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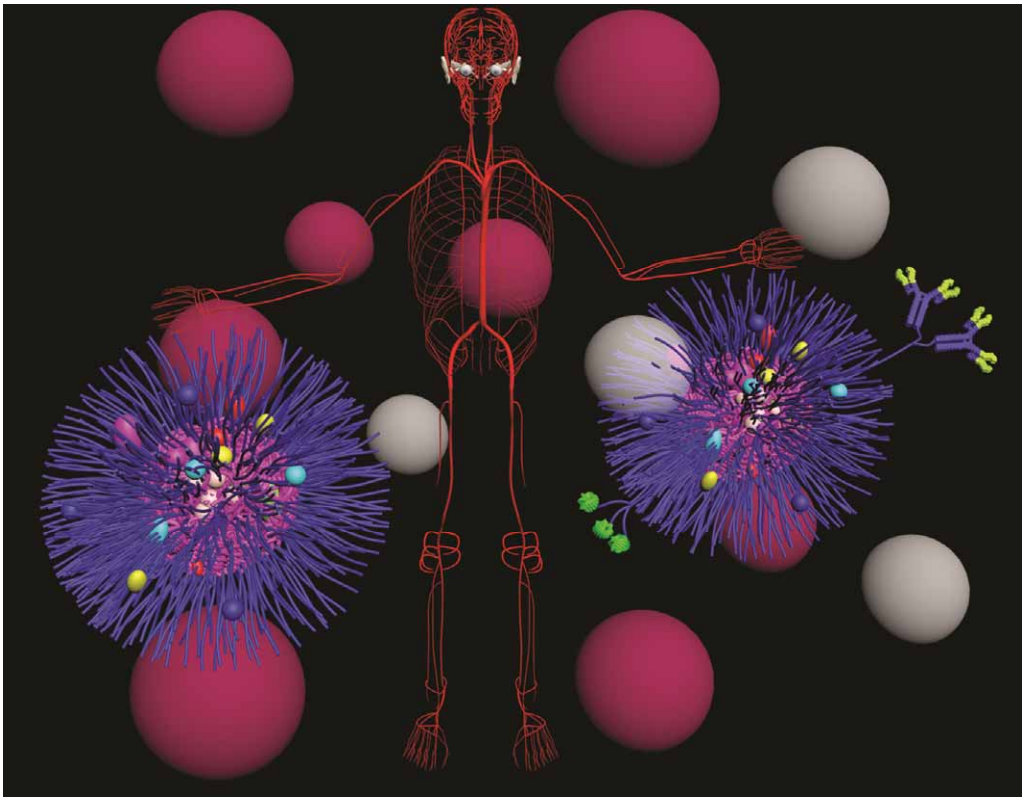

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CRITICAL REVIEW



# Multifunctional dendritic polymers in nanomedicine: opportunities and challenges†

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Nanotechnology has resulted in materials that have greatly improved the effectiveness of drug delivery because of their ability to control matter on the nanoscale. Advanced forms of nanomedicine have been synthesized for better pharmacokinetics to obtain higher efficacy, less systemic toxicity, and better targeting. These criteria have long been the goal in nanomedicine, in particular, for systemic applications in oncological disorders. Now, the "holy grail" in nanomedicine is to design and synthesize new advanced macromolecular nanocarriers and to translate them from lab to clinic. This review describes the current and future perspectives of nanomedicine with particular emphasis on the clinical targets in cancer and inflammation. The advanced forms of liposomes and polyethylene glycol (PEG) based nanocarriers, as well as dendritic polymer conjugates will be discussed with particular attention paid to designs, synthetic strategies, and chemical pathways. In this *critical review*, we also report on the current status and perspective of dendritic polymer nanoconjugate platforms (*e.g.* polyamidoamine dendrimers and dendritic polyglycerols) for cellular localization and targeting of specific tissues (192 references).

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## 1 Introduction

Nanotechnology is a rapidly advancing, innovative field of science. It involves interdisciplinary research that is aimed towards the production, characterization, development, and application of "molecular" materials with sizes ranging between $10^{-9}$ m (nanometre) and $10^{-6}$ m (micrometre).[1,3] A specific example is the application of nanotechnological

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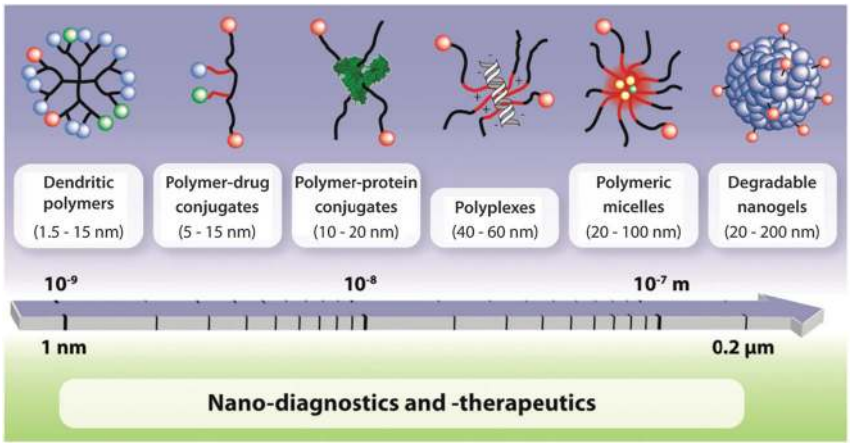


