophobicity.[50] One series of studies explored this phenomenon in-depth using a computational approach for three different polymer nanoparticles, polyethylene (PE), polypropylene (PP), and polystyrene (PS), which could readily penetrate into lipid membranes on account of their hydrophobic nature.[51,52] The authors performed molecular simulations using a MARTINI coarse-grained (CG) force field and atomistic simulations using the OPLS-UA force field for the interaction of the particles with both single-component, homogeneous 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC) lipid membranes and with phase-separated ternary mixtures containing dipalmitoylphosphatidylcholine (DPPC), dilinoleoyl-phosphatidilcholine (DLiPC), and cholesterol (CHOL) molecules. The particle chemistry was shown to dictate the exact nature of the particle interactions within the membranes, whereby PE showed a tendency to aggregate and form lens-shaped structures within the bilayer membrane, whereas PS and PP were more evenly dissolved and dispersed throughout the entire bilayer structure. In the single-component membranes, both PS and PP caused significant changes in membrane elastic properties and a decrease in lipid diffusion. In the ternary lipid models, distinct behaviors were observed. PS was found to partition strongly to cholesterol-poor regions, meaning local polymer concentration was high even at relatively low overall nanoparticle concentration, which has important implications for cellular toxicity. PS was also observed to significantly stabilize phase separation of lipid domains and alter their chemical composition, while PP had the opposite effect and stabilized phase separation. Due to its aggregated distribution within the membranes, PE was found to completely modify lipid distributions and cholesterol concentration within the cholesterol-rich domain. Overall, it was obvious that interactions of hydrophobic polymers with lipid membranes can impact crucial functions of cell membranes such as membrane sorting and trafficking, cell polarization, and signal transduction. Similarly, other groups have shown that computational modeling using molecular dynamics simulations is a valuable tool for identifying optimal nanoparticle design parameters, by further understanding the interactions between polymers and lipid membranes and the role of hydrophobicity in selective partitioning within the different regions.[53,54] One study examined surface grafted NPs using extensive microsecond unrestrained molecular dynamics simulations, and consistent with previous experimental evidence, a high density of surface hydrophobicity increased partitioning into the cholesterol-rich domains, whereas reducing the hydrophobicity shifted the preference to the opposite domain.[55] These interactions could be tuned, for example, by varying the length and density of the grafted surface chains or the charge density, and thus overall the preferred final localization of nanoparticles could be dictated by adjusting these factors. Apolar and nonpolar particles predictably located within the lipid bilayers; however, particle shape was also found to influence this, with disc-like shapes further increasing these interactions over rod or spherical particles. While in this case polymer hydrophobicity was perhaps not the most crucial factor, the modeling employed in this study is an important example of how computational tools can aid in the rational design of polymer particles with directed lipid membrane interactions or for permeating lipid layers, such as required for oral drug delivery. Another study investigated the impact of polymer hydrophobicity on membrane translocation and thus the ability to overcome biological membrane barriers without specific triggers (Figure 4).[54] Sommer and co-workers used coarse-grained molecular dynamics simulations with a simplified MARTINI model to analyze the lipid bilayer interactions of nanoparticles with varying degrees of hydrophobicity (H) which

