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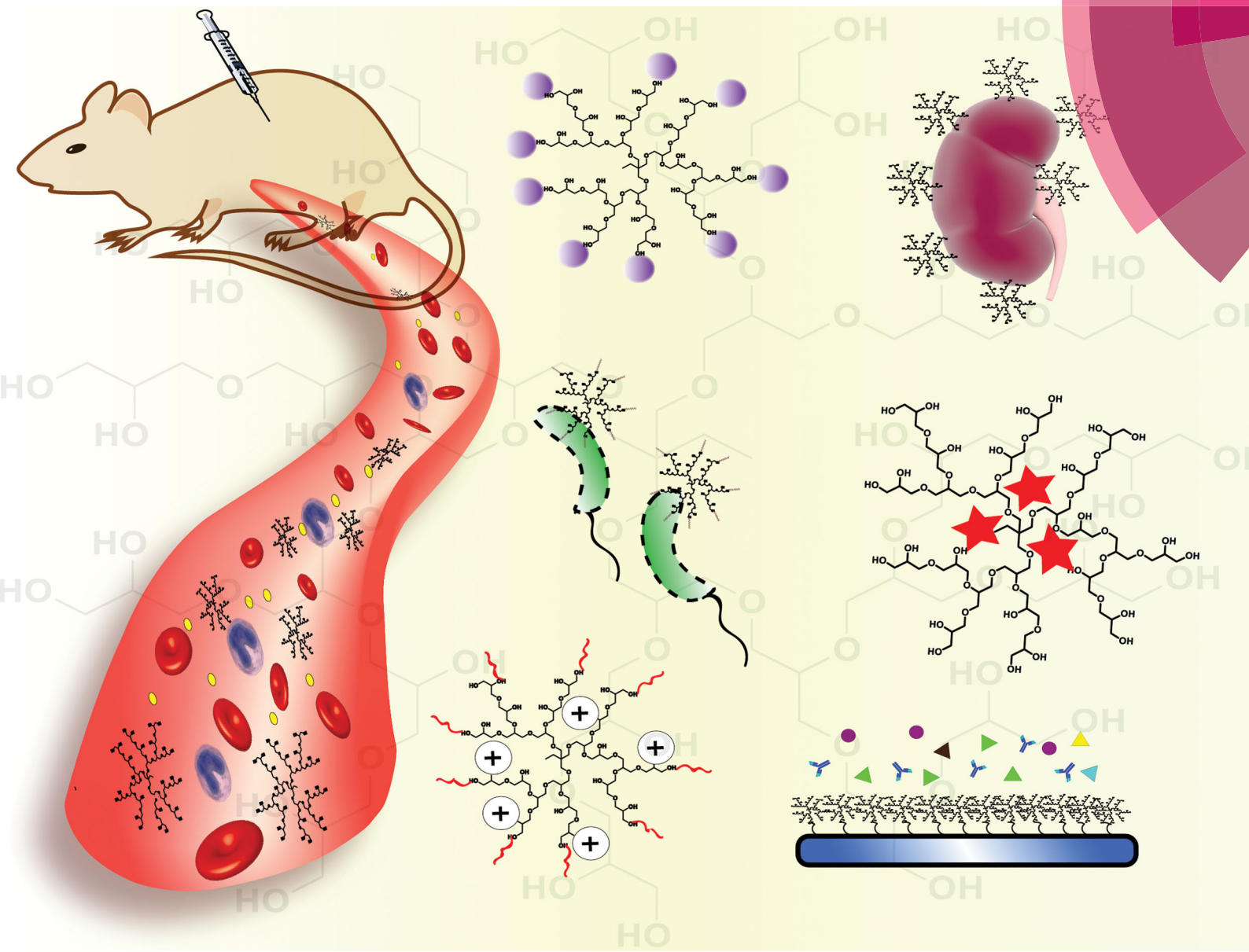
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Hyperbranched polyglycerols: recent advances in synthesis, biocompatibility and biomedical applications

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In the pursuit of dendrimer alternatives, hyperbranched polymers have found increasing interest from academia and industry in a broad range of fields due to their topological and synthetic advantages. Hyperbranched polyglycerol (HPG), as the name implies, is a hyperbranched polymer with about 50–65% dendrimeric structure. Due to its ease in synthesis, globular nature, versatility in terms of functionalization, and superb biocompatibility profiles HPG provides a promising class of materials suitable for numerous applications in nanomedicine and biomedical technologies. The structural features of HPG can be easily tailored by adopting different synthetic methodologies. In this review, we briefly explore the synthesis of HPGs starting from the traditional Lewis acid based approaches to recent advances including the development of high MW HPGs, biodegradable HPGs, co-block HPGs and sustainable or 'green' HPG synthesis. The robust history of HPG biocompatibility is extensively reviewed giving examples of both *in vitro* and *in vivo* models. In particular, HPG showed very minimal polymer accumulation in vital organs after intravenous injection compared to other polymers widely used for various biomedical applications. HPG is well tolerated in mice and rats, and has been found to be non-immunogenic to date. Due to its demonstrated safety profile and multifunctionality, HPG has been extensively studied for different biomedical applications including as macromolecular therapeutics, multivalent inhibitors/scavengers, in controlled drug delivery systems, in organ preservation, dialysis and cell surface engineering, as imaging agents and theranostics, in the development of anti-fouling surfaces and proteomics reagents. We highlight these applications along with its advantages. Finally, we conclude by providing a future prospective of HPG as one of the promising PEG alternatives with a great potential to enter clinical trials in the near future.

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1. Introduction

In modern materials chemistry polymers have become increasingly ubiquitous, forging applications in areas ranging from synthetic materials with tunable physical properties to biocompatible materials for medical applications.[1–7] In research, particularly involving biocompatible polymers, there is a significant focus on developing polymers for various biomedical applications including as polymer therapeutics,[8,9] drug delivery systems,[10] inhibitors or scavengers[11,12] and in regenerative medicine.[13] With the availability of advanced synthetic methodologies, polymers can be designed with a high degree of control over their physical properties and biological activity. Tight control over polymer properties can be achieved through several routes: variation in the chemical composition of polymer and molecular weight/size, the architecture (branched, linear, *etc.*), packing density of the monomers and pendant functionalities. Such tuning is critical in achieving desired biocompatibility profiles, pharmacokinetics and biological performance for *in vivo* applications.

Most polymeric architecture fall into four major overarching categories: linear, cross-linked, branched and dendritic architecture including dendrimers and hyperbranched polymers (HBPs).[14,15] As the name implies, linear polymers are macromolecules made up of a single, linear chain of monomers and have been extensively used in many biomedical applications.[16] As a subclass of dendritic polymers, dendrimers are generally defined by a monodisperse "tree-like" structure with high levels of branching symmetry in three dimensions generating a globular structure. While the structurally homogenous dendrimers are excellent candidates

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for biomedical applications, the level of structural control needed necessitates difficult and labor-intensive syntheses which hinder their large scale applications.[17] As a result, there has been an increased focus on the HBPs as dendrimer alternatives. HBPs contain a combination of linear, terminal and dendritic units, the relative ratios of which define the properties of such systems unlike the dendrimeric systems that contain only dendrimeric and terminal units.[18,19] The synthesis of HBPs is often a one-step process, affording rapid access to dendrimer type globular structures with low molecular weight distribution. In addition, the HBPs offer low chain entanglements, good solubility, low solution and melt viscosity and the availability of easily tunable functional groups. Since the first report of HBP in late 20th century by Kim and Webster, the field of HBP has been expanded tremendously. Having most of the features of dendrimers, HBPs became an attractive alternative to dendrimers and found great interest in both industry and academia. Recent reviews highlighted various aspects of HBPs in details.[18–24]

In recent years, hyperbranched polyglycerol (HPG) has emerged as one of the most promising, versatile, and well-studied hyperbranched polymer structures (Fig. 1). HPG attracted immense interest in the research community[25] owing to its dendrimer-like structure, chemical stability, excellent biocompatibility (similar or better than polyethylene glycol), low toxicity, tunable blood circulation, low intrinsic viscosity, compact size, multifunctionality and ease of synthesis. HPG contains terminal, linear and dendritic regions with a degree of branching around 0.5–0.6.[26] Its backbone is composed of ether linkages and its surface and core contain numerous reactable hydroxyl groups. It is highly hydrophilic, water soluble (>400 mg mL^{-1}) and its intrinsic viscosity, is similar to that of proteins. HPG is available in molecular weights ranging from a few hundred Daltons to about a million Daltons with low polydispersity ($Đ$ ~1.05 to 1.5). HPGs have been synthesized in grams to kilogram scale in the laboratory with good control of its properties.[27] The Haag group and Frey group have reviewed the synthesis and applications of

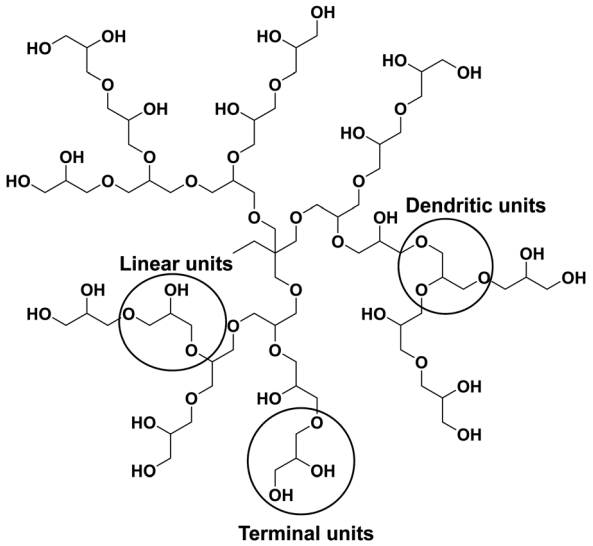


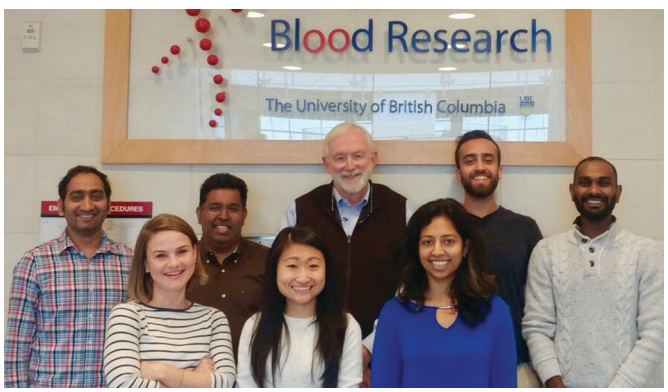
Fig. 1 A schematic representation of hyperbranched polyglycerol with linear, dendritic and terminal linkages.

HPG a few years ago.[28,29] Since then there have significant advances in the synthesis and use of HPG in various biomedical applications. Thus in this review, we summarize recently developed synthetic approaches, biocompatibility, and novel biomedical applications of HPG.

2. Synthesis of HPGs

2.1 Early synthetic routes of HPG

After Flory's theoretical representation of HBP from AB_2 type monomers, the first synthetic route for HPG synthesis was carried out by Sandler and Berg in 1966 using a variety of catalysts at room temperature.[30,31] Later, Dworak *et al.* demonstrated the first cationic ring opening polymerization of glycidol with two possible mechanisms: active chain end and activated



Back row (from left): Dr. Srinivas Abbina, Prof. Jayachandran Kizhakkedathu, Prof. Donald Brooks, Usama Abbasi and Prashant Kumar. Front row (from left): Erika Siren, Chanel La and Sreeparna Vappala

In the quest of finding solutions for unmet clinical needs using macromolecular approach, the Kizhakkedathu and Brooks research groups at the Centre for Blood Research, Department of Pathology and Laboratory Medicine & Department of Chemistry, University of British Columbia, are working at the interface of polymer chemistry, biology and medicine. We are focussed on the development of biocompatible materials for different biomedical applications. We are interested in understanding the fundamental interaction of synthetic materials with biological systems in order to generate clinically relevant polymers and materials for different therapeutic applications. Our research program spanned into four different themes polymer therapeutics, cell surface engineering, blood compatible surfaces and devices, and proteomics reagents. We work closely with engineers, clinicians, drug development experts and other collaborators to translate our discoveries to clinics. Dr Abbina is a postdoctoral fellow, and Usama Abbasi, Prashant Kumar, Erika Siren, Chanel La and Sreeparna Vappala are PhD students within the Kizhakkedathu group.

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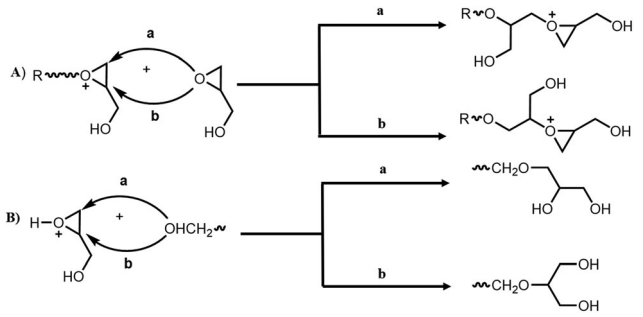


Fig. 2 Cationic ring opening polymerization of glycidol resulting in HPG. (A) Active chain end mechanism of cationic polymerization of glycidol. (B) Activated monomer mechanism of cationic polymerization of glycidol.

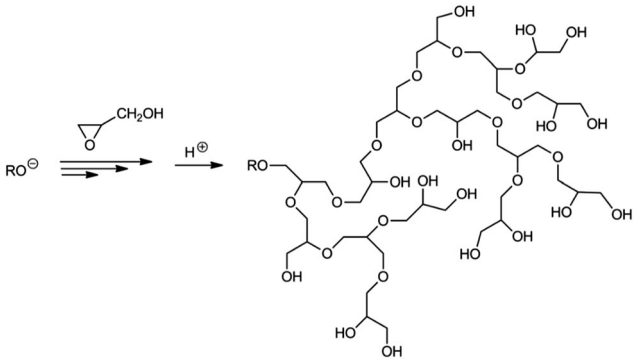


Fig. 3 General scheme for the anionic ring opening multi-branching polymerization of HPG.

monomer.[32,33] In this strategy, cationic ring opening polymerization of glycidol was initiated using Lewis (BF_3OEt_2 or $SnCl_4$) or Brønsted (CF_3COOH or CF_3SO_3H) acids (Fig. 2). Cationic polymerization processes are notoriously difficult to control due to the multiple side reactions that hinder propagation including chain transfer and chain termination. Though polymerization of glycidol was possible through cationic routes, inactivated propagating chains during the reactions resulted in lower molecular weight HPG (less than 10 000 Da) with high polydispersity.

Sunder *et al.* overcame the limitations of cationic polymerization of glycdiol by employing an anionic ring opening multi-branching polymerization (ROMBP) in combination with slow monomer addition.[26] In this approach, glycidol was added drop-wise to a partially deprotonated initiator, 1,1,1-tris(hydroxymethyl)propane at elevated temperatures (90–100 °C) (Fig. 3). The slow addition of monomer avoided the intra-cyclization of low molecular weight HPGs which was accountable for chain termination and successfully generated moderately sized HPGs with a narrow polydispersity ($Đ < 1.5$).[26] The mechanistic details of ROMBP and improvements on HPG polymerization conditions have been well described in a review written by Frey's group.[28]

2.2 Synthesis of high molecular weight HPGs

The synthesis of high molecular weight HPGs with controlled polydispersity by the ROMBP of glycidol possess a significant challenge. High molecular weight HPGs are attractive for various biomedical applications owing to their large hydrodynamic size, enhanced vascular retention and the availability of substantial number of functionalities per macromolecule. The solvent-free ROMBP of glycidol demonstrated by Sunder *et al.* generated well controlled HPGs with molecular weights up to 6500 Da, however, dramatic increases in the viscosity of the system during the polymerization process lead to poor mixing.[26] The resulting heterogeneity promoted intra-cyclization reactions that limited the synthesis of high molecular weight structures and yielded broad MW distributions. By modifying reaction parameters such as stirring intensity, stirrer geometry, monomer addition rate and introducing 1-methoxy-2-(2-methoxyethoxy)-ethane as an inert emulsifying agent, Frey and co-workers reported a synthetic procedure to generate medium molecular weight range HPGs up to 20 000 Da. However, beyond this molecular weight range broader distributions were still observed.[34] Further attempts were undertaken by Wilms *et al.* in which a new macroinitiator approach was reported to overcome the limitations associated with the synthesis of medium range molecular weights. The slow addition of glycidol to a partially deprotonated low molecular weight HPG–macroinitiator (500 and 1000 Da) resulted in controlled molecular weights up to 24 000 Da.[35] Recently, Moore *et al.* used a slightly modified approach to generate the high molecular weights (20 000–100 000 Da) by employing larger macroinitiators of HPG (2000 Da) (Fig. 4).[36]

The Brooks and Kizhakkedathu research groups made further significant progress in the pursuit of high molecular weight HPGs[37,38] by examining the utility of different solvents as emulsifying agents in ROMBP. Using dioxane as an emulsifying agent, Kainthan *et al.* successfully synthesized HPGs with molecular weight up to 700 000 Da ($Đ$ 1.1–1.4) in a controlled fashion with excellent yields (70–90%).[37] The obtained high molecular weight HPGs that were demonstrated to possess spherical geometries with compact structure consistent with dendritic polymers of similar molecular weights. The degree of branching was in the range 0.55–0.62, similar to low molecular weight HPGs. Interestingly, the high MW HPGs maintained a low intrinsic viscosity, a property that was found to be independent of MW and was similar to that observed for proteins. Unlike linear polymers of similar high molecular weights, high molecular weight HPGs were highly water soluble (>400 mg mL^{-1}) and did not form gels at high concentrations.