**Fig. 1** Multifunctional polymeric nanosystems under clinical consideration.

products for highly specific medical intervention at the macromolecular scale. The classical molecular bottom-up approach (1–100 nm) will be joined by a top-down approach. More recently, methods used to generate nanoscale structures and nanostructured materials are defined on the basis of either as "top-down" or "bottom-up". In the top-down approach, lithographic methods have been utilized to pattern nanoscale structures. While, in the bottom-up approach, interactions between molecules or colloidal particles are utilized to assemble the discrete nanoscale structures in two and three dimensions.[4] Recently, many devices have been conceptualized to fabricate with precision and to control the nanosize of materials using the top-down approach. De Simone *et al*. have reported the PRINT® process technology to obtain nanoscale control over the bulk heterojunction device architecture.[5] In this review we focus on the bottom-up approach, especially on soft matter polymeric systems as "nanotherapeutics".

Within the area of anticancer nanotherapeutics, it is anticipated that the nanotechnology based formulations will enhance the efficiency of the free form of drugs by imparting targeting to the desired organ/tissue/cell/cellular compartment and, concomitantly, reducing the systemic toxicity of the drug. Thus, it is not surprising that an emerging active area of academic and applied research in oncology is to identify the molecular targets which would facilitate the delivery of multifunctional nanosystems consisting of (i) polymeric nanocarriers, (ii) a targeting moiety possessing greater affinity or recognition for the cellular receptors, (iii) a therapeutic moiety, and/or (iv) an imaging probe. A parallel burst of interest has occurred in the production and characterization of new nanosystems, which require a highly interdisciplinary research environment. Some critical questions that have risen are whether the right nanosystems are being designed and evaluated? Which materials could be transformed into nanoforms (*e.g.* depots, nanoparticles) with the right inherent traits and suitability for

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nanocarrier systems? However, not enough polymer architectures are being designed and critically evaluated to deliver the drugs at the targeted site. To date, linear poly(ethylene glycol) (PEG), poly(lactide-co-glycolide) (PLGA), polysaccharide based, and amino acid based polymers have been successfully implicated, particularly to deliver the bioactives through the systemic circulation. More recently, advanced polymeric architectures such as hyperbranched molecules have been introduced and are now being evaluated for their safety and ability to deliver therapeutic agents.

A growing volume of literature indicates that an array of structurally diverse nanostructures of different sizes (*e.g.* quantum dots, nanoparticles, prodrug conjugates, nanospheres, nanotubes, nanocrystals, nanogels, liposomes, micelles *etc.*) are being developed for diagnostics or treatment related purposes (Fig. 1). Linear forms of PEG, multifunctional linear polymers like HPMA, dendritic polymers, and their conjugates are some of the nanostructural architectures that have been widely characterized and studied.[6–10] In this review we focus on the carriers and targeted conjugate forms of nanomedicines (polymer–prodrug conjugates and polyglycerol based nanogels). In particular, we discuss and compare the conjugation strategies of bioactives with PEG and dendritic polymers. The application of these conjugates for combating oncologic and auto-immune/inflammatory disorders (by virtue of their cellular localization and targeting the pertinent tissue) is also described. Towards the end, we delineate the current and future perspectives of such nanomedicines in therapeutics.

### 1.1 Definitions

*Nanocarrier*. The term ''nanocarrier'' is used to describe hybrid multifunctional systems with sizes typically ranging between 1–200 nm which may deliver the bioactive agent at the targeted site with improved therapeutic activity over the free form of bioactive agent. To date, they are also involved in long circulating liposomes, polymeric prodrug conjugates, polymeric micelle, nano/microgels, and nanocomplexes.

*Polymer therapeutics*. The term ''polymer therapeutics''[4–6] encompasses several different classes of polymeric systems including polymeric drugs, drug–polymer conjugates, polymeric micelles covalently linked to drugs, multicomponent polyplexes (including covalent unions), and prodrug–protein complexes.[11–13]

*Nanomedicine*. The term ''nanomedicine'',[13,14] is used to imply the application of nanotechnology (usually regarded within the size range of 1–200 nm) in the design of systems and devices that can facilitate better understanding, diagnosis, and treatment of pathological diseases. Nanodiagnostics and nanotheranostic technologies are other important fields of nanomedicine.[13,15]

### 1.2 Biophysical requirements

The interface between nanomaterials and biological systems is of great importance due to their associated toxicity and overall safety. As a result, the biophysicochemical interactions at the nano–bio interface can be thoroughly predicted due to the relationship between structure and activity and properties such as size, shape, surface chemistry, roughness, and surface coatings.[16]

The *in vivo* biocompatibility of nanoparticles based on their physical characteristics can be seen in a three-dimensional phase diagram (Fig. 2).[16] The qualitative biocompatibility trends are revealed after screening around 130 nanoparticles *in vivo* intended for therapeutic applications. The particle variables that determine *in vivo* biocompatibility are size, zeta potential, and dispersibility. The biocompatibility is shown with red representing toxicity, blue the safety, and blue-green-yellow intermediate levels of safety (in the same order). This also reconfirms that the traits of cationic particles with high surface reactivity would be more toxic (red hue) than the larger hydrophobic or poorly dispersed particles. In addition, they are rapidly and safely (blue hue) removed by the reticulo-endothelial system (RES). Therefore nanoparticles with sizes over 10 nm need to be biodegradable for effective clearance by kidney or biliary tract (Fig. 2). Interestingly, the particles with the most enhanced permeation and retention (EPR) in anticancer drug delivery systems are average sized and have a neutral surface charge.