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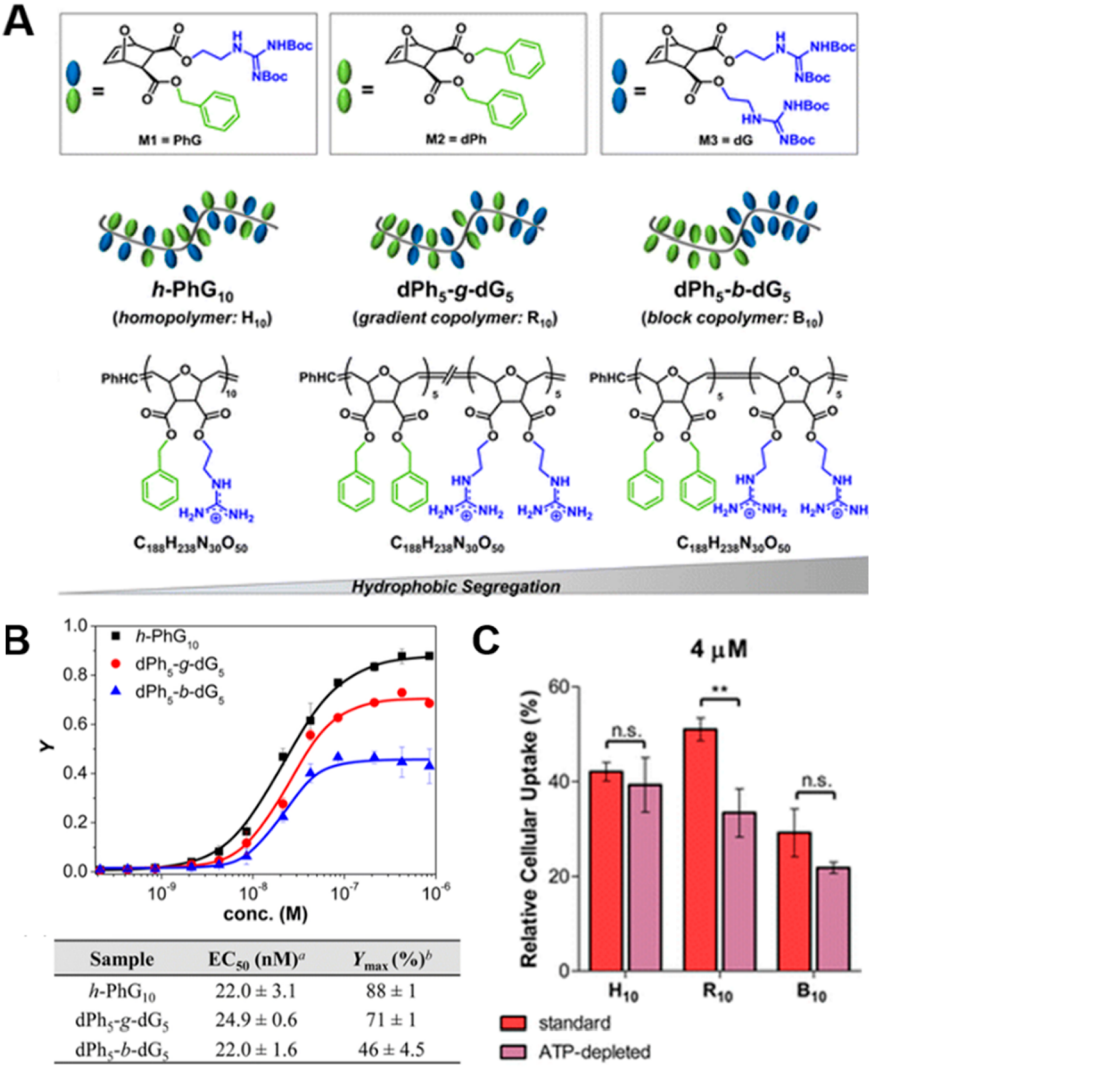

Figure 5. (A) Structures of monomers and related constitutional macromolecular isomers represented as chemical structures, representative cartoons of the polymeric isomers, and corresponding polymer chemical structures. Green and blue represent the hydrophobic and the cationic components, respectively. (B) Polymer-induced dye release from carboxyfluorescein (CF)-loaded liposomes. Fractional membrane activity (Y) vs polymer concentration with Hill equation fit; EC_{50}: effective polymer concentration needed to reach Ymax/2; Ymax: maximal fractional CF release relative to the total release obtained by Triton X-100. (C) Internalization in Jurkat-T cells after a 30 min incubation with 4 μM of NBD-labeled polymer (H_{10}: NBD-h-PhG10; R_{10}: NBD-dPh5-*g*-dG5; B_{10}: NBD-dPh5-*b*-dG5) at standard conditions (red bars) or after cellular ATP depletion (purple bars). Reproduced with permission from ref 56. Copyright 2014 American Chemical Society.