ul-Haq *et al.* later expanded this method of controlled generation of medium and high molecular weight HPGs to include different solvents including 1,4-dioxane, tetrahydropyran, ethylene glycol diethyl ether, and decane.[38] The role of solvent, solvent to glycidol ratio, monomer concentration and addition rate on polymerization process were studied. It was found that the molecular weight of the HPG formed in the medium decreased in the order 1,4-dioxane > THP > EGDE > decane (Fig. 5). The exchange of counter cations, potassium ions in this case, with the propagating species was demonstrated to be solvent dependent, leading to variation in the molecular weight of HPG formed. The microstructure, solution and thermal properties of HPG formed, however, was not depend on the nature of solvent used.[38]

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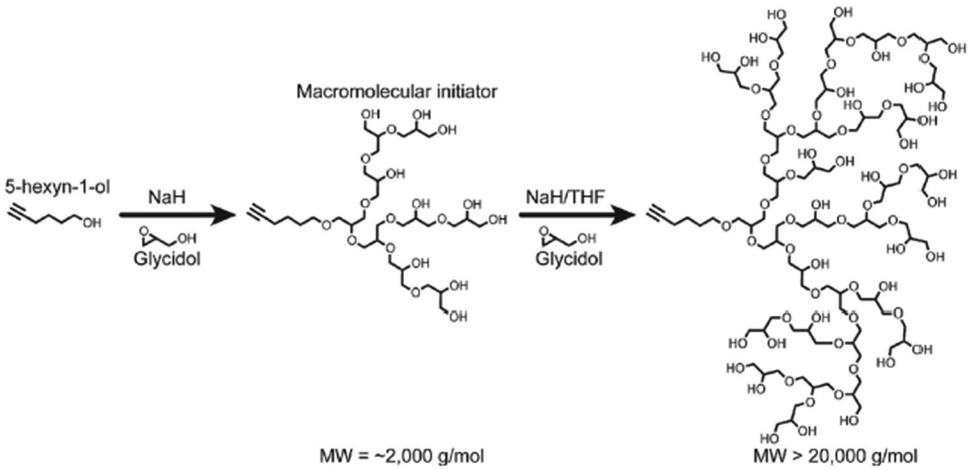


Fig. 4 Synthesis of high molecular weight HPG by macroinitiator approach.[36] [Reproduced with permission, Copyright 2016, Wiley-VCH.]

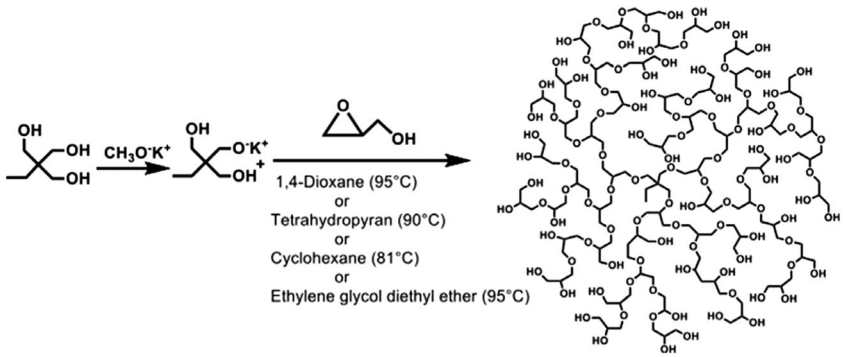


Fig. 5 Synthesis of high molecular weight HPG by emulsification method.[38] [Reproduced with permission, Copyright 2013, Wiley-VCH.]

2.3 Synthesis of biodegradable HPGs

One of the challenges of using high molecular weight polymers for biomedical applications is their bioaccumulation *in vivo*. Insufficient biodegradability *in vivo* can lead to the accumulation of high molecular weight polymers in organs and may impede organ function. The biodegradability of HPGs is an important concern in generating HPG-based therapeutics. Recently, incorporation of degradable moieties into the HPG scaffold including acetal, ketal, ester, disulfide linkages that respond to various physiological stimuli, has shown to be a viable approach to mitigate bioaccumulation. Kizhakkedathu and co-workers developed two distinct types of degradable HPGs by incorporating acid sensitive ketal moieties one within the polymer core and the other randomly distributed in the polymer.[39–42] By adopting the traditional ROMBP, ketal containing AB_2 type epoxide monomers were copolymerized with glycidol to arrive at degradable HPGs with randomly distributed ketal groups. They synthesized five different ketal containing monomers, 2-{1-methyl-1-[2-(oxiran-2-yl methoxy)ethoxy]ethoxy} ethanol (DMKM), 2-(1-(2-(oxiran-2-yl methoxy)ethoxy)cyclohexyloxy) ethanol (CHKM), 2-[1-(2-oxiranyl methoxy-ethoxy)-cyclopentyloxy]-ethanol (CPKM), 2-(2-methyl-4-((oxiran-2-yl methoxy)methyl)-1,3-dioxolan-2-yl)ethanol (GHBKM), (8-(oxiran-2-yl methoxy)-1,4-dioxaspiro[4.5]decan-2-yl)methanol (GCHKM) containing either linear or cyclic ketal moieties.[39] The incorporation of the ketal monomer into the polymer structure did not affect MW distribution and was well controlled in the adapted ROMP approach. It was demonstrated that the hydrolytic degradation profiles and the *in vivo* properties of biodegradable HPGs (BHPGs) were highly dependent on the type of degradable linkages in the monomer. This further provides opportunities for fine-tuning the polymer behavior by altering monomer structure.[41,42] For instance, the hydrolytic half-life of the dimethyl ketal containing BHPG is different from polymers containing endo-cyclic ketal monomers. In addition, the half-life can be varied from a few minutes to a few hundred days depending on the monomer used (Fig. 6).[39] This new class of polymers were demonstrated to be multifunctional, highly water soluble, non-toxic and biocompatible both *in vitro* and *in vivo*.[39,41,42] In another iteration, glycidol was added slowly to an acid sensitive initiator core containing dimethyl and cyclohexyl ketal groups to derive the core degradable HPGs.[40] The degradable core was synthesized by click-chemistry approach.

In another approach, Frey and co-workers successfully introduced acid sensitive acetal groups in the HPG backbone by the copolymerization of a new degradable monomer, 1-(glycidyloxy) ethyl ethylene glycol ether and glycidol by anionic ring-opening polymerization.[43] The resultant degradable HPGs showed pH

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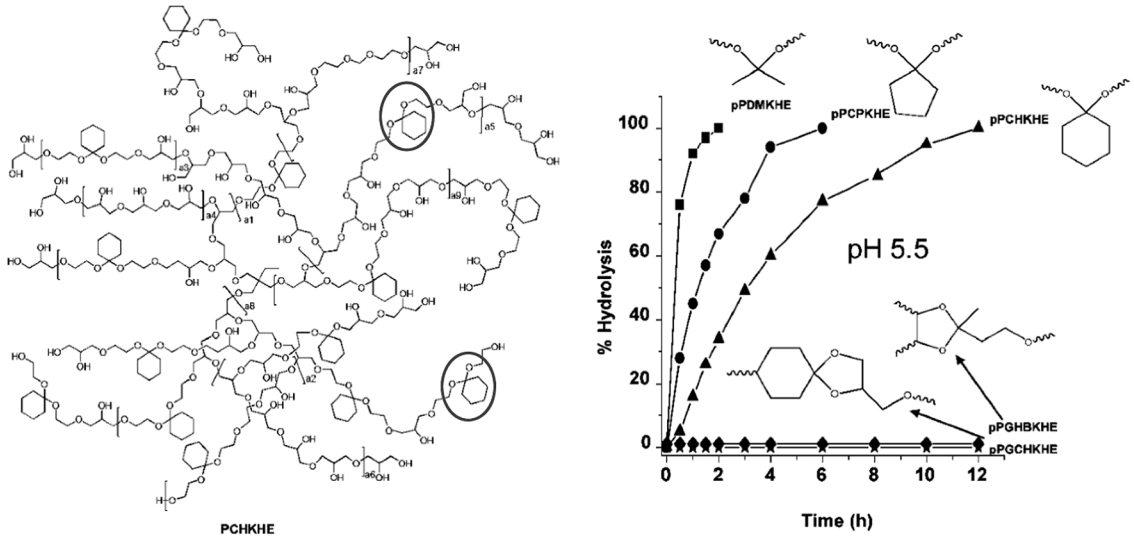


Fig. 6 An example of randomly distributed, acid sensitive ketal groups (shown in circles) mimicked biodegradable HPG (left) and degradation profiles of biodegradable HPG with different acid sensitive groups in the polymer backbone with respective to pH (right).[39] [Reproduced with permission, Copyright 2012, American Chemical Society.]

sensitive degradation kinetics; however, the molecular weights of the polymers were moderate.[43] In a similar fashion, Hu *et al.* reported another interesting class of degradable HPGs by incorporating ester functionality in the polymer backbone by oxyanionic polymerization of glycidol and glycidyl methacrylate. The resultant hybrid polymer showed good degradation profiles and low cytotoxicity.[44]

The redox sensitive disulfide moiety containing degradable polymers are well known for various drug delivery applications owing to their intercellular degradation. Kim and co-workers reported novel redox stimuli responsive HPGs.[45,46] ROMP of epoxide monomer bearing disulfide groups, *i.e.*, 2-((2-(oxiran-2-yl methoxy)-ethyl)disulfanyl)ethan-1-ol, yielded a series of redox-sensitive HPGs with different molecular weights (2000–11 000 Da) (Fig. 7).[46]

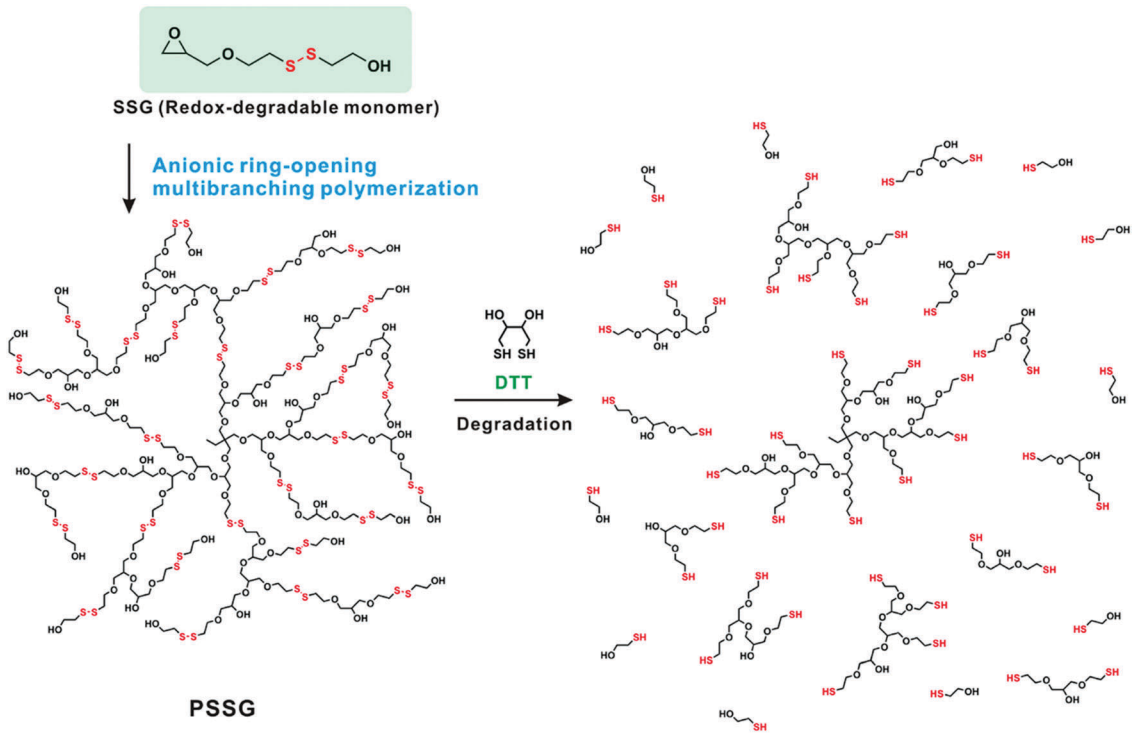


Fig. 7 Synthetic scheme of disulfide based biodegradable HPG and their degradation pattern upon the addition of a reducing agent.[46] [Reproduced with permission, Copyright 2015, American Chemical Society.]

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In continuation of their efforts on degradable HPG, Shenoi *et al.* also developed redox-degradable HPG by anionic ring opening copolymerization of glycidol with an epoxide monomer containing a disulfide bond, (2-((2-oxiran-2-yl methoxy)ethyl)disulfanyl)ethan-1-ol.[47] The resultant HPGs are highly biocompatible and are degraded into small fractions in presence of reducing agents such as tris(2-carboxyethyl)phosphine, dithiothreitol and glutathione.

2.4 Synthesis of HPG based copolymers

HPG is a highly hydrophilic polymer and may not be suitable for certain applications, for instance in the delivery of hydrophobic drugs *via* non-covalent encapsulation. To overcome these challenges, researchers have used co-polymerization approaches with different monomers to generate the polymers for desired applications. One of the early studies reported this approach by Brooks and co-workers is the co-polymerization of C_{8-18} epoxide (alkyl epoxide) with glycidol followed by the ring opening polymerization of methoxy PEG epoxide (Fig. 8).[48] Such modifications resulted in HPG polymers with hydrophobic drug binding properties with excellent biocompatibility.[48,49]

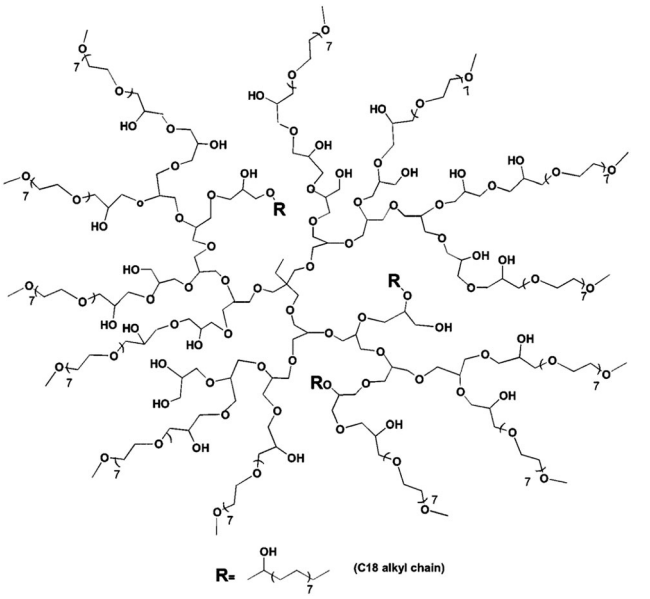


Fig. 8 A schematic representation of hydrophobically modified HPG.[48] [Reproduced with permission, Copyright 2007, Elsevier Ltd.]

Haag and co-workers further developed two different HPG based drug delivery platforms containing hydrophobic dendritic core–hydrophilic shell type polymers to encapsulate hydrophobic drugs and various dyes (Fig. 9A).[50–53] Different hydrophobic moieties such as biphenyl groups, perfluorinated alkyl chains at the core of HPG were introduced in a site specific manner by taking the advantage of the multifunctionality of HPG. Turk *et al.* modified the core of HPG to enhance the hydrophobicity of the system with biphenyl groups, by employing the Suzuki-coupling reaction to encapsulate the hydrophobic guest molecules such as nimodipine and pyrene.[50]

In another report, Kurniasih *et al.* modified HPG by the covalent attachment of an *m*-PEG shell either directly or through a hydrophobic phenyl spacer to the HPG scaffold by click chemistry protocols. Alternatively the HPG core was decorated with biphenyl hydrophobic segments located around the HPG core and mPEG chains as the shell to the presumably peripheral OH groups of HPGs (Fig. 9B).[51] This core–shell architecture showed a higher transport capacity for the hydrophobic guest molecules. It was found that the molecular weight of the HPG, degree of functionalization, location of the hydrophobic segments and PEG length influences the encapsulation of guest molecules.