More studies focused in this direction have been recently done by the Haag group, wherein the structure–activity relationship of dendritic polyglycerol (dPGs) derivatives is predominantly disclosed.[17,18] Interestingly, surface charge properties of different dPGs are highlighted in terms of surface functionalities and compared with amine and hydroxyl terminated polyamidoamine (PAMAM) dendrimers. Furthermore, the cell biocompatibility studies demonstrated that the dPGs are as non-toxic as linear PEG polymer or dextran.

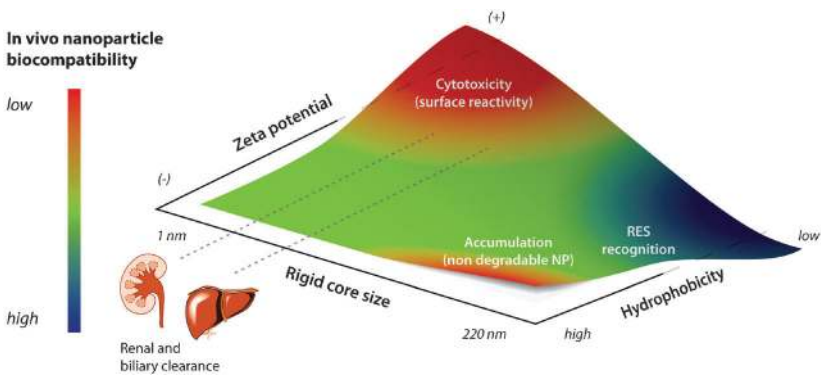


**Fig. 2** Physical characteristics of nanoparticles determined in terms of *in vivo* biocompatibility. Modified with permission from ref. 16, Copyright 2009 Macmillan Publishers Ltd: Nature Materials.

 

## 2 Current status of nanomedicines

The emerging field of nanotherapeutics has already had multiple success stories based on innovations related to covalent conjugation of polymers with drugs or proteins (Table 1). Although a variety of nanotherapeutics have been conceptualized in the form of drug delivery systems (liposomes, nanoparticles, micelles), polyplexes (*e.g.* DNA–polycation complexes), polymeric micelles, dendritic core–shell architectures as well as nanoparticle depots, the clinical success of nanomedicine is best exemplified by the utilization of polymeric conjugates to effectively deliver therapeutically relevant drugs, peptides, proteins, and antibodies (Fig. 3). Several polymers have been approved for use as a conjugate for delivering bioactive agents in the form of nanotherapeutics. However, poly(ethylene glycol) (PEG) remains the polymer of choice for "clinical" prodrug conjugation. This technology is now commonly referred to as 'PEGylation'.[10–12] The companies Nektar and Enzon have developed clinically successful PEG based protein conjugates such as PEGylated asparaginase (Oncospar®), PEGylated bovine adenosine deaminase (Adagen®), PEGylated interferons, PEGylated granulocyte colony stimulating factor, and PEGylated insulin. Companies, such as Pfizer, Schering Plough, Roche, and Amgen, have PEG-conjugates (PEGvisomant, PEG-intron, PEGasys, and Neulasta) that have been clinically applied as nanotherapeutics (Table 1). Besides the PEG-based technologies, several other polymer conjugates are also being evaluated for their potential to ameliorate the treatment of different human diseases. Of note, a conjugate of polystyrene-co-maleic acid and neocarzinostatin (SMANCS; marketed by Yamanouchi Pharmaceutical Company) is being used to treat hepatocellular carcinoma. The cumulative evidence from these clinical formulations has firmly established that nanoconjugates improve the therapeutic value of bioactives by any of the following approaches: (i) increasing the half-life of poorly bioavailable molecules, (ii) reducing the immunogenicity, (iii) decreasing the systemic toxicity, and (iv) exhibiting increased stability over the free form of the bioactive.

### 2.1 Implications and rationale for effective nanodelivery systems

An advanced area of nanomedicine is based on the potential utility of polymeric systems in the diagnosis and/or treatment of cancer. Indeed, various classes of polymer–drug conjugates, polymer–protein conjugates, nanoparticles, polymeric micelles, and multicomponent polyplexes have been extensively studied and some are routinely being used in clinical settings.[19–22] Currently, a plethora of highly potent anti-cancer drugs are available, but the selective targeting of these drugs to pertinent sites still remains a challenging task. Several important considerations are to be borne in mind when polymer–drug conjugates are being sought to deliver and specifically target anti-cancer drugs. These include the design of a stable covalent linkage between the drug and polymer, to ensure the uptake of pro-drug in tumor cells (*e.g.*, *via* an endocytic route),[12] improvement of the "pay load" and retention of drug within cancer cells, and utilization of ligands (*e.g.*, antibody, peptide, carbohydrate) to increase the targetability of the polymer conjugate.[23,24] The above parameters are critical for the targeted delivery of not only highly toxic small molecule anti-cancer drugs but also