showed a clear variation in nanoparticle distribution related to H (Figure 4A). The parameter of H was mapped to hydrophobicity values accessible in experimental models, by measuring the free energy of partitioning of nanoparticles at the boundary between water and lipophilic oil phases comprised of hydrophobic oligomers. Using this data, they were able to plot free energy profiles of different NPs based on their distance from the center of the oil phase (Figure 4C), which showed that for hydrophilic particles ($H = 0.4$), the lipid membrane is a potential barrier and acts as a potential trap for highly hydrophobic NPs ($H = 1$), while particles with moderate H values can directly translocate through the membrane, which can be explained by the relatively flat free energy landscape. Finally, the authors measured the free energy difference between water and oil phases for the nanoparticles, which gave a linear relationship between difference in free energy values and their degree of hydrophobicity, H. Given the free energy difference was directly related to the partitioning coefficient, these models can be used to further understand experimental results. The authors subsequently investigated the changes in membrane properties in response to particles which varied across this hydrophobicity scale. Following interactions with NPs, the membrane changed properties depending on the degree of hydrophobicity, disturbing the molecular order, and thereby affecting membrane permeability for water. In all, a moderate hydrophobicity resulted in the highest translocation rates for NPs, with a linear relationship between the hydrophobic parameter and the partition coefficient on the passage of NPs between the two phases.

In addition to fundamental investigations into polymer modulation of membrane properties, several studies attempt to deconvolute the effects of chemistry and hydrophobicity on lipid membrane interactions. For instance, PEO−PPO−PEO triblock copolymers are commercially available with two different hydrophobic block lengths: P188, a poloxamer in the form of PEO_{80}−PPO_{27}−PEO_{80}, and P181, in the form of

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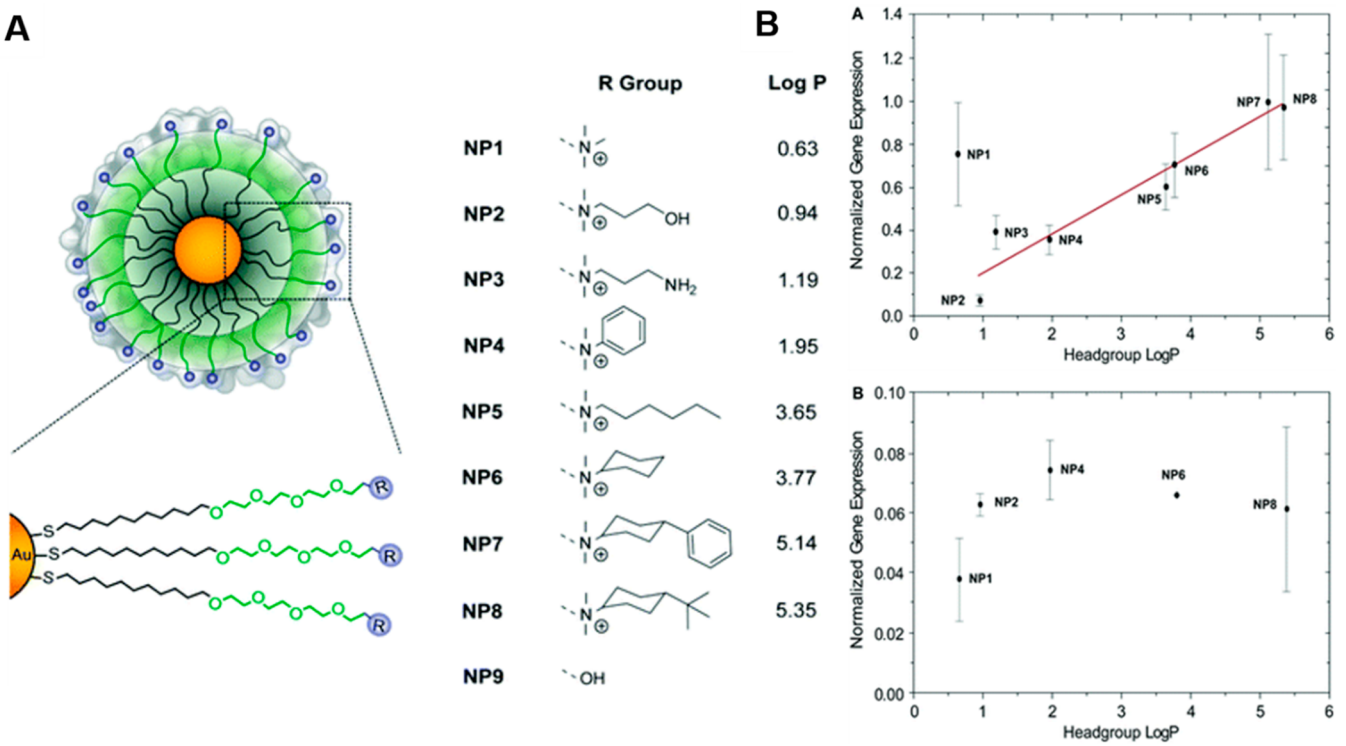