To further pursue the use of hydrophobically modified HPG in cellular uptake studies Paulus *et al.* developed HPG sulfates with a hydrophobic core.[54] The HPG copolymer was synthesized by adding glycidol derivatives containing mono or biphenyl moieties followed by glycidol addition. The shell of the polymer scaffold was then modified to sulfates using sulfamic acid. The hydrophobically modified HPG sulfates were able to encapsulate indocarbocyanine dye molecules. Endocytosis of these complexes and the release of the dye molecule indicated that these sulfated polymers can be developed as a potent drug delivery system.[54]

In another approach, Luo *et al.* reported dendritic core–shell architecture to develop thermoresponsive unimolecular polymeric micelles with a hydrophilic HPG core and poly(*N*-isopropylacrylamide) shell.[55] HPG (3000 Da) was functionalized as macro

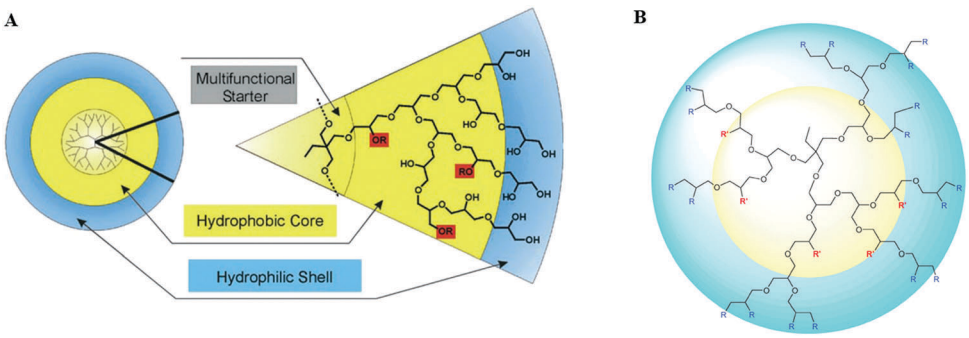


Fig. 9 Core–shell type architecture of the HPG: (A) HPG scaffold containing hydrophobically functionalized core (red),[50] [Reproduced with permission, Copyright 2007, Wiley-VCH]. (B) Dendritic HPG scaffold containing hydrophilic groups (PEG, blue) and/or hydrophobic functionality (biphenyl, red) which are arranged either in a random or site-specific manner.[51] [Reproduced with permission, Copyright 2011, Royal Society of Chemistry.]

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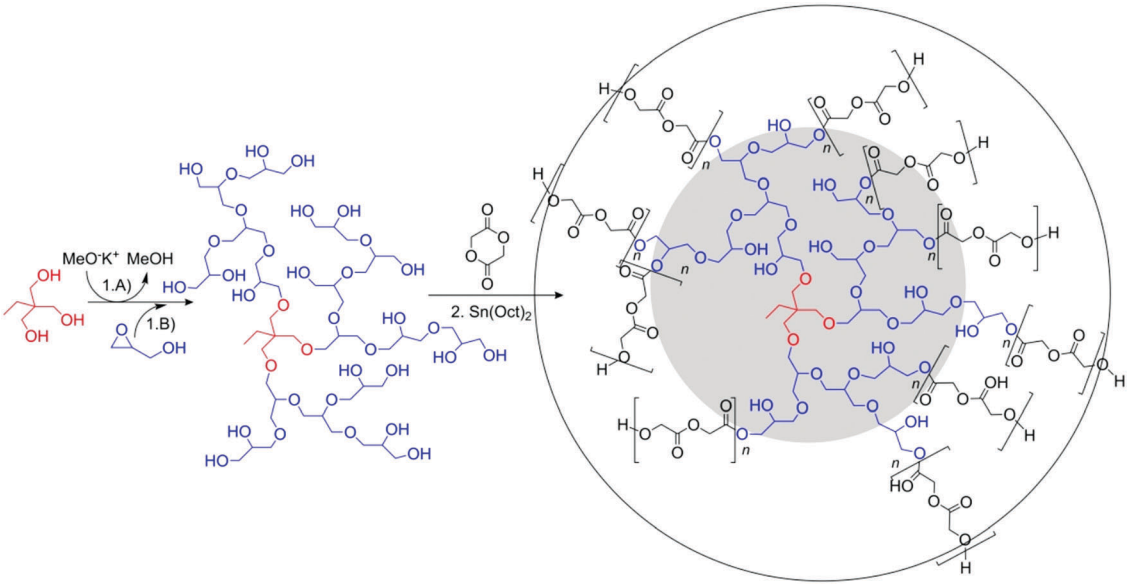


Fig. 10 A two-step synthetic protocol to generate HPG with PGA multi-arm star copolymers.[56] [Open access, *Beilstein J. Org. Chem.*, 2010.]

reversible addition–fragmentation transfer (RAFT) agent by decorating it with dithiobenzoate groups. The HPG-based macro RAFT reagent was grafted with poly(*N*-isopropylacrylamide) brushes by RAFT polymerization to generate the thermoresponsive star block copolymers, HPG-PNIPAM.[55] These polymers formed unimolecular micelles in aqueous solutions (<30 °C). In a similar fashion, Wolf *et al.* utilized the multifunctional core to synthesize a HPG with multiple poly(glycolic acid) (PGA) arms by a straight forward two step approach.[56] After the polymerization of glycidol, the polyether-polyols were used as macroinitiators for the ring opening polymerization of glycolide using $Sn(Oct)_2$ as a catalyst. Interestingly, the polymers obtained exhibited improved solubility in organic solvents compared to their linear analogs (Fig. 10).[56]

Amphiphilic block copolymers are of great interest due to their self-organization at the nano scale. The use of HPG as one of the blocks in a block copolymer was first reported by Wurm *et al.*[57] Diblock copolymers of HPG (3000–15 000 Da) were synthesized by linking a linear polyethylene oxide block with HPG (PEO-*b*-HPG). Furthermore, Frey and coworkers extended this approach by synthesizing triblock ABA polymer by adding one more block to the AB block type polymer. They synthesized the triblock polymer, HPG-linear PEO–HPG (HPG–PEO–HPG) in the molecular weight range of 6000–18 000 Da with varying block sizes.[58] Interestingly, all materials had a similar glass transition temperature (T_g) indicating miscibility of different segments in the amorphous state.

2.5 Green approaches for HPG synthesis

Advances in HPG research have warranted polymer scaffolds with versatile properties. Many of the established protocols for HPG synthesis, however, are not considered sustainable under the green chemistry guidelines. Recent advances in the synthesis of low molecular weight HPG have focused on the development of greener approaches to HPG production utilizing concepts in sustainability and green chemistry.[59,60] Harth and coworkers were the first to explore this approach, publishing the green synthesis of low molecular weight HPGs. The HPG synthesis was performed in PBS buffer (pH-3.5 to 8) by aqueous epoxide ring-opening polymerization of glycidol by taking advantage of the catalytic activity between water and glycidol at high temperatures (60–100 °C); it proceeds *via* the well-known activated monomer approach and is confirmed by different patterns of branching.[59] Recently, Mohammadifar *et al.* also reported another interesting green synthesis of low molecular weight HPG at room temperature.[60] Citric acid, an economic and biodegradable carboxylic acid, was used as the initiator as well as proton donor in the cationic polymerization of glycidol. The polymerization follows the activated monomer mechanism which includes the protonation of glycidol leading to the ring opening of activated glycidol epoxide (Fig. 11).[60] This citric acid core containing HPGs are quite suitable for cosmetics and biomedical applications.

3. Biocompatibility, blood circulation and biodistribution of HPGs

For polymers intended for biomedical applications, it is essential that these macromolecular structures are highly biocompatible. Polymers that demonstrate cell compatibility, blood compatibility and whole animal tolerance are needed. Thus it is important to investigate tolerance/toxicity of new materials for their eventual clinical use. As explained in the previous sections, HPG is a highly functional and water soluble polymer. Significant effort has been made to understand the biocompatibility of HPG in both *in vitro* and *in vivo* using cell culture, blood interaction and animal models (Fig. 12).[61–63]

The structural similarity of HPG with PEG (a well-known FDA approved polymer) motivated several researchers to examine their biocompatibility. Researchers performed studies with wide

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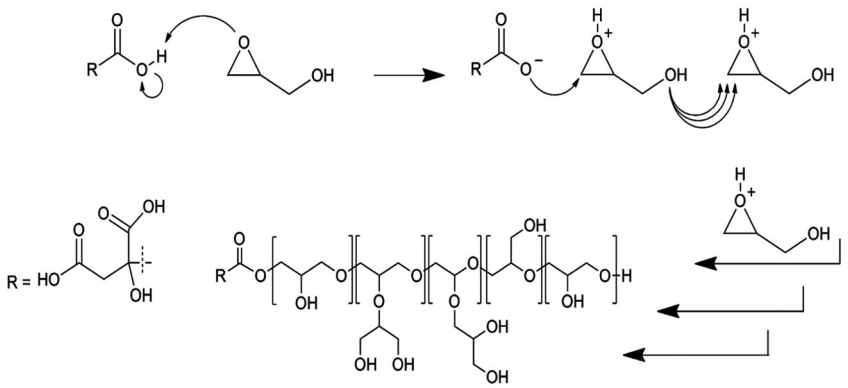


Fig. 11 Proposed mechanism of green synthesis of low molecular weight biodegradable HPG.[60] [Reproduced with permission, Copyright 2016, American Chemical Society.]

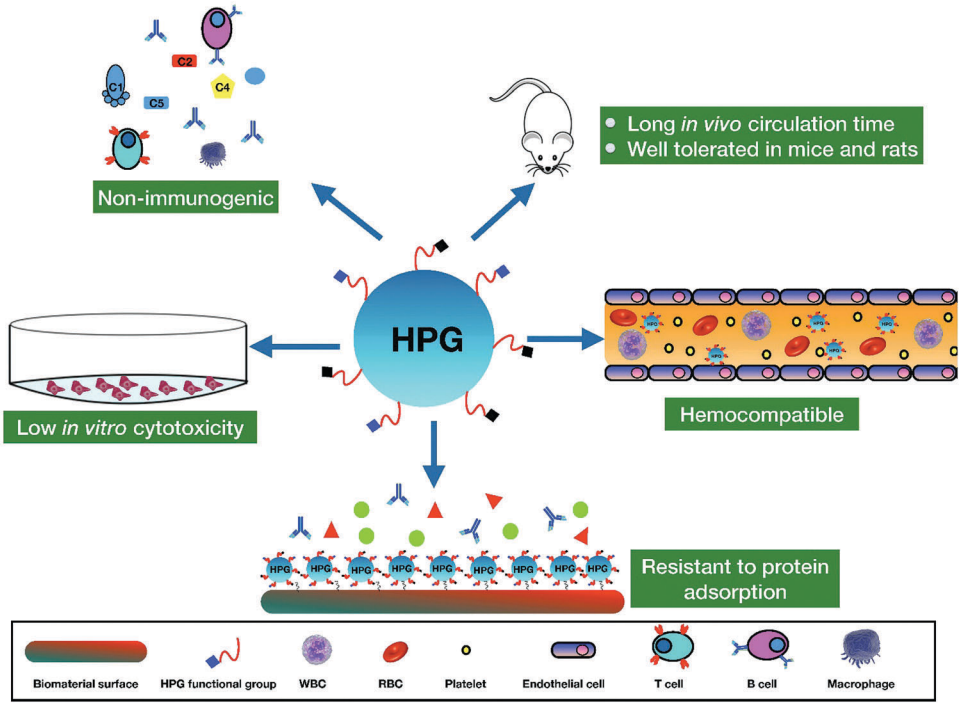


Fig. 12 An illustration depicting the overview of the biocompatibility profile of HPGs.

variation of molecular weights HPGs, biodegradable HPGs and modified HPGs. Our group contributed significantly to understand the biocompatibility of HPGs. These studies have concluded that HPG is highly blood compatible, apparently non-immunogenic and non-toxic. HPGs have equal or better biocompatibility profile compared to PEG. In the following section, we will describe in detail various studies published on the biocompatibility of HPGs.

The Brooks group was the first one to compare the biocompatibility of both branched and linear polyglycerols in the low molecular weight range (up to 6000 Da).[61] Unlike PEG, HPGs and their linear analogues had no effect on red blood cell (RBC) aggregation and hemolysis. Consistent with this result, whole blood variable shear rate viscosity studies showed that both types of polyglycerols did not alter blood viscosity beyond a very minor effect on suspending medium viscosity. Detailed blood compatibility studies were performed including its effects on blood coagulation, platelet activation and complement activation, and it was found that HPGs caused no adverse effects. In another study, Kainthan *et al.* investigated the *in vitro* blood compatibility of high molecular weight HPGs (100 000 to 540 000 Da).[63] High molecular weight HPGs appeared to have insignificant effects on various *in vitro* hemocompatibilty measures including activated thromboplastin time (APTT), prothrombin time (PT), compliment factor C3a generation and platelet activation. Effects were minimal even at very high concentrations (20 mg mL^{-1}).

In another important study, ul-Haq *et al.* compared the blood compatibility of linear polyglycerols (LPGs), HPGs and PEG of similar molecular weights (~100 kDa).[64] These studies confirmed the superior blood compatibility of LPG and HPG in

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comparison to PEG. Notability, PEG 100 kDa aggregated red blood cells in whole blood, however, LPG and HPGs showed similar profiles as that of control samples. In addition, at high concentrations, unlike LPG and HPG, PEG altered blood coagulation, activated the platelets and complement system (Fig. 13).[64] ul-Haq *et al.* further investigated the blood compatibility of hybrid co-polymers of LPG and HPG which also demonstrates the superiority of these polyglycerols compared to other hydroxyl containing polymers such as dextran, polyvinyl alcohol in addition to PEG.[65] Taken together, increasing the molecular weight of HPGs showed a considerable improvement in bio-compatibility compared to other linear polymers.[64] Shenoi *et al.* compared the blood compatibility of biodegradable HPGs with randomly distributed ketal groups, HPGs with core containing ketal groups and redox cleavable HPGs. Studies demonstrated that biodegradable HPGs were highly blood compatible and their degradation products also did not generate any unwanted toxicities.[39]

In addition to blood compatibility, the cell compatibility of HPGs was also investigated using cell culture models. Early studies demonstrated the low toxicity of low molecular weight HPGs to fibroblasts even at very high concentrations (10 mg mL^{-1}).[63] In a more recent study, ul-Haq *et al.* demonstrated the non-toxic nature of high molecular weight HPGs using human umbilical vein endothelial cells (HUVECs). In this study, they have shown that PEG 100 kDa is toxic at concentrations (>5 mg mL^{-1}) based on the cell viability data while HPGs were well tolerated.[64] In another study, Mendelson *et al.* and Du *et al.* have shown that low molecular weight HPG was well tolerated against human peritoneal mesothelial cells (HPMCs) in comparison to glucose.[66,67] Cytoplasmic vacuolation was observed for glucose incubated HPMCs while HPG did not show any such effects demonstrating its non-toxic nature. Very recently Gao *et al.* further showed that HPG is well tolerated against H9c2 cells and HUVECs in comparison to hydroxyethyl starch (HES).[68]

In vivo tolerance of HPGs has been performed in a couple of different animal models. A combination of the body weight of animals, histology of organs/tissues and blood chemistry were used as measure of tolerance of HPG in animals. Kainthan *et al.* have shown that low molecular weight HPGs are well tolerated in animals.[62] In this study, they intravenously injected up to 1 g kg^{-1} dose of 3 kDa HPG in Balb/c mice; animals tolerated the material well. In another study, hydrophobically modified HPG was injected in mice and it was demonstrated that the C18 alkyl chain modified HPG was also non-toxic. In a recent published study, Du *et al.* went further and investigated the tolerance of low molecular weight HPG (1 kDa) in a chronic peritoneal dialysis (PD) model.[69] Rats were injected daily with peritoneal dialysis solution (10 mL) containing 6 wt% HPG intraperitoneally for 3 months. It was found that rats tolerated these injections well without any unwanted toxicity even after systemic exposure of high doses of HPG (6% w/v) for long time periods. Minimal effect was observed on the peritoneal membrane, internal organs and blood chemistry upon exposure to HPG (Fig. 14).[69] It was also shown that modified HPGs such as drug conjugates,[70,71] polycationic HPGs,[72] and C10 alkyl chain modified HPG[73] were also found to be well tolerated demonstrating the non-toxic nature of the HPG scaffold *in vivo*.

An important aspect of polymers used for drug delivery and drug conjugation applications is the information regarding pharmacokinetics, distribution of the polymers in vital organs and its clearance/elimination of the body. Brooks and co-workers

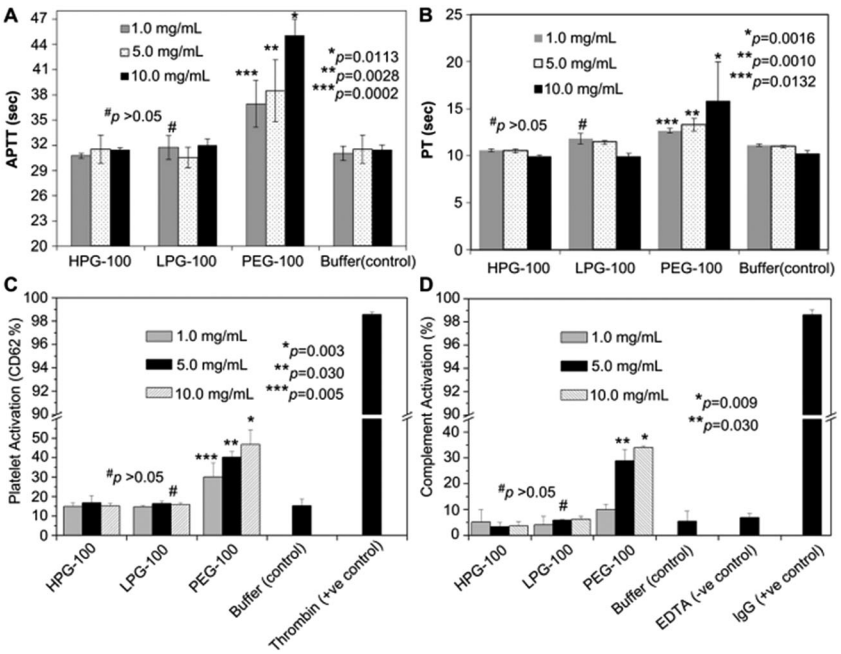


Fig. 13 (A) Effect of HPG, PEG and linear polyglycerol on activated partial thromboplastin time, (B) prothrombin time, (C) platelet activation estimated from CD62 expression, measured by flow cytometry, (D) complement activation is measured using CH50 analysis.[64] [Reproduced with permission, Copyright 2012 Elsevier Ltd.]