**Table 1** PEG, dendrimer, and other nanocarrier platforms (*e.g.*, liposome, nanoparticles) in clinic

| Nanomedicine | Company | Form | Indication | Delivery route |
|---|---|---|---|---|
| Pegasys, Peginteron | Nektar Hoffmann-La Roche | Linear PEG (40 kDa) conjugated with interferon α 2a | Hepatitis C | s.c. |
| Mylotarg, Gemtuzumab ozogamicin | Wyeth | Recombinant humanized IgG4, kappa antibody conjugated with calicheamicin | $CD33^+$ acute myeloid leukemia | i.v. or i.m. |
| Xyotax, Paclitaxel (37 wt%) | Cell Therapeutics | Poly L-glutamic acid (40 kDa) | Non-small cell lung cancer | i.v. or i.m. |
| Oncospar | PEG Asparginase | mPEG (5 kDa) | Acute lymphocytic leukemia | i.v. or i.m. |
| VivaGel | Starpharma Holdings | Dendrimer gel | Vaginal microbicide for prevention of HIV and genital herpes | Vaginal gel |
| Aurimune (CYT-6091) | CytImmune Sciences | Colloidal gold nanoparticles coupled to TNF and PEG-thiol | Solid tumors | i.v. |
| Somavert | Nektar Pfizer | Pegvisomant (PEF-hGH) | Acromegaly | s.c. |
| Macugen | OSI Pharmaceuticals Pfizer | Pegylated anti-VEGF aptamers | Neovascular age-related macular degeneration | Intravitreal |
| Lupron Depot (Leuprolide acetate) | Abbott Endocrinology | Depot suspension | Advanced prostate cancer | Bolus |
| Elestrin (Estradiol gel–calcium) | BioSante Phosphate | Nanoparticles | Moderate to severe flashes in menopausal women | Transdermal |
| Abraxane (Paclitaxel bound albumin) | Abraxis | Nanoparticles size | Metastatic breast cancer | i.v. |
| Doxil/Caelyx (Doxorubicin HCl) | OrthoBiotech | PEG stabilized liposome | Ovarian cancer Kaposi's sarcoma | i.v. |
| DaunoXome® (Daunorubicin) | Gilead Sciences | Liposomal emulsion | Advanced HIV-related Kaposi's sarcoma | i.v. |
| Certolizumab Cimzia® | UCB Inc. | Pegylated form of antibody | Crohn's disease | Subcutaneous |

PEG: polyethylene glycol; HIV: human immunodeficiency virus; TNF: tumor necrosis factor.

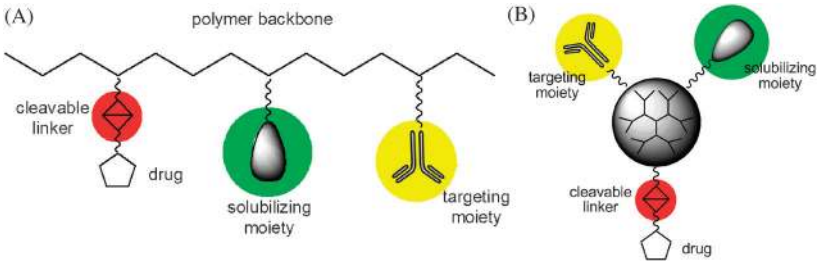


**Fig. 3** Different nanoconjugate architectures: (A) linear polymer–prodrug conjugate and (B) dendritic polymer–prodrug conjugate.

macromolecular oncologic therapeutics encompassing peptides, oligonucleotides, and antibodies.[25,26]

Although many nanodelivery systems have been adroitly synthesized, each platform technology needs to be critically evaluated in a specific therapeutic application prior to its being labeled as 'nanomedicine'. Because of the inherent cellular and molecular complexities of myriad human diseases, this remains a challenge. At the same time, this presents several opportunities. In the past, many startup companies and research laboratories have successfully introduced nanoplatforms. As a specific example, Lupron depot is now routinely used for treating prostate and other hormone dependent cancers.[27] Another example is Mylotarg, a nanomedicine platform consisting of antibody–drug conjugate, which is prescribed for acute myeloid leukemia. Several other nanoplatforms including Oncospar, PEGASYS, Neulasta, and Somavert can now be deemed as nanomedicine technology (see Table 1 for additional nanomedicines in clinic).