Figure 6. (A) Chemical structure of the monolayer-protected 2 nm core diameter gold nanoparticles. To generate the profiles for the SAR studies, functionalities (blue) are tuned at the ligand termini to control the surface hydrophobicity. Log*P* represents the calculated hydrophobic values of the head groups and (B) cytokine gene expression as a function of nanoparticle headgroup Log*P*. (top) TNFα (a representative pro-inflammatory cytokine) *in vitro* gene expression and (bottom) IL-10 (a representative anti-inflammatory cytokine) *in vivo* gene expression as a function of the calculated AuNP headgroup Log*P*. Adapted with permission from ref 61. Copyright 2012 American Chemical Society.

PEO_2−PPO_{32}−PEO_2, which result in opposing effects on lipid membrane integrity. The overall more hydrophilic polymer acts as a membrane protectant, whereas the more hydrophobic copolymer is a membrane permeabilizer. Through DLS and ITC measurements, the authors were able to show that hydrophobic interactions directed both copolymers to the lipid membrane but that a higher degree of hydrophilicity allowed weak adsorption at the membrane surface without penetrating into the bilayer core, whereas the more hydrophobic copolymers insert into the lipid bilayer, disrupting lipid packing and enhancing membrane permeability.[57]

The ability to understand in detail the mechanisms behind polymer interactions with lipid membranes and how to specifically modulate these is of crucial importance to the progress of biomimetic polymer chemistry. In a pivotal example, Tew and co-workers exploited the affinity of amphiphilic polymers for lipid membranes in order to construct synthetic protein transduction domains, mimicking cell penetrating peptides that can transport various macromolecules across the cellular membranes into cells (Figure 5).[56] The authors synthesized a series of polymers maintaining the same chemistry but altering the distribution of the hydrophobic segments (Figure 5A) and subsequently investigated the role of hydrophobic segregation on efficacy of membrane transport. They initially used a standard liposomal dye leakage assay, which suggested the greatest membrane activity for the homopolymer compared to the block and gradient polymers, as indicated by an increase in fluorescence as the encapsulated carboxyfluorescein was released (Figure 5B). Cellular toxicity and uptake studies in Jurkat-T cells revealed that decreasing hydrophobic segregation improved cell internalization without impacting cytocompatibility. The authors used 7-nitrobenz-2-oxo-1,3-diazol-4-yl (NBD)-labeled polymers, which provided the ability to distinguish between internalized and membrane-bound polymer, as NBD is quenched in the presence of membrane-impermeable reducing agent dithionite. In all experiments, the homo- and random copolymers outperformed the block copolymers, which indicated that strong hydrophobic segregation had a negative impact on membrane translocation (Figure 5C). The authors showed that the amphiphilic polymers did not tend to self-assemble in solution but that the presence of proteins and subsequent polymer−protein interactions drove formation of protein transduction domain complexes.[58] Finally, the positive implications of this learning were demonstrated in the study of protein and antibody delivery into cells facilitated by the mimic protein transduction domains, achieving successful intracellular delivery even in traditionally difficult-to-transfect cell types.[59,60]

INTERACTIONS WITH CELLS AND PROTEINS

In order to put the above lipid membrane studies into context, within the body, lipid bilayers form the double layered cell walls of all cell types. In addition to this, various proteins with hydrophobic regions are found throughout the bloodstream and within cell membranes, playing crucial roles in immune response, cell growth, and cell adhesion. Therefore, upon entry into the body, polymeric materials must navigate a variety of possible interactions while avoiding modifications to cell behavior and function, with particularly significant consequences for biomaterial clearance from the blood (through detrimental interactions with serum proteins and red blood cells) and in tissue engineering.

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For example, polymer hydrophobicity was shown by Rotello and co-workers to direct immune response of splenocytes using a set of engineered gold nanoparticles with tunable hydrophobicities (Figure 6).[61] The authors constructed a series of nanoparticles which differed only in the surficial ligand headgroup and computationally calculated the log*P* values to rank in order of hydrophobicity. They analyzed the expression of immunological reporter cytokines against the log*P* values of the surface functional groups to explore structure−activity relationships (SARs) at biological interfaces. Using this data, a linear increase in immune activity as hydrophobicity increased was observed both *in vitro* and *in vivo* in a mouse model, giving crucial insights into immune system activation. This is particularly promising for understanding molecular mechanisms of immune cell activation and, in particular, the role of hydrophobicity in directing immune response within biological systems.