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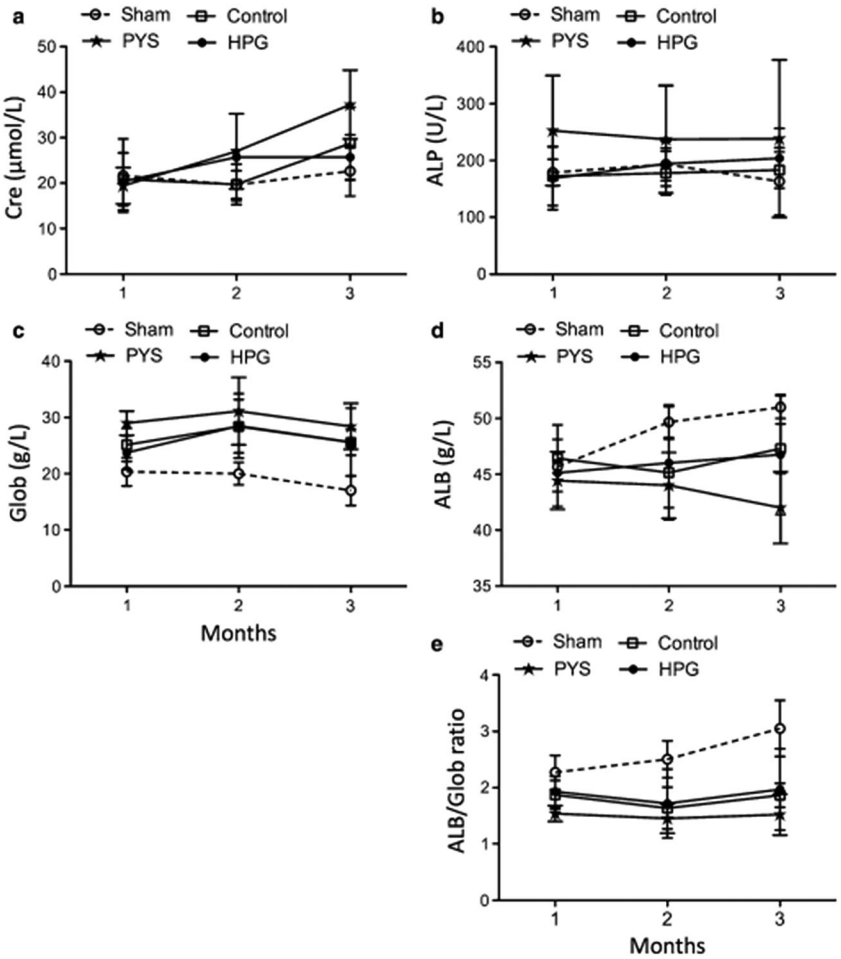


Fig. 14 Male Wistar rats received once-daily intraperitoneal injection of control (N = 7), physioneal solutions for PD (N = 7) or HPG (N = 8), or needle puncture as Sham control (N = 3) and the chemical changes in the blood of rats after 3 months of intraperitoneal injection.[69] [Reproduced with permission from Du *et al.*, Copyright 2016.]

were the first to report the circulation time, biodistribution and excretion of high molecular weight HPGs.[62] Radio-labelled HPG was used for these studies; tritium labeled methyl iodide was used to label hydroxyl groups of HPG as methoxy groups for this purpose. The circulation half-life of a stable tritium labeled HPG (540 kDa) was found to be ~57 h.[62] ul-Haq *et al.* compared the circulation time of LPG and HPG of 100 kDa molecular weight and showed that HPG has long circulation half-life (~40 h) compared to LPG and linear PEG of similar molecular weights.[64] The compact nature of HPG and its inability to repetat is thought to be the reason for its long circulation.[62] Importantly, the circulation time of HPG can be fine-tuned from few hours to many days in mice by changing the molecular weights. In addition to this, the circulation time of HPGs can be altered by incorporating biodegradable linkages. Shenoi *et al.* have demonstrated that by incorporating ketal linkages, circulation half-life of HPG can be altered from a few hours to several hours. This approach could provide an additional approach to change the blood circulation behavior of HPG.[41,42] These reports also suggest that vascular residence time of HPG can be controlled by choosing the chemistry of the co-monomers used for the synthesis.[42]

The immunogenicity of polymers is an important consideration for its use *in vivo* applications especially for long circulating therapeutics. The immunogenicity of HPG was investigated indirectly with both PEG–HPG constructs and using RBC conjugated HPG as model systems. Chapanian *et al.* conjugated HPG on the surface of red blood cells and followed its circulation time after multiple injections in Balb/c mice.[74] HPG conjugated RBCs circulated in mice for long periods of time (50 days) after initial injection. They followed the circulation time of HPG modified RBC in same mice after a second injection and showed that the circulation time of the conjugate did not change. These results show that the HPG coupled to a surface is potentially non-immunogenic. If antibodies were developed against HPG conjugate, the circulation time of the modified RBCs in the second injection would have been shorter.[74] Although preliminary proof-of-concept has been achieved, detailed studies are warranted to investigate the immunogenicity HPGs.

Although, there are advantages of using high molecular weight polymers, studies have noted an increased accumulation in liver and other organs.[71] Glomerular filtration limit for linear polymers are in the range of 50–65 kDa.[75] Therefore, higher

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molecular weight polymers are likely to be retained in the organs. Biodistribution and excretion of HPG in mice were also reported using radio-labelled HPGs. It was shown that low molecular weight HPGs were exclusively eliminated *via* kidney without organ accumulation.[41,76] High molecular weight HPG showed some accumulation in some vital organs (*e.g.* liver, spleen)[71] although much lower than other polymers of similar molecular weights (~100–700 kDa).[77,78] The bioaccumulation of HPG increased with increase in molecular weight of the polymers.[71] The bioaccumulation HPG can be altered by incorporating biodegradable groups within HPGs. Shenoi *et al.* recently demonstrated a strategy to alter the biodistribution HPG using biodegradable linkages incorporated within HPGs. They have shown that the biodistribution and excretion of HPG can be altered by changing the type of ketal linkages incorporated within HPGs; dimethyl ketal containing generates faster degradation *in vivo* compared to a cyclic ketal (Fig. 15).[41,42]

In short, with excellent biocompatibility and tremendous possibilities for multi-functionalization, HPG has been shown to have great potential in the field of biomedicine and nanomedicine. Compact structure, low intrinsic viscosity, high oxidative stability makes HPGs superior to its linear equivalents or other linear polymers. Now that the molecular weight can be controlled with narrow polydispersity and it can be biodegradable, HPG holds the key to a number of biomedical applications. Thus in the next section we discuss novel biomedical applications of HPGs recently reported in literature.

4. Biomedical applications of HPG

With excellent biocompatibility and the ability to incorporate multiple functionalities, HPG has been recruited for a number of biomedical applications, and has shown significant potential

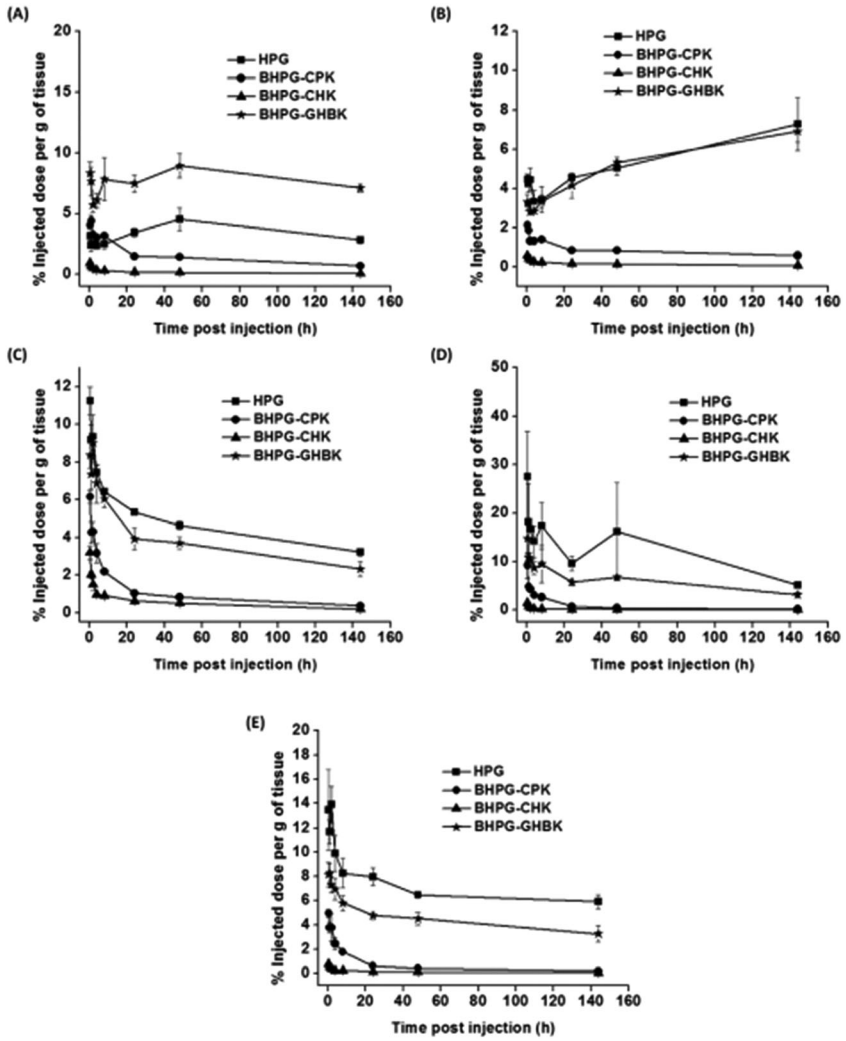


Fig. 15 Organ distribution of tritium-labeled high molecular weight biodegradable and nondegradable HPG after intravenous administration in female Balb/c mice (N = 3): (A) liver; (B) spleen; (C) kidneys; (D) lungs; (E) heart. BHPG-CPK and BHPG-CHK showed negligible accumulation (<1%) where as BHPG-GHBK showed a nearly similar biodistribution pattern to that of nondegradable HPG of comparable molecular weight.[42] [Reproduced with permission, Copyright 2016, American Chemical Society.]

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to translate to clinics in the near future. Using a wide set of chemical tools, the hydroxyl groups of HPG have been converted into a wide array of functionalities including carboxylic acids, sulfates, sulfonic acids, aldehydes, azides, amines, alkyl halide, alkenes and alkyne groups for various applications. The compact, dendritic structure of HPG allows for high accessible presentation, displaying ligands in a multivalent fashion on the polymer. Such display of ligands has shown significant advantage for the design of biological inhibitors as spatial organization of ligands is an important factor in such applications. The dendritic architecture of HPG also brings significant advantage; as such HPG has shown relatively weak interactions with cell surfaces and proteins, unlike linear polymeric structures. This prevents cell aggregation and clumping in presence of HPG solutions. The relatively low intrinsic viscosity of HPG is also advantageous in the development of free flowing HPG based injectable drug formulations at high doses or its use at high concentrations *in vivo*. This has been limiting for linear polymers as the viscosity of such formulation increases strongly with molecular weight and concentration. We will discuss a number of novel applications where HPG has shown great success in this review (Fig. 16).

4.1 Polymer therapeutics

4.1.1 Bioactive HPGs. Polymeric therapeutics encompass distinct therapeutic strategies where the molecular architecture and size of the polymers become advantageous. In this strategy, the entire polymeric system is acting as a therapeutic agent rather than the polymer scaffold delivering small molecule active agent. The scaffold architecture can provide pre-assembled geometries of ligands presented to substrates (cell surface or proteins) to enhance binding taking advantage of multivalency. The resulting high affinity structures have found use as competitive inhibitors for biological processes which depend on multivalent interactions.[79] The architecture and multivalent presentation of certain ligands or active agent are being used to design novel inhibitors of biological reactions or as scavengers of a biologically active moiety.[80–83]

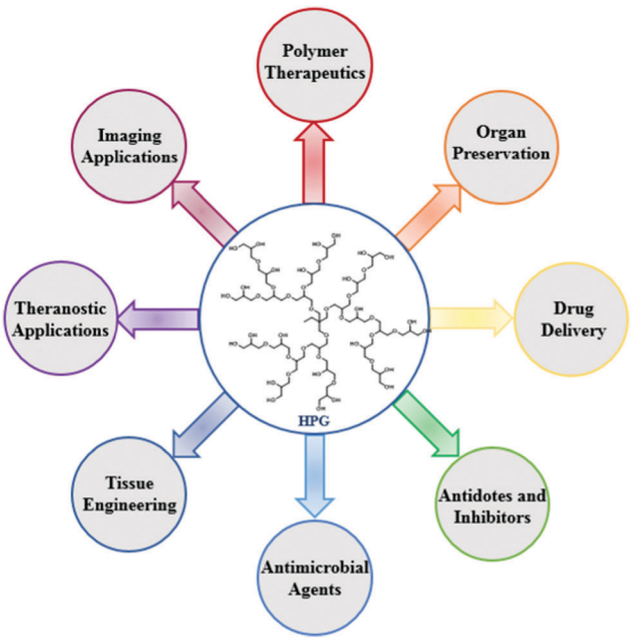


Fig. 16 A cartoon displaying diverse biomedical applications where HPG has been used as a primary scaffold.

An excellent example of a polymer therapeutic where HPG has been used as a scaffold is the universal heparin reversal agent (UHRA) (Fig. 17).[72,84,85] Heparins are negatively charged polysaccharides used clinically as anticoagulants to prevent thrombosis related conditions. Although this class of drugs is corner stone of clinical medicine, bleeding associated with heparins is important limitation of their use in the clinic. To circumvent these issues, Shenoi *et al.* has developed a universal antidote named UHRA which neutralizes all the heparin based drugs used in the clinics.[72] The general structure of UHRA is an HPG core decorated with positively charged heparin binding groups (HBGs) protected by layer of polyethylene glycol. Preclinical studies and other extensive biological evaluation demonstrated its efficacy in preventing bleeding associated with heparin, low toxicity and excretion from animals (Fig. 17).[72] Recently Kalathottukaren *et al.* further studied these molecules and demonstrated UHRA is superior to protamine, the only unfractionated heparin antidote, and could prevent protamine associated toxicity in animals.[84] The molecular design of the antidote design based on HPG is described recently.[85]

Another example of a polymer therapeutic using HPG is in the inhibitor for polyphosphates (PolyPs). PolyPs released from platelets are known to be prothrombotic and are an important target for developing novel antithrombotics without bleeding side effects. Travers *et al.* demonstrated that a subclass of UHRAs are excellent in preventing procoagulant properties of polyPs and could prevent thrombosis in animal models.[86] These UHRAs allow much less bleeding than heparins used as control.

Another classical example of HPG based polymeric therapeutics are the heparin analogues. Turk *et al.* have developed a heparin analogue from dendritic polyglycerol sulfates. Dendritic polyglycerol was functionalized with negatively charged sulfate and carboxylate groups.[87] Relative to unfractionated heparin (UFH), these molecules showed moderate anticoagulation activity and strong anticomplement activity.