In general, only a fraction of macromolecular agents reach their biological targets *in vivo*. Thus, to enhance the therapeutic activity, it is vital to increase the intracellular penetration of drug-bearing nanoconjugates which reach their biological target *in vivo*.[28] Typically, the cellular plasma membrane serves as a barrier which occludes the transport of molecules based on the molecular weight, size, polarity, and charge of the macromolecule. Nanocarriers, by virtue of their internalization or shielding of anti-cancer agents, genes, and proteins, can "break" this barrier, cross into the cytoplasmic region, and increase the probability of heightened therapeutic response. Notably, the internalization of the nanocarriers into the cancer cells is achieved most efficiently by simple diffusion or receptor-mediated endocytosis.[29] Interestingly, several polymeric candidates, designed to augment the therapeutic response of a drug, may not be biocompatible due to their unsuitable polymeric architecture, higher surface charge, and inappropriate molecular weight.[17,20] Furthermore, the physico-chemical characteristics (*e.g.*, immune response, pH dependency profile, p$K_a$) of the polymeric candidates may also limit their potential use. Accordingly, these factors need to be considered when developing a nanomedicine based platform. Thus, critical determinants for nanodelivery systems include: (i) identification of specific molecular target(s), (ii) selection of suitable nanopolymer candidate(s), (iii) design of the nanocomponent delivery system, (iv) characterization of the nanoform, and (v) *in vitro* and *in vivo* biological activity and pharmacological evaluation.

To date, several different approaches (including the use of membrane-permeable peptides such as Tat protein and non-arginines) have been adopted to increase the intracellular uptake of nanotherapeutics.[30] As a specific example, cell penetrating peptides have been attached onto liposomal carriers and micelles which results in enhanced uptake of the polymeric carriers.[31] The augmented expression of cell-surface receptors—in particular of the receptors which are molecular mediators of disease—could also be exploited to increase the intracellular uptake of nanodelivery systems. For example, in oncologic indications, the vascular endothelial growth factor (VEGF) receptor, which is prominently present on the surface of several tumor cells, has served as an "internalization-facilitator". VEGF plays a major role in tumor initiated angiogenesis.[32] Furthermore, the largest class of oncologic drugs that block angiogenesis are the multi-targeted tyrosine kinase inhibitors (TKIs) targeting the VEGF receptor (VEGFR).[33] Anti-vascular endothelial growth factor therapies and, in particular, bevacizumab as monoclonal antibody against vascular endothelial growth factor, have demonstrated antitumor efficacy, although the mechanism of action in the latter is not fully understood. In this context, only a few vectors and molecular transporters show immense potential for breakthrough therapy as they deliver the drugs at intracellular locations after facilitation of their transport across the biological barriers.[26]

### 2.2 Enhanced permeability and retention effect (EPR) and types of targeting

Water soluble polymers are now routinely used to prolong the drug circulation and residence time within affected cells, enhance the solubility of drug, and reduce the systemic toxicity of drug.[29,34,35] Back in the 1980s, Maeda *et al.* and Jain observed that covalent conjugates of water soluble polymers with cytotoxic drugs were more effectively targeted to the tumor tissue than to its free form of cytotoxic drug.[36] Maeda described his finding using the term "enhanced permeability and retention (EPR) effect". The EPR effect, which leads to an increased "passive" accumulation of macromolecules in the tumor tissue, is governed principally by the hyper permeability of tumor vasculature. This hyper permeability allows selective extravasation of macromolecules into the tumor and poor lymphatic drainage and resultant increased retention of macromolecules in the tumor (Fig. 4).[37–41]

In order to exploit the EPR effect, due consideration needs to be given to the size and other physicochemical traits of the polymeric delivery system. The following examples will illustrate this view point. Abraxane® and Doxil® were two of the first nanocarriers to be approved by the FDA for cancer treatment. Given their relatively large sizes (130 and 150 nm, respectively), it is unlikely that these nanodepots will deeply penetrate into a tumor mass.[42] Therefore, the size of these nanocarriers needs to be critically optimized.[43] Indeed, in a recent study, Sisson *et al.* demonstrated that the polyglycerol (PG) microgel particles with

 

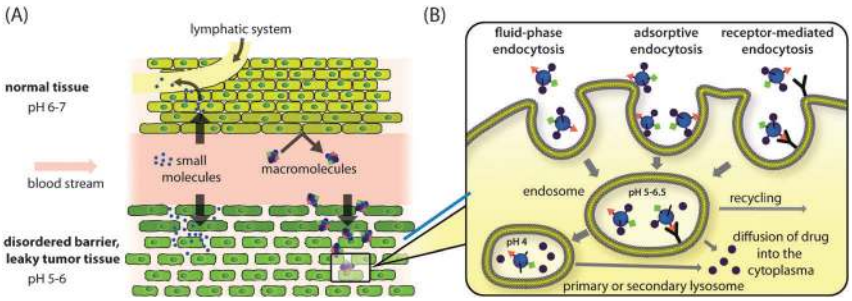


**Fig. 4** Schematic representation of the (A) EPR effect and the further (B) cellular uptake mechanism.[7,36,37]

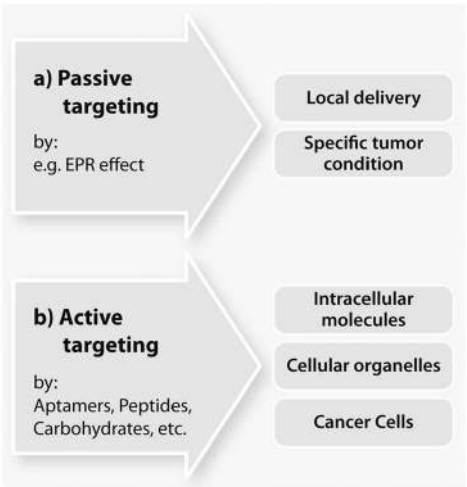


**Fig. 5** Types of tumor targeting: passive and active targeting.

diameters ranging between 25–50 nm are very efficiently and non-disruptively uptaken by the cancer cells.[44] These studies highlight the importance of an "optimal" size for at least a partially efficient passive accumulation of polymeric delivery systems in the disease/distressed tissues.[6,7,45,46]