Polymer hydrophobicity is an important consideration in tissue engineering and cellular regeneration applications, as literature has shown that varying the hydrophobicity of scaffolds can result in different interactions with cells and proteins.[62] In particular, polymer hydrophobicity has been shown to play a crucial role in directing cell attachment, spreading, and viability within biological systems. One study explored the impact of altering hydrophobicity of polymer films while maintaining similar chemistries in terms of functional groups, using different ratio combinations of poly(ε-caprolactone) and poly(lactic acid) and poly(2-hydroxyethyl methacrylate) (PHEMA) and ethyl methacrylate (EMA) as exemplar pairs. The hydrophobicity of the copolymer films was quantified by water contact angle, whereby PCL was the most hydrophobic and PHEMA the least.[63] On moderately hydrophobic surfaces, cell viability was highest within 3 days, whereas moderately hydrophilic surfaces reached the highest cell viability at prolonged culture periods up to 2 weeks. When analyzing cell morphology, it was observed using scanning electron microscopy (SEM) that on moderately hydrophobic PCL/PLA blended surfaces, cells adhered and proliferated in the early stages but were round in shape and preferentially attached to each other rather than the polymer surface. In comparison, the onset of cell attachment and proliferation was delayed on the more hydrophilic p(HEMA-*co*-EMA) surface; however ultimately the cells exhibited a flatter shape and had improved contact with the polymer surface. The authors hypothesized this was likely influenced by competitive absorption of proteins to the biomaterial surface; albumin had a high affinity for the more hydrophobic surface and therefore protein fouling occurred rapidly which prevented cell adhesion, whereas the more solvated hydrophilic surface could prevent this attachment, and therefore cell adhesion and proliferation were promoted.[64] The affinity for albumin and hydrophobic polymer surfaces is well-documented and is an important consideration when developing polymeric materials for systemic administration or for prolonged exposure in the body. In particular, materials which also feature surficial cationic groups have a strong propensity to interact with negatively charged serum proteins, and therefore, when these constructs enter the body, they can be readily associated with proteins which limits their therapeutic efficacy and increases undesirable toxicity.[65] For example, two polycations which had been quarternized with two lengths of alkyl chains were investigated for their interactions with negatively charged BSA compared with a hydrophilic polycation control.[66] Consistent with other literature reports, the protein adsorbed more strongly onto the material with the longer pentyl chain than the ethyl side chain polymer or control polymer, as a result of the greater hydrophobic interactions with the hydrophobic clefts of the protein. The interactions of BSA with quarternized cationic polymers from poly(chloromethylstyrene) were also studied in detail using SANS and circular dichroism, again demonstrating that the hydrophobic domains within polyelectrolytes are an important contributor to interactions with the protein and can be enhanced as polymer hydrophobicity increases.[67] A particularly important finding in this study was that the polymer complexation with BSA critically compromised its secondary structure, whereby its α-helical conformation was lost, and such protein denaturation is known to cause harmful effects in the body.[68] The implication of this is not only can protein binding cause biomaterial fouling and reduction in therapeutic efficacy but also it further increases nonspecific toxicity. Shi and co-workers examined this process and the relationship between protein binding and hydrophobicity by employing mixed-shell micelles with patchy thermoresponsive behavior.[69] Their mixed-micelle design was used to form two series of coassemblies: micelles with PEG as the fixed hydrophilic chain with either poly(*N*-isopropylacrylamide (PNIPAM), poly(*N*-isopropylacrylamide-*co*-*n*-*tert*-butyl acrylamide) P(NIPAM-*co*-NTBA), or poly(2-(2-methoxyethoxy)ethyl methacrylate) ($PMEO_2MA$) as the thermosensitive block or micelles with PNIPAM as the fixed thermosensitive block and PEG or poly(2-methacryloyl-oxyethyl phosphorylcholine) (PMPC) as the hydrophilic block. Due to the introduction of the thermosensitive blocks, protein interactions could be selectively turned on and off by varying temperature across the LCST. In the hydrophobic state, proteins could be captured and prevented from intermolecular aggregations and thermal denaturation and then released upon transitioning to their more hydrophilic state. They found that the reversibility of this process was highly dependent on a delicate balance of hydrophobicity, whereby weakly hydrophobic segments could not sufficiently trap the proteins, whereas strong hydrophobic interactions caused denaturation and difficulties in protein release. A combination of p(NIPAM) and PEG was identified as the micelle composition that could capture proteins and assist in refolding during release in a reversible nature and thus was the hydrophilic/hydrophobic balance needed to achieve the optimum function.