Widely observed in nature, multivalency is a key characteristic of strong and selective substrate binding. In this process, weakly binding functionalities present in large numbers can cooperatively enhance surface binding based on their spatial organization on the presenting surface.[88] Haag's research group has contributed extensively in the design of HPG-based therapeutics based on multivalent interactions. Possessing a defined architecture and maximal presentation in comparison to linear polymer scaffolds, HPGs are an ideal candidate for multivalency. These high affinity multivalent structures have found use as powerful competitive inhibitors for biological processes which depend on multivalent interactions.[89] For example, viruses and leukocytes use multivalent interactions to control cell recognition and uptake[90] and the Haag research group has extensively explored HPGs as macromolecular binding agents to both viral and immune inhibition in response.[89–92]

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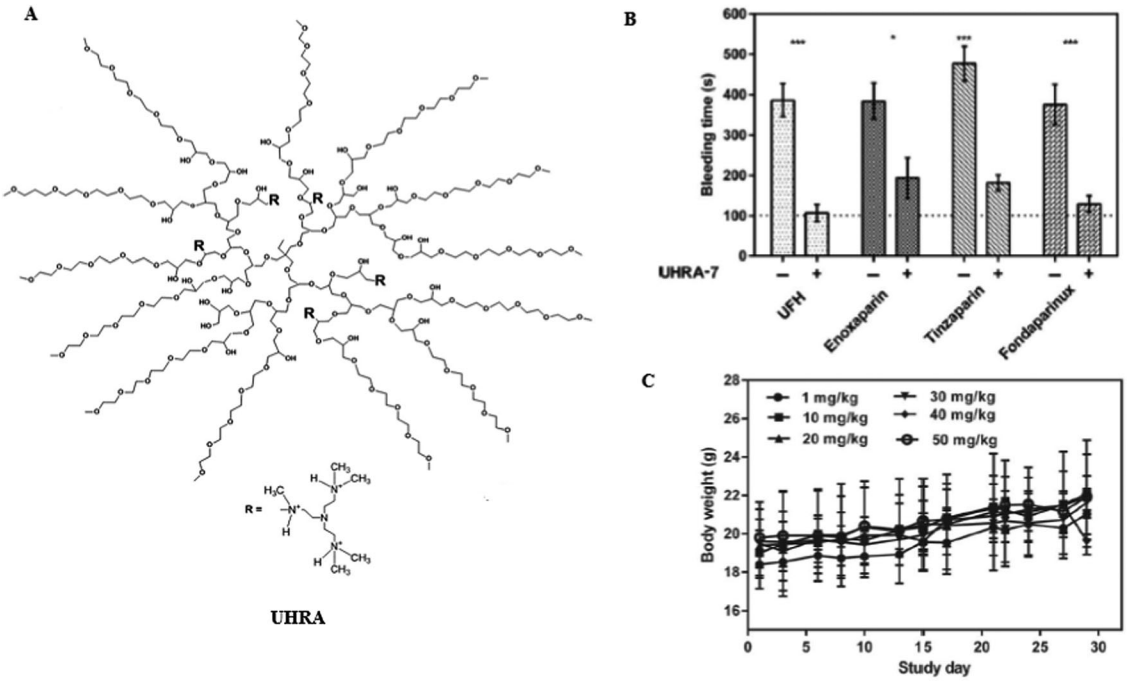


Fig. 17 (A) Schematic representation of general structure of UHRA, (B) UHRA-7 (23 kDa, 20 R groups) showed very minimal bleeding profils in comparison with other anticoagulants (enoxaparin, tinzaparin and fondaparinux) in mice, (C) UHRA-7 was injected in Balb/c mice with varying doses and monitored over a period of 29 days. No signs of toxicity or major body weight loss was observed. [Reproduced with permission, Copyright 2014, AAAS.]

In one implementation of multivalent inhibitors, HPG sulfates showed potent inhibition of leukocyte mediated inflammation reactions.[93] On the leukocyte surface, receptors known as L-selectin use multivalent interactions to tether leukocytes to cell surfaces, triggering inflammation.[94] These receptors selectively recognize and bind negatively charged glycoclusters presented on cell surfaces. A series of HPG bearing polyanions (carboxylates, sulfonate, phosphonate, phosphate, sulfate) were tested for competitive L-selectin inhibition. While most polyanions demonstrated inhibitory concentrations (IC_{50}) in the mM range, HPG scaffolds presenting sulfate groups (dPGS) were able to competitively bind L-selectins in the nM range, inhibiting leukocyte uptake and subsequent inflammation (Fig. 18).[93,95]

Further mechanistic investigation revealed that dPGS structures presenting high charge densities were found to be the most potent inhibitors.[96] Intriguingly, a non-linear change in charge density was found on high MW dPGS (480 and 800 kDa) which were significantly higher than low MW analogues. This observation was attributed to the increased flexibility of the dPGS backbone in higher MW structures. In high MW dPGS, the increased scaffold flexibility allowed for more extensive conformational changes of the presented sulfates, resulting in charge clustering that elicited enhanced L-selectin binding in a multivalent manner.[96] Selectin inhibition has also been demonstrated with HPG-based glycoconjugates with scaffolds bearing both sulfate and galactose moieties exhibiting IC_{50} values of 1 nM.[92]

It has been shown that HPG sulfates accumulate at the sites of inflammation. In addition to being a potential therapeutic for chronic inflammation, HPG sulfates also have the potential as a diagnostic tool for imaging inflammation. *In vivo* studies using an arthritis rat model with joint inflammation demonstrated differential dye uptake depending on the acuteness of the inflammation.[97] In addition, Schulze-Tanzil and co-workers investigated the influence of sulfated HPG on knee osteoarthritis in an instability rat osteoarthritis model.[98] Rats did not show clinical signs, joint swelling, macroscopical alterations, and pain when treated with this polymer. The authors concluded that while the mode of action remains a matter of debate, this polymer did in fact improve the condition in osteoarthritis.

The Haag research group further explored the use of HPG as a macromolecular binding agent in both viral and immune inhibition.[89–92] Papp *et al.* presented mannose derivatives of hyperbranched polyglycerols which showed high inhibition, in nM range, with concanavalin A in multivalent fashion.[99] Multivalent HPGs bearing negative charges or carbohydrates have also been used for inhibition in the binding and uptake of influenza A,[92] controlling mRNA splicing events,[91] as anti-thrombotic agents[100] and most recently, as an approach for targeted antibacterial photodynamic therapy.[101] As multivalent binding is an essential aspect of biological interactions and assembly, it is expected that the utility of multivalent HPG scaffolds will be far reaching in nanomedicine.

4.1.2 HPG conjugates (drugs, proteins and peptides). Often small molecule drugs, peptides and antibody-based drugs and protein therapeutics suffered from weak solubility, short blood circulation times, non-specific interactions, enhanced immunogenicity and toxicity. Conjugation of such moieties to polymers can be used as a viable strategy to overcome these limitations. For instance, in the field of iron chelation therapy (ICT), polymer conjugation with low MW iron chelators has proved

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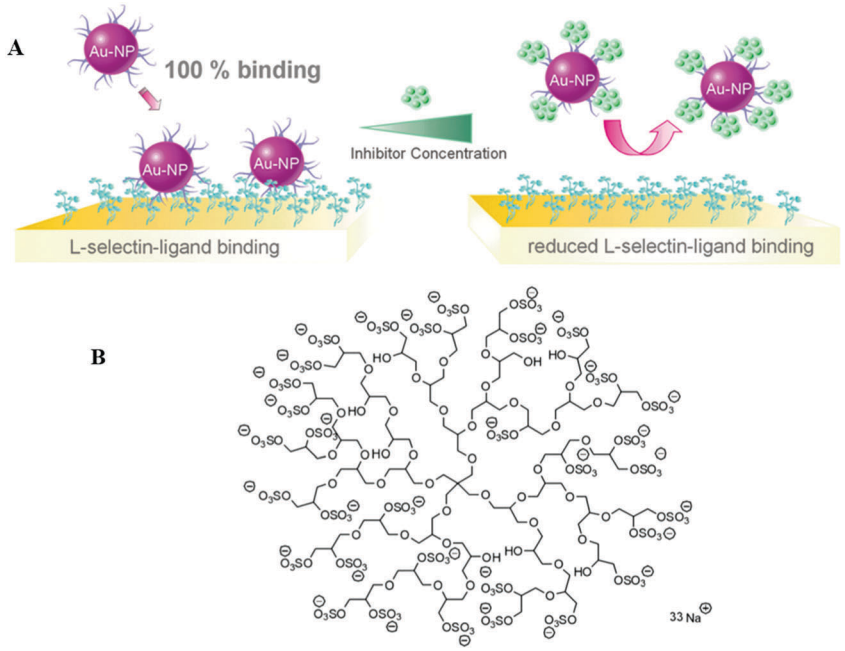


Fig. 18 dPGS mediated inhibition of L-selectin binding; (A) AuNP decorated with L-selectin have a reduced ability to bind to surfaces following incubation with dPGS, (B) representative structure of dPGS.[95,96] [Reproduced with permission, Copyright 2011, American Chemical Society.]

to be a successful means to mitigate toxicity, enhance bio-kinetics and bio-distribution, and significantly improve drug efficacy.[102–104] Kizhakkedathu and colleagues recently reported the design and development of long acting macromolecular chelators to address the current limitations in ICT.[70,71,105] They took advantage of the biocompatibility, multifunctionality and long circulation times of HPG. The HPG deferoxamine (DFO) conjugate developed showed significantly improved pharmaco-kinetic properties as well as iron excretion efficiency in iron overloaded animals in addition to long circulation. They were able to increase the circulation half-life of DFO from 5 minutes in normal mice to almost 44 hours which is the longest circulation time reported for DFO conjugates (Fig. 19). This would allow the possibility of once a week/two weeks injection of DFO conjugates compared to the current clinical regime of ~8 h daily subcutaneous injection of DFO. In addition, the dose tolerance of HPG–DFO was also significantly increased based on both mouse and zebra fish models.[71,104] This nanopolymer based chelating system is potentially of high clinical utility for the treatment of transfusion dependent iron overload.

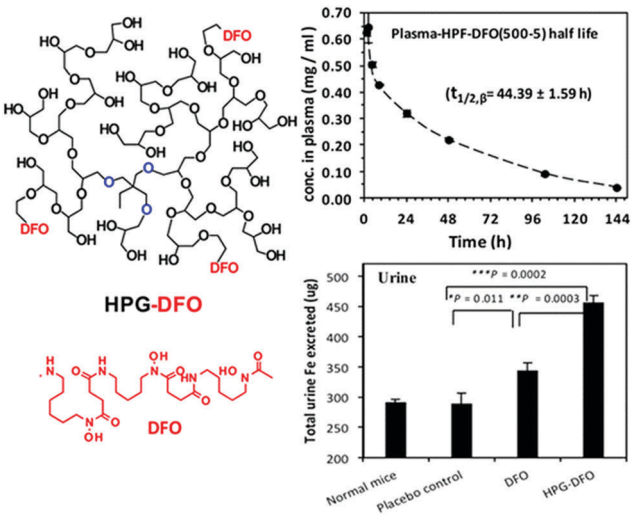


Fig. 19 Pictorial representation of HPG–DFO derivative and plasma concentration in mice and iron excretion efficiency of HPG–DFO in comparison to DFO in iron overload model.[70] [Reproduced with permission, Copyright 2013, American Chemical Society.]

Peptides are important biomolecules involved in critical biological processes such as immunity, targeted imaging, anti-microbial activity, platelet function and mRNA splicing.[106–110] Freund and co-workers utilized the multivalency effect on HPG by conjugating many peptides to the HPG scaffold.[110] The proline rich peptide used is a binding partner to FBP21, a protein involved in splicing of pre-mRNA in the nucleus of eukaryotic cells. The binding affinity was enhanced by nearly 9-fold once the peptides were conjugated to HPG compared to free peptides. In contrast, Zhang *et al.* conjugated a very short peptide, RGD (arginine–glycine–aspartic acid), to HPG polymer for its use in antiplatelet therapy.[109] RGD peptide is known to bind to platelet integrin GPIIbIIIa and interrupts platelet-fibrinogen binding, and platelet aggregation during thrombosis.[109] Conjugation of the RGD peptides to high molecular weight HPG increased the platelet inhibitory function by 2–3 orders of magnitude (Fig. 20). Moderate levels of conjugated peptides could lead to suitable candidates for the development of an antithrombotic drug strategy.

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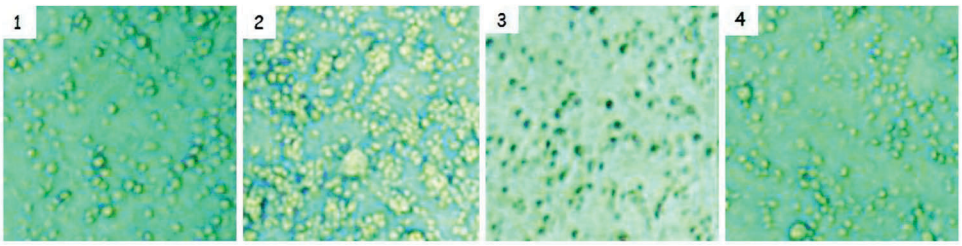


Fig. 20 Decreased platelet aggregation in the presence of HPG–RGD conjugate.[109] Panel 1, resting platelets; panel 2, activated platelets; panel 3 and 4, platelets incubated with HPG–RGD conjugate. [Reproduced with permission, Copyright 2008, American Chemical Society.]

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In addition, Haag and co-workers conjugated a tolerogenic peptide to HPG to increase the bioavailability and efficacy in vaccination strategies.[107] Tolerogenic peptides have an ability to provide immunological tolerance. The tolerogenic peptide, pOVA is presented *via* the major histocompatibility complex II (MHCII) of the antigen presenting cell (APC) and recognized by T-cells. The uptake, storage and stability of the HPG–pOVA conjugates were dependent on the linkage chemistry. The degradable ester linked conjugates had superior properties compared to amide linked polymers. The authors also speculated that the linker structure might additionally target the conjugates to specific APC and favor a tolerogenic T-cell response. These conjugates are promising novel candidates for vaccines that could be used for the treatment of autoimmune diseases and allergies.

Additionally, Kumar *et al.* decorated moderate molecular weight HPGs with different densities of an antimicrobial peptide aurein 2.2t.[108a] Upon conjugation, the antimicrobial activity of the peptides and the alpha helical content decreased by 3 fold compared to free peptide. Increased peptide density resulted in a favorable biomembrane interaction. On the other hand, the conjugates showed excellent blood and cell biocompatibility when compared to the free peptides (Fig. 21).[108] The size of the conjugates and peptide density influence the biocompatibility of antimicrobial conjugates. In the following studies, Kizhakkedathu and Straus research groups showed that conjugation of more active aurein type peptides to HPG lead to enhancement in activity of the HPG–peptide conjugates with decrease in molecular weight of HPG. The optimal activity with good biocompatibility was obtained by conjugating the peptides to HPG with moderate molecular weights (22 kDa). Intriguingly, the HPG (22 kDa)–peptide conjugates were resistant to proteolytic digestion and retained antimicrobial activity compared to the free peptides which were inactive after proteolytic digestion. These results indicate that the conjugation of antimicrobial peptides to HPG could be a good way to achieve desirable therapeutic properties.[108b]

In recent years, the application of protein therapeutics has been developing rapidly for the treatment of various disorders such as cancer, hormone deficiency, virus infections and other metabolic diseases. The main challenges in protein therapeutics are their poor stability and low bioavailability.[111] Polymer conjugation approaches such as PEGylation have been used for decades to overcome some of these challenges. Hyperbranched polymers show

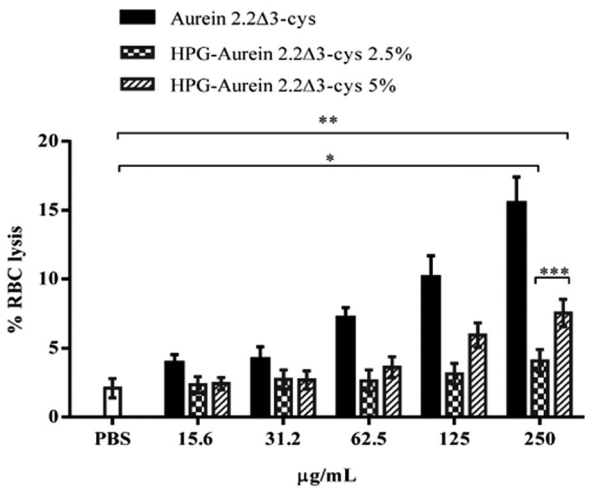


Fig. 21 Cell biocompatibility of aurein peptides and their conjugates against human umbilical vein endothelial cells.[108] [Reproduced with permission, Copyright 2015, American Chemical Society.]

many advantages over linear polymers and especially, HPG has been utilized as a candidate in both covalent and non-covalent conjugation of various proteins.[112–115]

Frey and coworkers designed a novel linear-hyperbranched PEG–HPG heterotelechelics with a single amino moiety at the alpha position which was subsequently used to attach biotin utilizing the high specificity of pentafluorophenyl esters of biotin. The biotinylated PEG–HPG was used for non-covalent conjugation with streptavidin. Biotin–PEG–HPG formed a tetramer complex with the multiple binding site of streptavidin (Fig. 22).[115] This approach has many advantages as a variety of functional groups can be introduced and covalent conjugation with other proteins and peptides is feasible.

In protein conjugation approaches, Klok and coworkers covalently attached BSA and lysozyme to various hyperbranched and linear-*block*-hyperbranched polyglycerol copolymers (Fig. 22).[114] The specific linkage of the protein to the PEG–HPG (and other copolymers) was facilitated by the squaric acid diethyl ester functionality (Fig. 23).[114] Although relatively low molecular weights (1–2 kDa) HPG–lysozyme conjugates showed high activities, HPG copolymer did not have any obvious structure–function relationship in this case. Interestingly, mid-functional polyglycerol lysozyme conjugates in the 10–20 kDa had significantly higher activities compared to their linear counterparts with

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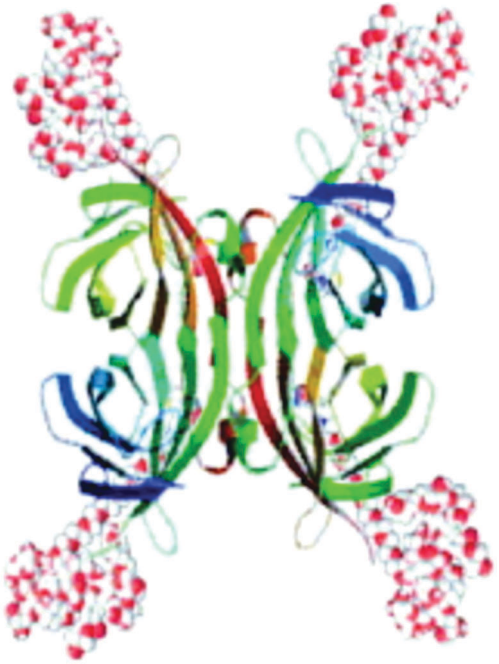


Fig. 22 Streptavidin–(Biotin–PEG–HPG)$_4$ non covalent conjugation *via* the biotin–avidin interaction.[115] [Reproduced with permission, Copyright 2009, American Chemical Society.]

similar molecular weight. The conjugation strategy can be further developed for protein delivery.