Tumor invasion, metastasis, and resistance to chemotherapeutic drugs as well as radiation are major obstacles for the successful treatment of cancer.[47] Some of these limitations can be overcome by therapeutic strategies that increase the specificity and efficacy and, at the same time, reduce the toxicity of the anti-cancer drugs. One of these approaches includes targeting the polymeric delivery systems specifically to the cancer cells.

The targetability of polymeric forms of nanodelivery systems to the cancer cells and tumor can be achieved by adopting one of the following two approaches: (1) passive targeting and (2) active targeting (Fig. 5).[48]

**2.2.1 Passive targeting.** In the passive targeting approach, the localized delivery of a drug is achieved, due to environmental conditions in tumors and/or tumor bearing organs.[48] It is well recognized that, in comparison to the normal tissues, tumor microvascular endothelium exhibits an enhanced leakiness which results in markedly elevated permeability to macromolecules.[48] Furthermore, the tumor tissue is characterized by ineffective lymphatic drainage.[36] The combination of the above characteristics, along with the hypervascularization evident in the tumor microenvironment, leads to an accumulation of low molecular weight drugs coupled with high molecular weight nanocarriers in tumors. Thus, the aforementioned accumulation of macromolecules in tumor tissues has the potential to "passively" deliver the chemotherapeutic agent to the tumor. Notably, the existence of this predicted EPR effect has been experimentally confirmed by many types of macromolecular anti-cancer nanodelivery systems.[49] Theoretically, any high molecular weight water-soluble drug carrier, including water-soluble polymers, liposomes, and polymeric drugs, should display passive tumor targeting. However, the degree of accumulation of a polymeric nanodelivery system in the tumor will be a function of size, molecular weight, overall charge, and hydrophobic–hydrophilic characteristics of the delivery system.[17,20] Subsequent to its accumulation in the tumor tissues, the macromolecular form of a drug can act as a depot by slowly releasing the low molecular weight active drug. The conjugation of therapeutic agents to the polymeric nanocarriers could potentially afford further beneficial effects. For example, multi-component macromolecular pro-drug delivery systems may influence the drug distribution in the body, with enhanced bioavailability due to controlled and/or delayed release (Fig. 5). Such pro-drug systems often demonstrate reduced systemic toxicity in comparison to the free form of the drug. One of the earliest studies involving macromolecular carriers reported the utilization of DNA as a carrier for two oncologic drugs: daunorubicin (DNR) and doxorubicin (DOX).[50] It has been clearly established that DNA has a limited carrier ability due to potential genomic alterations.[47] In follow-up studies, the authors conjugated DNR to human serum albumin (HAS) *via* degradable peptide spacers. This conjugate showed a 200% increase in the life-span of mice inoculated with L1210 leukemia cells.[51] Later in 2005, paclitaxel was successfully bound with human protein albumin (brand Abraxane) to deliver a highly water insoluble drug in chemotherapy. This albumin based nano formulation could eliminate the use of chemical solvents (like Cremophor) causing a hypersensitivity reaction.

In an alternative approach of passive targeting, the molecular conditions in an organ bearing a tumor and/or in tumor environment are exploited to facilitate the drug release from the nanodelivery system.[52] These conditions may include, but are not limited to, a particular pH, and the existence of certain enzymes and/or microflora in a specific organ or tumor. For example, drug delivery to the colon might be targeted by formulating tablets with a specific coating that is destroyed in the colon by colon-specific pH and/or colon-specific bacteria.[53–55] An important limitation of this approach is the targeting of the entire organ and not just the tumor itself. This can potentially cause severe organ cytotoxicity,

unless the selective stimuli of the tumor itself (*e.g.* lower pH) are utilized.

The third passive tumor targeting approach is based on a direct local delivery of polymeric nanocarrier conjugated anti-cancer agents directly into the tumor site.[52] This delivery technique has the obvious advantage of excluding drug delivery from the systemic circulation. While topical delivery for some tumors may be achieved by injection or surgical procedures, other tumors, for instance, in lung cancers, are difficult to access for local drug delivery. To overcome this, several aerosolized technologies have been developed to locally deliver anti-cancer agents to the lung.[56]

All of the above "passive" approaches for targeting the polymeric forms of nanodelivery systems can be utilized to enhance a tumor-specific delivery of drugs. However, these approaches are rarely used as the predominant methodologies in current cancer therapies. The preferred and more routinely employed technique involves an 'active targeting' of the polymeric forms of nanodelivery systems.