CONCLUSIONS AND OUTLOOK

The literature overwhelmingly suggests that achieving the correct balance of hydrophobicity and hydrophilicity within biomaterial polymers is crucial for achieving optimal functionality in biological systems, but it is often a fine balance to increase beneficial hydrophobic interactions and decrease detrimental ones. For instance, within the antimicrobial polymer field, materials are specifically designed to interact with bacterial membranes and thus must be carefully considered to achieve selectivity over mammalian cells. For tissue engineering, attachment of the target cell type to the scaffold or implant is crucial for healing and regeneration, but nonspecific attachment of proteins must be avoided to limit an immune response. In drug delivery, nanomedicines must be shielded from serum proteins and blood cells during circulation to prevent premature clearance, but such stealth behavior can have a negative impact on cell internalization at the target site. This is compounded when the therapeutic site of action is intracellular, requiring the construct to evade some biological membranes but actively transport through others. Given the complex functionalities that

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must be achieved by any polymer within biological applications, and the essentially limitless design chemistries that can be employed, it is unsurprising that optimum design elements have yet to be identified within each application field.

Despite this, as has been discussed here, there are a few reports throughout the literature that attempt to achieve clarity on the biomaterial properties that best suit specific applications. The implication of this is a whole host of data which, taken together, could potentially contribute to more streamlined knowledge on the chemistries and polymer properties that are ideal for achieving the optimum hydrophobicity of polymers and their self-assemblies. Almost certainly a standardized and consistent process of analysis, including computational models and statistics, would greatly contribute to future experimental design, interpretation of results, and a more streamlined overall understanding of how chemistry and hydrophobicity direct biological interactions. For the most part, studies in this field employ the partition coefficient log*P* as the quantitative measurement of hydrophobicity, which is certainly a valuable parameter when considering small molecules and single chemical moieties (e.g., polymer end groups). However, studies have shown that this parameter loses its predictive power when translating into larger polymer systems.[70,71] Mathers and co-workers advocate for the solubility parameter log*P*/SA which takes into account additional factors such as number of repeat units and polymer surface area, and indeed, this has recently been demonstrated to yield meaningful correlations against polymer properties such as water solubility, lower critical solution temperature (LCST), ability to self-assemble, and hydrolytic degradation.[72] This approach could certainly contribute to data analysis for all aspects discussed in this review, from using the Log*P*/SA parameter to analyze antimicrobial polymer activity against polymer structural elements such as monomer chemistries, block length, or copolymer ratios, through to correlation of cellular regeneration scaffold hydrophobicity and resultant cell growth and shape. Such computational analysis will likely prove vital for ultimate progress in the rational design of materials for biomedical applications; while this review has specifically focused on hydrophobicity, the importance of polymer structural properties and their interplay with hydrophobicity cannot be ignored.

We have also highlighted studies that employ laboratory-based methods to measure partition coefficients experimentally, as the use of theoretical modeling in combination with practical validation represents an extremely powerful tool to yield meaningful analysis and improve the predictive power of these models. Particularly for synthetic polymers, these experiments can be readily performed using materials tagged with UV-active or fluorescent molecules, allowing for sensitive and quantitative characterization in a water/oil biphasic model system. Applying structure−activity modeling to experimental data has become the norm over past years in various biological fields including drug discovery and drug formulation, and we envision therapeutic polymer materials to be the next field to encompass this methodology more universally. Overall, we suggest that the introduction of more standardized methods of quantifying polymer hydrophobicity in conjunction with rigorous modeling of structure−activity relationships is the key element required to rapidly expand knowledge and understanding in this area, leading to faster development of functional, efficacious materials in the biomedicine field and the achievement of future translational efforts.

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The manuscript was written through contributions of all authors.

Notes

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