4.3 HPG as an osmotic agent in organ preservation and peritoneal dialysis

In biological systems, the colloid osmotic pressure imparted by proteins plays an essential role in controlling water movement between tissues and the surrounding environment.[116] The movement of water between different cell compartments is important for maintaining cell volume and changes in the concentration of osmotically active macromolecules present in the cytoplasm. In solution, HPG exhibit colloidal properties and contribute to the colloid osmotic pressure when added to biological systems.[66–68] With a low intrinsic viscosity similar to that of proteins, HPGs can be utilized in high concentrations without eliciting toxic effects.[67] With this, low molecular weight HPGs (1 and 3 kDa) have been employed as an alternative osmotic agent for therapies meant to control cell swelling.

The use of HPG as an osmotic agent was first demonstrated by Brooks and co-workers in the development of a substitute for human serum albumin (HSA), a protein responsible for 80% of the colloid osmotic pressure in the vasculature.[49] During severe blood loss, it is essential for HSA to be replaced to maintain blood volume. Recombinant HSA is currently used for HSA replacement, however, the production of HSA is expensive and susceptible to prion or virus contamination.[117] Synthetic alternatives to HSA like hydroxyethyl starch have been studied as an HSA replacement, however, these solutions have high plasma viscosity and cannot transport metabolites or scavenge waste molecules like native HSA.[118] Kainthan *et al.* synthesized an improved, low viscosity HSA substitute using HPG derivatized with hydrophobic groups for fatty acid binding and transport, and short PEG chains to enhance circulation times and protection from the immune system.[49]

Low molecular weight HPG was also used as a replacement for HES in cold organ preservation solutions, a common strategy used to minimize organ injury prior to transplantation.[119] Minimizing tissue edema and endothelial cell swelling is essential for improving viability and outcomes of donor organs.[120] HES is the most widely used osmotic agent for organ preservation solutions however, the high viscosity of concentrated solutions of HES promotes blood coagulation which impairs immediate donor function after transplantation.[121] In a study by Gao *et al.* HPGs of various MW were used as a replacement for HES in the cold heart preservation.[68] It was found that heart transplants preserved with 3% 1 kDa HPG demonstrated better functional recovery, less tissue injury and neutrophil infiltration compared to HES. HPGs larger than 8.7 kDa demonstrated lower levels of

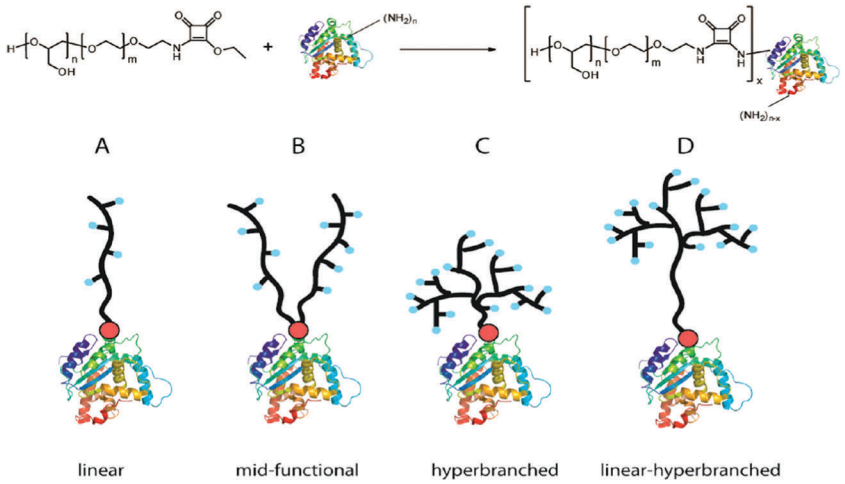


Fig. 23 Squaric acid mediated coupling of copolymers to proteins (top). Schematic representation of the different protein–polymer conjugates.[114] [Reproduced with permission, Copyright 2012, American Chemical Society.]

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cell protection, illustrating a MW dependence of the therapeutic effect. The improved HPG based organ preservation solution was further demonstrated to have a similar efficacy in kidney transplants.[122] In addition to cold storage, kidney is generally perfused with organ preservation solution before transplantation to avoid ischemic injury. Analysis of histological sections of kidney by Li *et al.* after perfusion revealed that the HPG solution had a better perfusion outcome compared to University of Wisconsin (UW) solution, the current gold standard for organ preservation or the other commercially available solution, histidine tryptophan ketoglutarate (HTK) solutions.[123] This result was attributed to the low intrinsic viscosity of HPG which prevented cell aggregation and damage to capillaries during perfusion (Fig. 24).[123] HPG fortified preservation solutions are currently in advanced preclinical studies to test its efficacy in different animal models.

To the same effect, low molecular weight HPG solutions have also been utilized as an osmotic agent in peritoneal dialysis (PD) solutions.[66,67] PD is an home based renal replacement therapy used in the management of end stage renal diseases. PD is a convenient and low cost alternative to hemodialysis and rapidly gaining popularity in the treatment of renal disease. Currently, glucose with high concentrations is used as an osmotic agent in the majority of FDA approved PD solutions. In collaboration with Du, Mendelson and da Roza, Kizhakkedathu and co-workers developed PD solutions containing low MW HPG as a glucose replacement which demonstrated good osmotic activity compared to control solutions.[66,67] In a rat model of peritoneal dialysis, the HPG containing PD solution protected the peritoneal membrane while having minimal impact on systemic and local toxicity compared to glucose based solution. It is remarkable that HPG showed better tolerance profiles in cells and in animals compared to glucose. HPG was also well tolerated in a chronic PD model where the HPG PD solution was injected daily for 3 months into the peritoneum of rats.[69]

4.4 HPG as a macromolecular crowder for cell-surface modification

The colloidal behavior of HPGs may also be used to enhance biological reactions. Inside the cells, proteins are present in high concentrations (200–300 mg mL^{-1}) creating an environment able to drive association processes and changes in protein conformation.[124,125] This effect can be recapitulated outside the cell in the form of macromolecular crowding with polymers. Crowding operates through a volume-exclusion effect which favors macromolecular associations onto surfaces, enhancing reaction rates and association constants of biological reactions.[126,127] With this, crowding based approaches are able to limit the amount of macromolecular reagent required by increasing association events without increasing the concentration of the active substrate.[125] This strategy has great potential to reduce costs of surface modification strategies. High concentration 'crowding' solutions of HPG have been used to great effect in enhancing reactions on a cell surface.[125,128] In a study by Rossi *et al.* the attachment of cell-surface reactive HPG and PEG polymers was enhanced 10-fold in the presence of 230 mg mL^{-1} HPG (30 kDa).[128] The cell compatible technique was demonstrated in the case of four different cell types-red blood cells (RBC), leukocytes, platelets, and Jurkat cells (Fig. 25).[128] Recently, Chapanian *et al.* have shown that enzyme reactions on cell surface can also be significantly enhanced using HPG as a crowder.[125] In the presence of HPG crowder (30 kDa), the activity of glycosidases that cleave A and B blood group antigens on red blood cells were enhanced as high as 500-fold compared to control reactions in PBS.

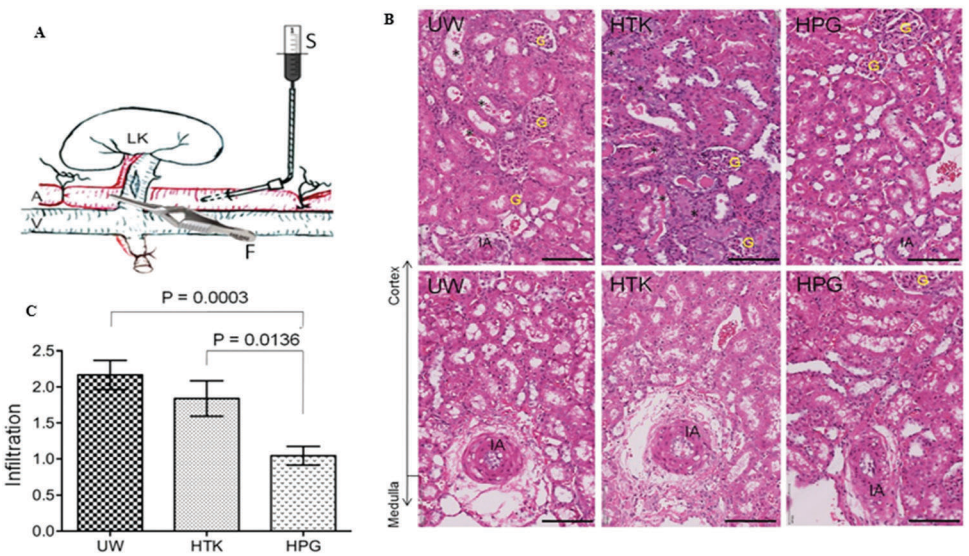


Fig. 24 HPG solution exhibited minimal cellular infiltration in kidneys: (A) left kidney of the Naïve adult Fischer 744 rat (male) was kept on ice for 1 h and perfused with three different ice-cold organ preservation solutions (HPG, UW or HTK) followed by reperfusion with blood; (B) the harvested kidney's sections were stained with hematoxylin and eosin, and the cellular infiltration in the cortex was scored under the microscope; *damaged tubules (necrotic tubules, tubular dilation, intratubular cast formation and tubular vacuolation), ^{G}glomerulus, ^{IA}interlobular artery; (C) a semi-quantitative analysis of at least 20 randomly selected views of two separate sections of a kidney.[123] [Reproduced with permission, Copyright 2016 Elsevier Ltd.]

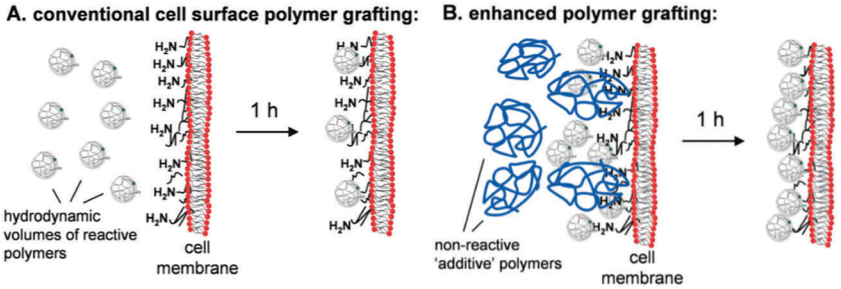


Fig. 25 Crowding-assisted attachment of polymers to RBC surfaces: (A) in the absence of additive inert polymers, cell-surface reactive polymers (grey) have low association with cell surface; (B) the addition of inert HPG crowders (blue) enhance cell surface association.[128] [Reproduced with permission, Copyright 2010, American Chemical Society.]

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The crowding based approach has also been demonstrated to increase the activity of a range of other glycosidases.[129] While linear polymers like PEG can also serve as macromolecular crowders, the highly viscosity of concentrated PEG solutions cause irreversible protein aggregation and cell damage.[128] In contrast, the compact branched polymer structure of HPG maintains low viscosity at high concentrations, making this polymer the best candidate demonstrated thus far for macromolecular crowding applications targeting a cell surface.

4.5 Controlled drug delivery applications

Controlled drug delivery is defined as the process of administrating a pharmaceutical compound in a controlled fashion to achieve a desired effect. Some of the most common drug delivery vehicles including liposomes, micelles, and polymers are commonly referred to as nanomedicines.[130] HPG has been utilized in the development of many nano-drug delivery systems owing to its compact shape, multi-functionality and biocompatibility. In the field of nanomedicine, HPG has been used as a drug delivery system in three different ways: (i) the covalent attachment of therapeutic molecules through biodegradable linkages (polymer–drug conjugates, polymer–gene conjugates), (ii) physical encapsulation of guest molecules within appropriately designed binding pockets within HPG (mostly polymeric micelles), and (iii) HPG based liposome systems. In most of these cases, the controlled release of drug from polymer scaffolds happens, in response to an external stimuli, such as a change in heat, light, temperature or pH.

4.5.1 Controlled drug delivery from HPG-conjugates. Among its many potential applications, HPG was used as a drug loaded dendritic core-multi-shell (CMS) nanocarrier for the treatment of skin disease. Topically applied nanocarriers are subject to degradation and conventional amide-based HPG-CMS nanocarriers risk the production of toxic polyglycerol amines. To circumvent this issue, Honzke *et al.* have developed a new tailored, biodegradable, HPG ester-based CMS nanocarrier with varying alkyl chain length of the lipophilic inner shell.[131] To test the efficacy of this nanocarrier, the HPG ester-based CMS was loaded with Dexamethasone (DXM), a potent drug used for the treatment of inflammatory skin diseases which, has shown success in an inflammatory skin disease model with distinct anti-inflammatory effects, superior to commercially available DXM cream. This study demonstrates the potential of an HPG based CMS nanocarrier as an efficient and biocompatible delivery system for the treatment of chronic skin diseases.[131]

Due to the enhanced retention and permeation (EPR) effect of tumor tissues, HPG finds many applications in anticancer drug delivery and therapy. HPG and poly(ethylene oxide) (PEO) block copolymers have been conjugated with a hydrazine linker to doxorubicin (DOX), an anticancer drug, to enhance biocompatibility, increase water solubility and decrease the clearance of the drug.[132] This new class of double hydrophilic block copolymer underwent spontaneous self-assembly to form core–shell type micellar aggregates in aqueous condition. Due to the low in pH in tumour, the labile hydrazone linkage was cleaved and released DOX, thereby targeting the tumour tissue. Cytotoxicity of the system was evaluated and it confirmed the biocompatibility and nontoxic features of this copolymer.[133]

A further application of HPG as a drug nanocarrier is demonstrated by the HPG–morphine system, where morphine is covalently attached to HPG by a cleavable linker, releasing the morphine in injured tissue while preventing blood–brain-barrier permeation. This system has shown selective release to produce analgesia in inflamed rat paws, likely due to leukocytes esterase activity without major side effects and without crossing the blood brain barrier, verified by analysis of the concentrations of morphine in the brain, blood and paw tissue.[134]

HPG has a flexible, not biodegradable polyether backbone which could be a major problem in long term applications. Hence the development of biodegradable HPG (dHPG) is crucial in drug delivery. Hu *et al.* used an ester containing dHPG for controlled release of anticancer drugs (Fig. 26A).[44] Methotrexate (MTX), an anticancer drug was conjugated to dHPG. The amphiphilic nature of dHPG-MTX enabled the conjugates to self-assemble into polymeric micelles (Fig. 26B).[44] The micelles could be easily internalized by cancer cells, releasing MTX in mild acidic conditions and exhibiting high anticancer efficacy. The dHPG–MTX conjugate showed similar anticancer activity against HeLa and CAL27 compared to MTX only.[44]

4.5.2 HPG-micelles. The other popular approach for drug delivery is non-covalent drug encapsulation and release. One of the common approaches is the development of hydrophobically modified HPGs (hHPG) to generate a hydrophobic pocket within the HPG core (Fig. 27).[73] Such an approach has been

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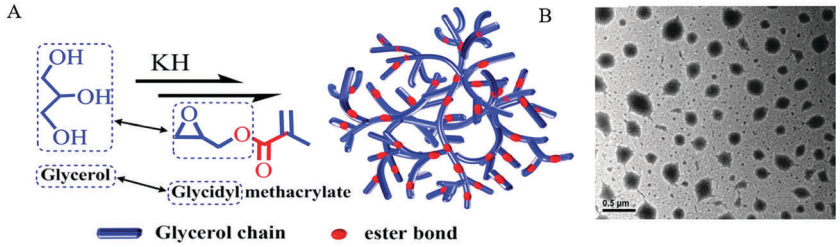


Fig. 26 (A) Oxyanionic initiating hybrid polymerization; (B) TEM of dHPG-MTX micelle.[44] [Reproduced with permission, Copyright 2012, American Chemical Society.]

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extensively studied for the use of loading and release of hydrophobic drug molecules.[135–137] Initial studies involved incorporation of paclitaxel and/or docetaxel in hHPG for cancer treatment. The HPG-C10-PEG formulation ford carrying paclitaxel was significantly more effective in reducing tumor growth in the bladder compared to paclitaxel alone (Fig. 27).[73] Later, amine groups were introduced in the shell of the same polymer architecture (HPG-C8/10-MePEG-NH_2) to encapsulate the docetaxel (DTX) in the hydrophobic pockets of the HPG-C8/10-MePEG-NH_2. The formulation of intravesical DTX (up to 1 mg mL^{-1}) in HPG-C8/10-MePEG-NH_2 was significantly more effective in controlling the tumor growth of the orthotopic model of bladder cancer than the commercially available formulation of Taxoteres. Other examples of successful HPG–drug encapsulation includes amphiphilic HPG (HPG-C18) loaded with hydrophobic docetaxel (DTX), a clinical antitumor drug, to inhibit breast cancer cell line MCF-7 cell proliferation. HPG-C10-PEG/HPGs systems were also used successfully to load and release docetaxel for the treatment of breast and pancreatic cancers.[138–140]

In another study progesterone was loaded to hHPG using the same technique as paclitaxel.[137] Interestingly, the maximum binding capacity of progesterone was dependent on the degree of functionalization on HPG. The progesterone release from hHPG correlated strongly with the bound water in the dendritic domain of the polymer indicating possible design of polymers with some control over loading and release. The release of progesterone was slower in human plasma compared to buffer.