**2.2.2 Active targeting.** An active tumor targeting of a nanodelivery system is usually achieved by coupling a targeting component on polymeric delivery system that provides preferential accumulation of the entire drug-delivery system or only of the drug in an organ bearing a tumor, in the tumor itself, in cancer cells, or in intracellular organelles of specific cancer cells.[52] The active targeting approach is based on the interactions between a ligand and its cognate receptor or between specific biological pairs (*e.g.*, avidin–biotin, antibody–antigen, sialic acid–carbohydrate).[57] In most cases, a targeting moiety in a nanodelivery system is focused on the specific receptor or antigen overexpressed in the plasma membrane or intracellular membrane in tumor cells.

This type of targeting is only possible when specific molecular receptors are present in malignant human tumor cells. For example, cancer cells often overexpress specific tumor associated antigens, carbohydrate epitopes, or growth factor receptors on their cell surfaces.[23,58,59] Incorporation of a biorecognizable moiety into the polymer carrier structure affords an actively targeted nano drug-delivery system. So far, the potential targeting moieties that have been explored include monoclonal antibodies, polyclonal antibodies and their fragments, carbohydrates (galactose, mannose), peptides/proteins (melanocyte stimulating hormone, transferrin, lutenizing hormone-releasing hormone, growth factors), glycolipids, vitamins, and other ligands.[44,58,59] Using these targeting moieties, active polymer–drug conjugates can be selectively transported into tumor tissues.

The concept of active tumor targeting has been illustrated by several approaches (Fig. 5). Many of these studies have utilized chemo-immunoconjugates wherein either a drug is directly conjugated with a monoclonal antibody or a drug–macromolecule conjugate is formed with a monoclonal antibody using a polymeric carrier. For example, the anti-cancer agent neocarzinostatin (NCS) has been conjugated with a murine monoclonal IgG1 antibody against a human colon cancer-associated cell-surface antigen. The NCS-monoclonal antibody conjugate showed significant suppression of tumor growth in patients with colon and rectal carcinoma and lower acute toxicity than with free NCS.[60,61] In separate studies, NCS has been covalently conjugated with TES-23, a highly specific anti-tumor tissue endothelium-specific monoclonal antibody.[62,63] The TES-23-NCS conjugate induced tumor hemorrhagic necrosis showed marked anti-tumor activity against rat/mice KMT-17 fibrosarcoma. Furthermore, mice treated with this immunoconjugate exhibited improved survival with no observable side effects.

These and other observations clearly demonstrate that active targeting enhances the overall accumulation of a polymeric nanodelivery system by the cancer cells thereby increasing the amount of the applied dose to actually penetrate the cancer cells. This may in turn lead to a substantial increase in the cytotoxicity of the drug and thus to a more effective anticancer activity.

### 2.3 Cellular localization and imaging ability of nanocarriers

Polymeric carrier platform involving prodrug delivery system (PDS) is likely to possess greater cellular entry and extracellular interactions compared to its free counterpart(s). This can be attributed, at least in part, to the supplementary characteristics of the polymeric carrier system. Indeed, the nanocarriers exhibit some of the critical features (*e.g.*, size, solubility, and molecular mass) required for a total PDS to facilitate the elicitation of enhanced efficacy. Furthermore, polymers (*e.g.* PEG) act as a penetration enhancer and improve the cellular internalization of a drug more than its free form.[64]

The plasma membrane has a dynamic structural functionality that segregates the chemically distinct intracellular milieu (the cytoplasm) from the extracellular environment by regulating and coordinating the entry and exit of small and large molecules.[65] For example, many small molecules, such as amino acids, sugars, and ions, can traverse the plasma membrane through the action of integral membrane protein pumps or channels. In contrast, larger macromolecules are carried into the cell through membrane bound vesicles by the "invagination" process and/or the plasma membrane process recognized as "endocytosis". This process of endocytosis encompasses two distinct mechanistic features: (a) phagocytosis (the uptake of large particles) and (b) pinocytosis (the uptake of fluid and solutes). Phagocytosis is an active and highly regulated process which involves specific cell-surface receptors and signalling cascades mediated by Rho-family GTPases.[63] Of note, phagocytosis occurs in specialized mammalian cells. Pinocytosis, which occurs in all cells, can take place in any of the following four forms: (i) macropinocytosis, (ii) clathrin-mediated endocytosis (CME), (iii) caveolae mediated endocytosis, and (iv) clathrin and caveolaein dependent endocytosis (Fig. 6). Each of these aforementioned endocytic pathways is known to affect a number of molecular processes including signal transduction, spatial organization, cell migration, and polarity. Intriguingly, the role of clathrin accessory proteins and the mechanisms that regulate clathrin-independent endocytosis are being extensively investigated.[65–67] Nevertheless, it is well-established that each pathway of endocytosis is regulated by the nature of the cargo molecule and its receptor.