4.5.3 HPG coated liposomes. Although conventional liposomes are a useful class of drug delivery platforms, they often suffer from low colloidal stability and rapid removal from the blood stream by the mononuclear phagocytic system (MPS). Attaching PEG to liposomes often provided stealth liposomes with improved pharmacokinetic properties, increased stability of the formulations, better blood circulation times and mimimized MPS uptake. Several PEGylated liposome formulations are FDA approved in particular in the field of cancer treatment. However, major disadvantages of PEGylated liposomes include the lack of additional of functionalities on PEG chains to further modify the properties of the assembled liposomes and the generation PEG antibodies. HPG coated liposomes are as one of the viable alternatives to PEG coating to overcome these limitations. Frey and co-workers developed the synthesis of hydrophilic HPG based liposomes by varying the molecular weight of HPGs and studied their stability in comparison to

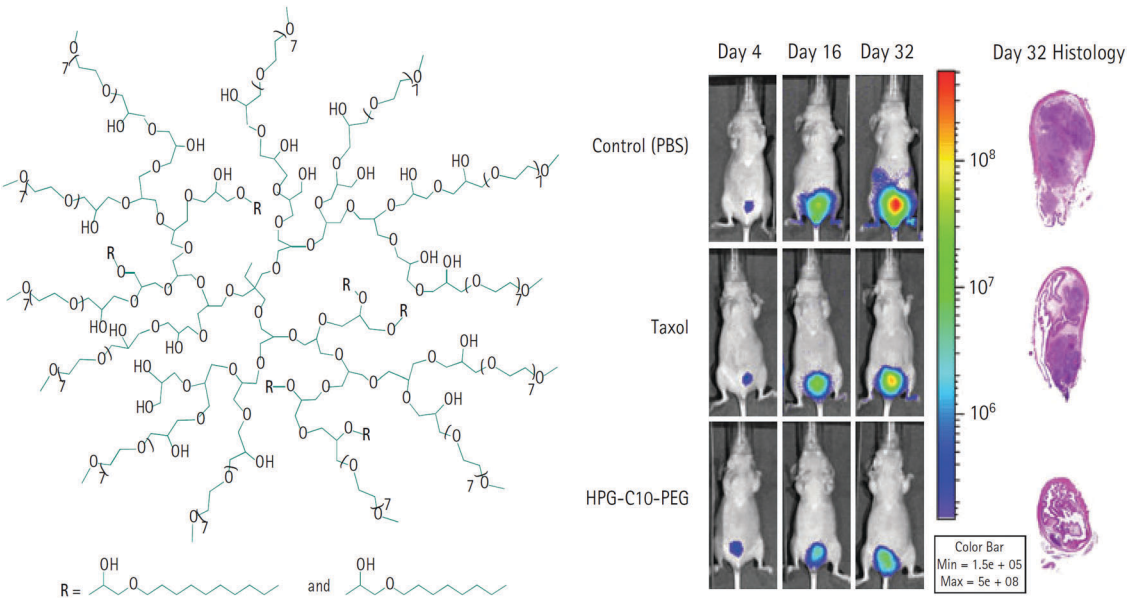


Fig. 27 (A) Typical hHPG molecule (HPG-C10-PEG); (B) reduction of tumor growth in mice models.[73] [Reproduced with permission, Copyright 2008, Wiley-VCH.]

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PEG systems.[141] Recently, Yuba *et al.* formulated hydrophobically modified HPG (3-methyl-glutarylated HPGs) and 1-aminodecane into a pH sensitive liposome for drug delivery applications.[142] In their pilot study, these HPG–liposomes delivered pyranine into immature murine dendritic cell lines more effectively than their PEG counterparts.[142] In their subsequent studies, the HPG–liposomes were used as a vaccine carrier to deliver ovalbumin which was encapsulated in liposomes to Murine bone marrow-derived dendritic cells. This encapsulated delivery system was successful in inducing an immune response in cell as well as a hormone mediated response in *in vivo* models.[143]

4.6 HPG as nucleic acid delivery agents

RNA interference (RNAi) is a mode of sequence based gene silencing that plays a key role in translational regulation. The clinical potential of this biological process is that, synthetic small interfering RNAs (siRNA) can be used for silencing the expression of a particular gene. This has therapeutic implications in various diseases such as cancer, infection and genetic disorders. However, it is challenging to deliver siRNA into the cell as these negatively charged nucleic acids are nearly impenetrable to the hydrophobic lipid bilayer. Traditionally, they are carried either by viral systems or non-viral vectors across the cellular membrane. Viral based systems have safety concerns and non-viral vectors are seen to be less efficient in transfection. Desirably, highly biocompatible HPGs offer solutions to the current limitations. Several studies have shown that functionalized HPGs can successfully deliver siRNAs.

The first class of nucleic acid binding HPGs were synthesized by Kizhakkedathu group in which, HPG was decorated with tris(2-aminoethyl)amine and capped with PEG chains.[144] Ethidium bromide (EtBr) displacement assay showed the binding of this system to DNA in the nanomolar range. Later, Fischer *et al.* developed several nanocarriers with an HPG core and amine shell for siRNA delivery.[145] One of the carriers they explored is a biodegradable version of an HPG–amine shell wherein, the shell is linked to the core with a carbamate bond (Fig. 28)[145] that is cleavable by enzymes. At physiological pH, these macromolecules are positively charged with high binding affinity for siRNA as can be seen from EtBr displacement assays. Their results indicate that by fine tuning the number of amines in the shell, it is possible to achieve a better transfection/toxicity ratio. Furthermore, the same group looked at the *in vivo* delivery of siRNAs into the tumor sites using these nanocarriers.[146] The nanocarriers successfully assisted the release of siRNA and silencing of the target gene at the tumor site in a luciferase expressing mouse model.

Although these macromolecules displayed a high transfection/toxicity ratio, both *in vitro* and *in vivo*, they were cytotoxic at higher concentrations, thus, limiting their administration to a narrow therapeutic window. To do away with this, Zeng *et al.* functionalized HPGs with amino acids as a safe alternative.[147] Tryptophan and histidine functionalized HPGs mimicked histones. These amino acid functionalized HPG turned out to be promising candidates with a broader therapeutic window and high transfection efficiency. In summary, HPG based nanocarriers have now made siRNA delivery possible in an efficient and less toxic way. HPG-siRNA therapeutics will be indeed a big leap in the field of biomedicine.

4.7 Cell surface engineering and regenerative medicine applications

The cell surface is a highly heterogeneous environment with a critical role in governing cell fate including: regulating cell–cell interactions, cell-niche communication and intracellular signaling

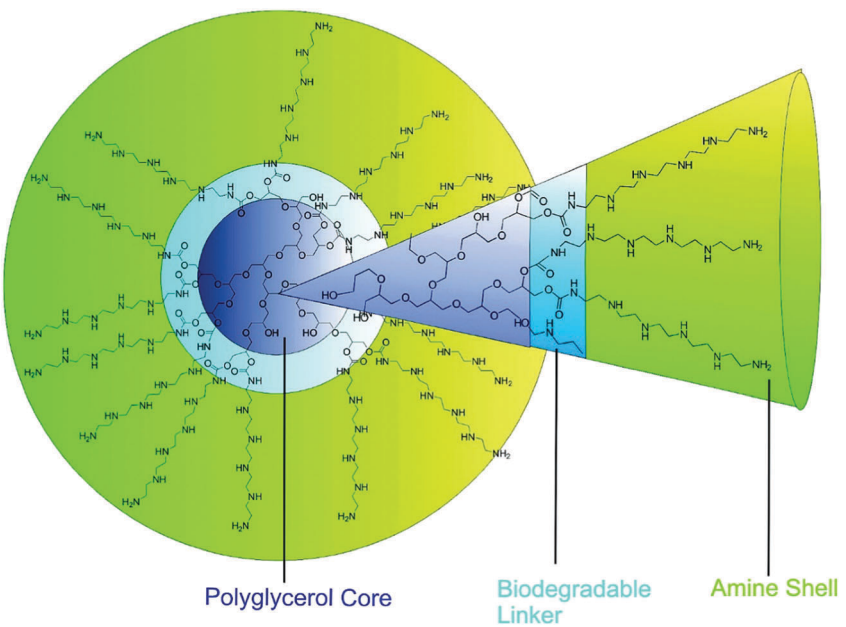


Fig. 28 Biodegradable HPG based siRNA nanocarrier. A carbamate bond linking HPG core and amine shell is subjected to enzyme mediated cleavage intracellularly.[145] [Reproduced with permission, Copyright 2010, American Chemical Society.]

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pathways.[148–152] Engineering the cell membrane with biomaterials, proteins and nanoparticles allows for new opportunities in controlling cellular functions which may be advantageous in drug delivery, cell-based therapeutics, transfusion and tissue engineering. Polymer-mediated cell surface engineering is especially powerful in immunocamouflage, an engineering strategy which attenuates the immune response to foreign cells. In this process, the introduced polymer coating on the cell surface acts as a shield to sterically prevent antibody mediated recognition of cell-surface antigens. PEG is currently the gold standard for immunocamouflage, however, issues related to PEG associated antibody generation have ignited a search for other immunocamouflaging polymers.[153–155]

HPG has a structure suitable for immunocamouflage but is less susceptible to undesirable effects such as protein aggregation.[74,125,128,156–159] Chapanian *et al.* developed antigen protected RBCs by grafting amine-reactive NHS ester functionalized HPGs onto the surface of RBCs (Fig. 29).[157] Subsequent HPG modification resulted in a significant reduction in binding of blood group antibodies to engineered RBCs compared to control RBCs.[128,157,158] In comparison to PEG, HPG provided significantly higher levels of CD47 self-protein accessibility and greater protection of certain antigens on the RBC surface without changing the native properties of RBCs. Chapanian *et al.* further investigated the *in vivo* circulation of HPG grafted RBCs in mice, demonstrating a normal circulation behaviour for HPG modified RBCs.[74,159]

Overall, cell surface engineering of RBCs using covalent grafting of polymers has shown promising results in production of antigen protected RBCs aimed at developing towards universal blood donor cells. It is important to note that multiple factors contribute to successful covalent modification of the cell surface, especially in polymer mediated immunocamouflage. The grafting density and depth of polymer brushes on cell membrane are vital in controlling the efficacy of immunocamouflage.[157,160,161]

In another approach for cell-surface modification, the Brooks and Kizhakkedathu groups developed zwitterionic HPGs capable of electrostatic mediated cell-surface modification. Hyperbranched polyglycerols (HPGs) were decorated with choline phosphate (CP), a neutral zwitterionic polymer which electrostatically interacts with the phosphatidyl choline end groups on cell-surface lipids in cell membrane without imposing cytotoxic effects (Fig. 30).[162] Various groups have adopted this methodology to modify the cell surface for different applications.[163–165] The Brooks group further extended this approach by developing sulfabetaine/quaternary ammonium modified HPG for hemostatic applications.[166]

Hydrophobic insertion into the cell membrane can also be used to attach polymers to the cell surface.[167,168] In a study by Jeong *et al.* to improve cellular homing, bioactive HPGs decorated with vasculature binding peptides (VBP) were conjugated with hydrophobic octadecyl chains and incubated with mesenchymal stem cells (MSCS). A surface coating suitable for targeted delivery to the vascular endothelium was generated using this approach (Fig. 31).[169] The bioactive polymer coating significantly enhanced the cellular affinity for the vascular endothelial adhesion molecule (VCAM), a protein which is over expressed in inflamed blood vessels.[169]

4.8 Imaging applications

Although there has been tremendous advancements in area of biomedical imaging, multiple imaging modalities are essential for precise detection of tumors. The current imaging modalities including X-ray, MRI (magnetic resonance imaging), SPECT (single photon emission computed tomography), PET (positron emission tomography) CT (computerized tomography) and US (ultrasound scan) have been widely used for different applications.[170] The use of a single carrier molecule with multiple functionalities can do away with the requirement of administering different probes having different pharmacokinetics their complementary properties offer precise tumor detection. Multifunctional HPG can serve as a useful platform to incorporate multiple imaging molecules on a single macromolecule. Its high water solubility, biocompatibility and long circulation time makes HPG a superior carrier molecule.

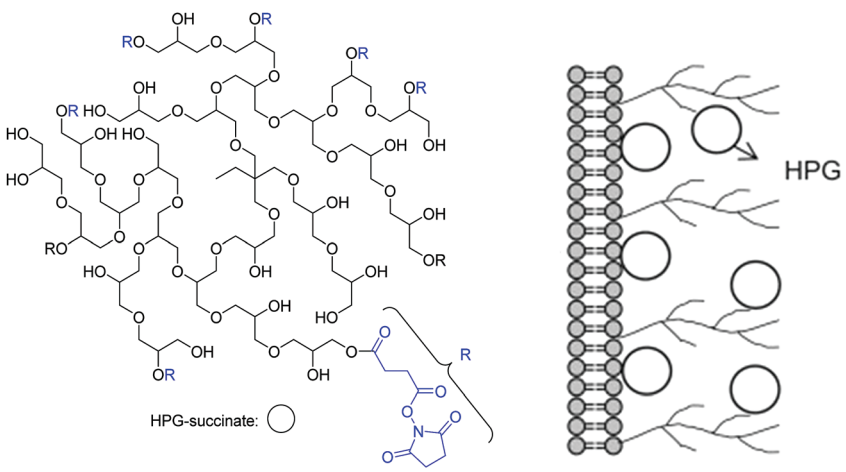


Fig. 29 Pictorial representation of covalent modification of cell surface with HPG *via* NHS ester–amine coupling. Reactions were performed under physiological conditions.[157] [Reproduced with permission, Copyright 2012 Elsevier Ltd.]

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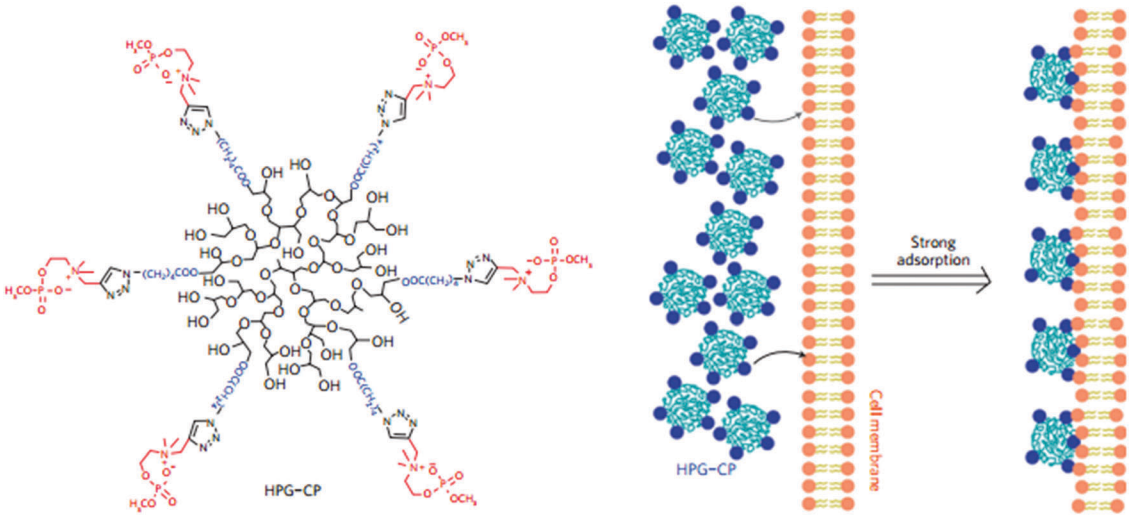


Fig. 30 An illustration representing the structure of polyvalent choline phosphate molecules contained hyperbranched polyglycerol (HPG-CP) and schematic representation of adsorption of HPG-CP on cell membrane.[162] [Reproduced with permission. Copyright 2012, Nature Publishing Group.]

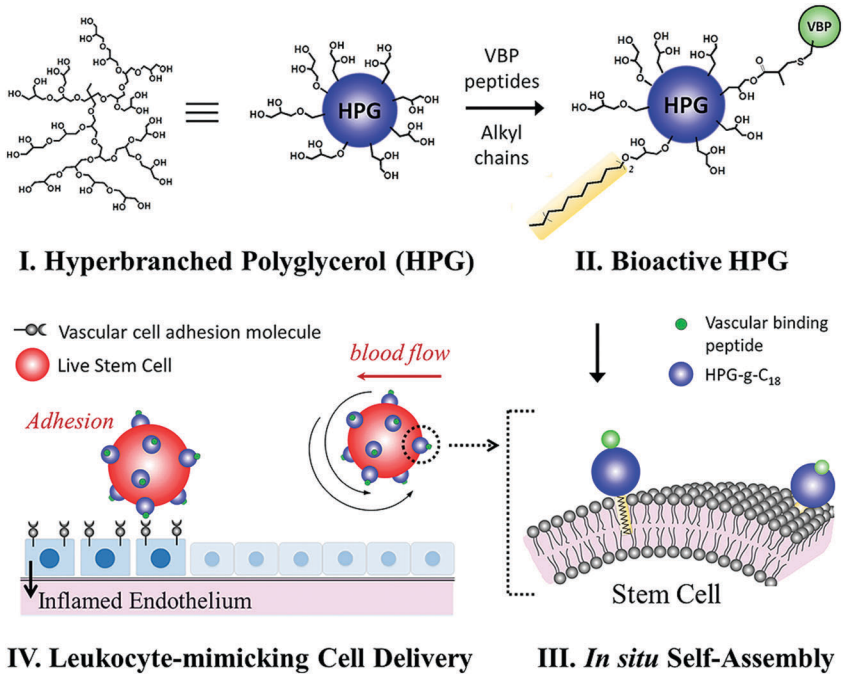


Fig. 31 Stem cell modification *via* hydrophobic insertion of modified HPG. A bioactive HPG modified with octadecyl chains and vasculature binding peptides was utilized for the modification of stem cells as a novel cell-guidance molecule and guide them to defective vasculature. *In vitro* studies demonstrated the proof-of-concept.[169] [Reproduced with permission, Copyright 2013, American Chemical Society.]

Saatchi *et al.* have shown the use of HPG in trimodal imaging for tumor detection.[171] HPG was functionalized with MRI, SPECT and fluorescent imaging probes with well-established approaches. HPG was functionalized with a chelator 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) that becomes paramagnetic and radioactive when coordinated with both Gd^{3+} and In^{3+} metal ions respectively. Also, attaching a fluorescent probe to HPG makes it useful for optical *in vivo* imaging. *In vivo* studies in mice have shown a differential distribution of modified HPG in the tumor. This could help in determining intra and intertumor heterogeneity. In addition to trimodal imaging, HPG can be grafted with supramagnetic Fe_3O_4 nanoparticles as a contrasting agent in MRI.[172] HPG has been also used for cardiac pool imaging. Cardiac pool imaging is a technique to see how well the heart is pumping blood to the rest of the body. Traditionally, this is done by isolating RBCs from a person, radiolabeling with gamma emitter ^{99m}Tc to form RBC-^{99m}Tc and intravenously injecting them back. The radioactive signals from RBC-^{99m}Tc can be detected by planar equilibrium radionuclide angiography (ERNA) or SPECT. However, there are disadvantages associated

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with this imaging approach. Firstly, blood may come into contact with pathogens during radiolabeling; this poses an elevated risk of infection. Secondly, the materials used in this labeling technique do have substantial interaction with other drugs/substances such as chemotherapeutic drugs or anticoagulants that may be present in potential patients. This can result in low efficiency of labeling and erroneous cardiac ejection fraction calculations. To avoid these issues, Saatchi *et al.* functionalized HPG with a positron emitter ^{68}Ga complex of DOTA for cardiac pool imaging *via* PET.[173] Modified HPGs were quite successful for imaging cardiac contractibility and measuring ejection fraction in rats.[174] Functionalizing HPG is less time consuming than compared the conventional method. Moreover, unlike RBCs which have to be labeled fresh each time, functionalized HPGs can be stored and used readily.

Another example of HPG modification for imaging is its conjugation with carbon dots. Carbon dots are an excellent tool for fluorescence imaging and have found applications in bio-imaging and bio-sensing. But, poor pharmacokinetics makes carbon dots undesirable for clinical use. Li *et al.* has developed a system carrying carbon dots at the inner core and HPG at the outer shell to form HPG conjugated carbon dots (Fig. 32).[175] The HPG conjugate showed improvement in quantum yield, biocompatibility and strong fluorescence. The cells were shown to readily uptake the conjugated dots and become fluorescent. The additional advantage of HPG conjugation is that one could add more functional groups to the dots.

4.9 Theranostic applications

Theranostics is the new paradigm in biomedicine that combines both diagnostic and therapeutic capabilities of a drug or conjugate. Once the drug is administered, it is important to be able to monitor how well it is performing inside the body. One way to approach this is by developing a multifunctional drug that can be both imaged as well targeted simultaneously. As HPGs can be functionalized with diverse groups, they are excellent candidates for theranostic applications. Krüger *et al.* developed a theranostic molecular prodrug whose drug release inside the cell can be monitored by Förster resonance energy transfer (FRET).[176] It is composed of the HPG scaffold carrying multifunctional coupling linker linking doxorubicin to the HPG *via* pH sensitive hydrazone bond and indodicarbocyanine dye (IDCC) which is spatially close to doxorubicin. Under physiological pH, the fluorescence emission maximum of doxorubicin at 500 nm is quenched by IDCC *via* FRET (Fig. 33).[176] But at the intracellular pH, the hydrazone bond will be cleaved and doxorubicin becomes fluorescently active with an emission maximum at 600 nm. This fluorescence on and off enabled to precise quantification of the uptake of the doxorubicine in HeLa cells. Thus, carrying two probes, both therapeutic as well as imagining candidates, on a single system is a useful strategy to monitor drug uptake and localization in real time. Moreover, unlike previous techniques, the cellular uptake kinetics can be now be studied easily.

Another interesting theranostic application of HPG is reported by Jahandar *et al.* who functionalized HPG with both superparamagnetic iron oxide nanoparticles (SPIONs) and an anticancer drug, curcumin.[177] Curcumin is a hydrophobic molecule and its medical use is often limited by its low water solubility. Functionalizing HPG with curcumin increases the solubility of the drug. The SPION-HPG system loaded with curcumin is shown to have an impressive *in vitro* contrast enhancement in MRI. Also, the functionalized material decreased the cell viability of MCF-7 breast cancer cells *in vitro* somewhat, demonstrating the activity of released drug.

4.10 Antifouling surfaces

Biofouling, the phenomena of adsorption of biological macromolecules onto a surface either by specific or non-specific interactions, remains one of the primary obstacles to overcome

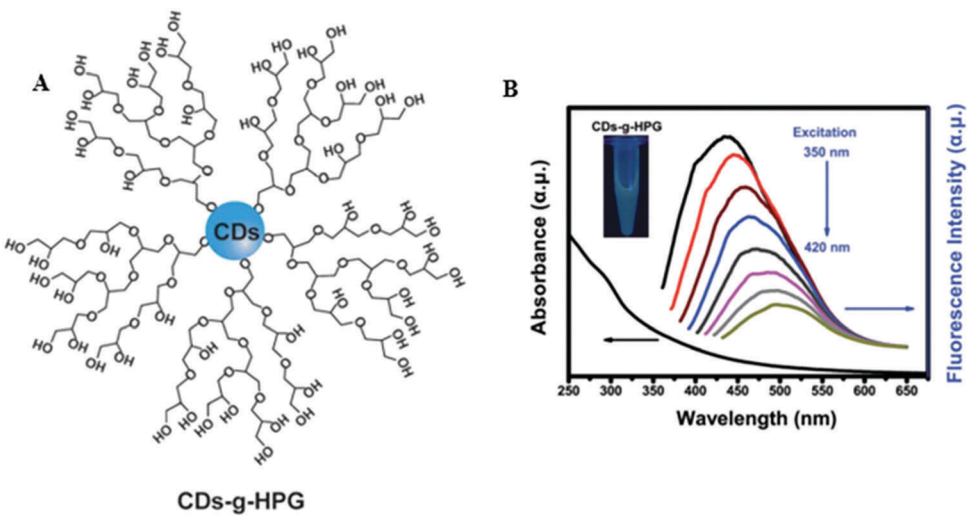


Fig. 32 (A) Schematic representation of an HPG-carbon dot conjugate. The carbon dot is the inner core and HPG forms the outer shell, (B) optical properties of the polymer carbon dot conjugate are shown.[175] [Published by The Royal Society of Chemistry, 2017 on behalf of the Centre National de la Recherche Scientifique (CNRS) and the RSC.]

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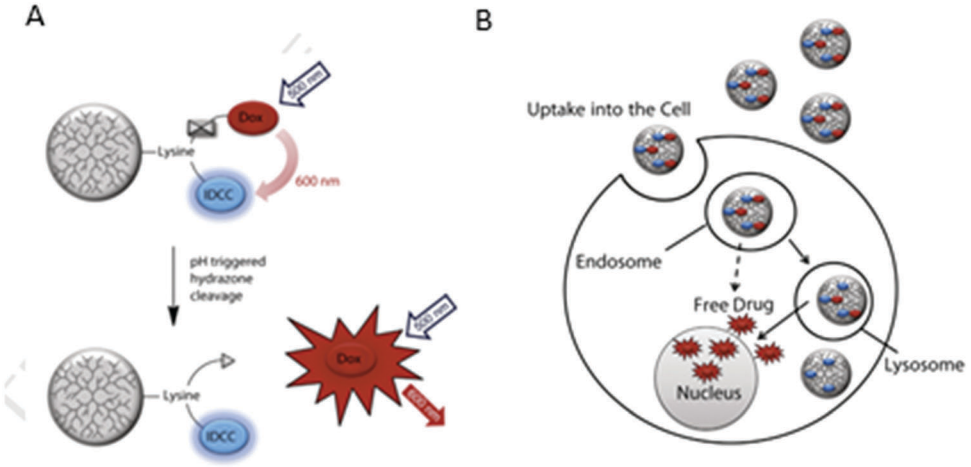


Fig. 33 Monitoring drug uptake by FRET: (A) doxorubicin fluorescence is quenched by IDCC when both are in close proximity. Once, the hydrazone bond is cleaved doxorubicin becomes fluorescent as it is spatially separated from IDCC; (B) pH sensitive hydrazone cleavage happens in the endo-lysosomal compartment of cells and free doxorubicin is translocated to the nucleus.[176] [Reproduced with permission, Copyright 2014 Elsevier Ltd.]

the design and fabrication of advanced surfaces for applications ranging from biosensors and biomedical implants to marine equipment.[178–180] Protein adsorption on synthetic surfaces can lead to increased cell and bacterial adhesion, forming a biofilm on medical device surfaces such as catheters, prosthetic devices, and contact lenses. A major strategy for adhesion resistance is the inclusion of an antifouling surface coating or functionalization of surfaces with an antifouling layer, typed experimentally by PEG in a self-assembled monolayer (SAM).[181–183]

Although PEG is currently the most commonly used moiety to impart antifouling properties to a surface, it has certain limitations such as the tendency to form aldehydes and ethers in the presence of oxygen, as well as poor thermal stability, causing the PEG coated surfaces to lose their protein resistance properties.[184,185] Thus, alternate macromolecules and strategies have been designed and studied.

HPG exhibits properties necessary for protein resistance when attached on a surface. In a study published by Wyszogrodzka *et al.* a series of HPG dendrons were modified by alkanethiols to allow generation of a coating on gold surfaces to study their interaction with proteins (*e.g.* fibrinogen, lysozyme, albumin and pepsin).[186,187] The SAMs of these HPG-thiols showed similar protein resistance to that achieved with PEG in a 24 hour time frame. Yeh *et al.* reported a comparison between PEG and HPG (similar molecular weights) coated surfaces against protein resistance.[188] They have shown that due to the compact and branched structure, HPG offers better protection against protein adsorption. Further, hydrophilic HPGs synthesized with biomimetic anchors (N-dopamine maleimide, tannic acid, dopamine) tethered to solid stainless steel surfaces.[189] These surfaces demonstrated substantial reduction in bacterial adhesion (Gram-negative *Pseudomonas* sp. NCIMB 2021 and Gram-positive *S. epidermis*) and inhibition of biofilm formation. This along with the comparable biocompatibility of HPG and PEG, and improved thermal stability of HPG compared to PEG, provides the initial evidence for HPG as a good alternative for PEG in antifouling biomaterials. Charged HPG thin films without the thiol functionalization have been systematically studied by Frey *et al.* and demonstrated protein resistance.[190] HPGs with opposite charges were synthesized and characterized (Fig. 34)[190] and cationic/anionic

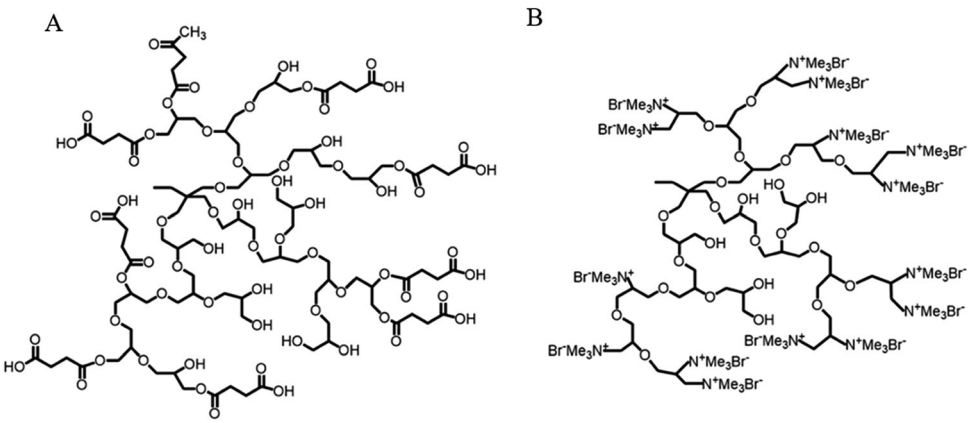


Fig. 34 Structures of (A) carboxyl-functional HPG and (B) cationic HPG, synthesized by Frey *et al.* as an antifouling thin film.[190] [Reproduced with permission, Copyright 2015, American Chemical Society.]

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HPG layers were fabricated by electrostatic interaction on various oppositely charged surfaces under physiological conditions.[190] The quantity of adsorbed protein was characterized and compared by surface plasmon resonance (SPR). Optimal films of HPG were shown to prevent non-specific protein adsorption. This work provides a simple strategy to fabricate HPG based antifouling surfaces *via* electrostatic interaction.

4.11 HPG based proteomics reagents

Analysis of N-termini of proteins and identification of protease cleavage sites have diverse applications in molecular and cellular biology. Isolation and identification methods based on chromatography, post-translational modifications, isotope and affinity based tagging approaches have been widely used to enrich and aid the quantitative analysis of the desired proteins or peptides. Recently, the Kizhakkedathu and Overall groups developed a hyperbranched polyglycerol (HPG) aldehyde based approach, named terminal amine isotopic labeling of substrates (TAILS) to overcome the limitations associated with the current proteomics strategies and have proved useful to distinguish protease cleaved N-termini peptides from their original N-termini protein.[191–194] In the TAILS approach, HPG aldehydes (100–500 kDa) were used to pull out the unblocked N-terminal tryptic peptides by an ultrafiltration method from a complex pool of N-terminal peptides including methylated, acetylated and cyclized peptides, isotopically labeled peptides and protease scissored naive N-terminal peptides. Later, all the above unbound proteins are analyzed by tandem mass spectrometry. This new approach helped to identify roughly 731 acetylated and 132 cyclized N-termini as well as 288 matrix metalloproteinase (MMP)-2 cleavage sites in mouse fibroblast secretomes.[192] In subsequent studies, the shell of HPG was modified with aldehyde groups by cleavable ester linkages.[195] The HPG supported aldehydes react with amine groups of the peptide by reductive alkylation and are removed from complex peptide/protein mixture by an ultrafiltration method to separate them from unbound peptides. The ester linkages between peptide and HPG were hydrolyzed and the released peptides with anchor was characterized by mass spectroscopy.[195] The HPG support was very efficient compared to commercially available resin supports owing to its very low non-specific binding and high reactivity.

5 Conclusions and future perspectives

In this review, we have described the increasingly relevant utility of hyperbranched polyglycerol polymers in translational research. Due to relatively simple synthesis protocol, scalability of synthesis, excellent biocompatibility and ease of functionalization, HPG and its derivatives have emerged as one of the most promising classes of biocompatible nanomaterials in the 21st century. In comparison to the most widely used polymers in medicine such as PEG, polyethyloxazoline, and HES, HPG has proved to elicit lower toxicity in biological systems with lower immunogenicity *in vivo.* Progress in the synthesis of HPGs including the development of high MW polymers, biodegradable scaffolds, hydrophobic HPGs, copolymers and HPG micelles have made HPG a versatile material for numerous applications. As bioactive agents and macromolecular therapeutics, HPGs have found utility in areas including drug delivery, treatment of thrombosis, dialysis, transplantation, imaging and as bactericides.

Although HPG based therapeutics have yet to reach clinical trials, many HPG based products have proved safe and effective in large animal models providing much hope for eventual clinical translation; such studies are ongoing. This review has highlighted significant advances in HPG research, however, improvements are always necessary from both biological and synthetic perspectives. The long-term (or chronic) development of HPG immunogencity *in vivo* will be a time intensive process, however; it is a worthwhile pursuit in elucidating the safety of HPG-based therapeutics. The development of greener strategies for HPG synthesis is a relatively new endeavor and an increasingly important aspect of modern chemical synthesis. While current strategies are limited to low MW HPGs, advancement in this sub-discipline toward higher MW species could have a significant impact on the translational aspects and commercialization of HPGs.

Conflicts of interest

JNK and DEB are inventors on a number of patent applications based on HPG.

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