The affinity of polymeric molecules with cells and the consequent cellular dynamics are of special interest in drug discovery research. It has been demonstrated that nanocarriers in the interstitial space of healthy tissue and tumor tissue are easily uptaken by cells *via* an endocytosis mechanism at rates

 

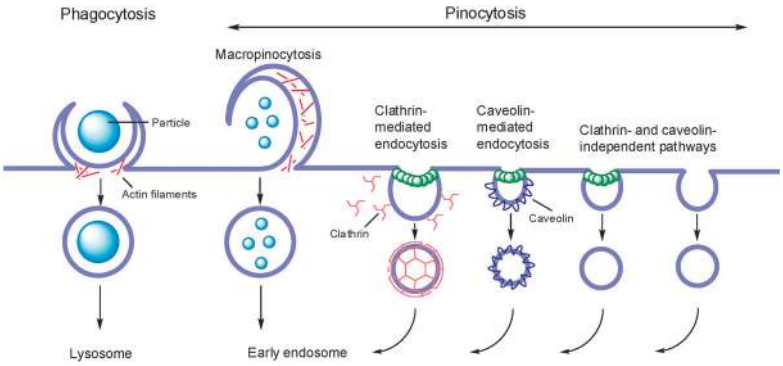


**Fig. 6** Mechanisms of cellular entry by phagocytosis (the uptake of large particles) and pinocytosis (the uptake of fluid and solutes). The endocytic pathways differ with regard to the size of the endocytic vesicle, the nature of the cargo (ligands, receptors and lipids), and the mechanism of vesicle formation.[65]

that are critically dependent on the affinity of the polymer for the cell surface.[34] Notably, most inert synthetic polymeric carriers are known to be taken up by fluid-phase pinocytosis.[67] Once in the cell, these polymers are trafficked to late endosomes and lysosomes for their degradation. However, many polymers do not exhibit degradation by the lysosomal environment and instead get accumulated in vesicles within the cell.[67] Although not well understood, this accumulation could be significant and result in toxicity over a period of time.

Several groups have sought to elucidate the distribution and cellular dynamics of targeted nanosystems. In particular, Minko and colleagues have performed a series of elegant studies to understand the kinetics, internalization, and colocalization of labelled lutenizing hormone-releasing hormone (LHRH) nanocarriers containing fluorescent probes.[23] It is well-documented that LHRH receptor expression is substantially more pronounced in cancerous ovarian tissue than in normal ovarian tissue.[23] Consequently, the LHRH peptide has been extensively used as a targeting moiety to direct the entire conjugate specifically to cancer cells and to enhance its penetration and intracellular uptake.[23,68,69] In earlier studies, the cellular dynamics of LHRH peptide and PEG polymer as a targeting moiety and a delivery vehicle was shown on human ovarian carcinoma cells expressing LHRH receptors (Fig. 7).[23,68,69] In subsequent studies, it was demonstrated that the rhodamine labeled LHRH peptide accumulated predominantly in the plasma membrane and part of the cellular cytoplasm adjacent to the outer cellular membrane.[23,68] In contrast, the FITC-labelled PEG polymer can be equally distributed in the cellular cytoplasm and nuclei.[65]

Among other physical attributes, the size of a polymeric carrier plays a crucial role in the cellular uptake of a drug.[70] Furthermore, depending on the size, polymer particles possess different velocities, diffusion characteristics, and adhesion properties. In general, particles less than 200 nm are considered optimal for intravascular applications due to their better circulation half-life compared to larger particles.[71,72] Overall, there are some established rules of thumb with respect to particle internalization into the cell: particles with diameters $>1$ μm are internalized by phagocytosis and those with diameters between 0.2–1 μm are internalized by endocytosis. Interestingly, two recent studies reported that particles as large as 5 μm can be endocytosed through receptor mediated endocytosis thereby heralding a new window to targeted delivery to the vasculature.[73–75]

It is interesting to note that linear PEG polymers of varied molecular weights internalize at different time intervals.[64] In this important study, two labelled PEG polymers with molecular masses of 3000 Da and 20 000 Da were used (Fig. 8). Intensive internalization of lower molecular mass polymer started within 20 min after the beginning of incubation (Fig. 8B). Forty-five minutes after the beginning of the exposure, 3000 Da FITC-labelled polymer was distributed almost homogeneously within the cancer cell (Fig. 8C). Intensive fluorescence, comparable with fluorescence in the medium, was observed both in the cellular cytoplasm and nuclei. PEG polymer with a molecular mass of 3000 Da easily penetrated into cellular cytoplasm and nuclei. However, the internalization of the PEG polymer with a molecular mass of approximately 20 000 Da occurred much slower than with the smaller polymer. Indeed, significant accumulation of the polymer in the cellular cytoplasm occurred only after 2–4.5 h after the addition of the polymer to the medium (Fig. 8E and F).[64]

In line with the afore mentioned imaging study, the Haag group has recently observed a molecular mass and size dependent cellular uptake of dendritic polyglycerols indicating a molecular weight/size optimum around 200 kDa/12 nm.[18] It is important to note that the internalization of nanocarriers is not solely dependent on the molecular mass or size of the polymer but is also influenced

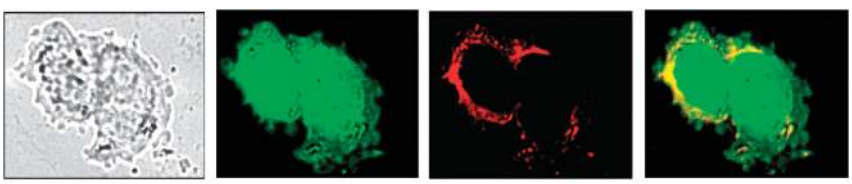


**Fig. 7** Localization of LHRH and PEG in cancer cells expressing LHRH receptors. Modified from ref. 68.

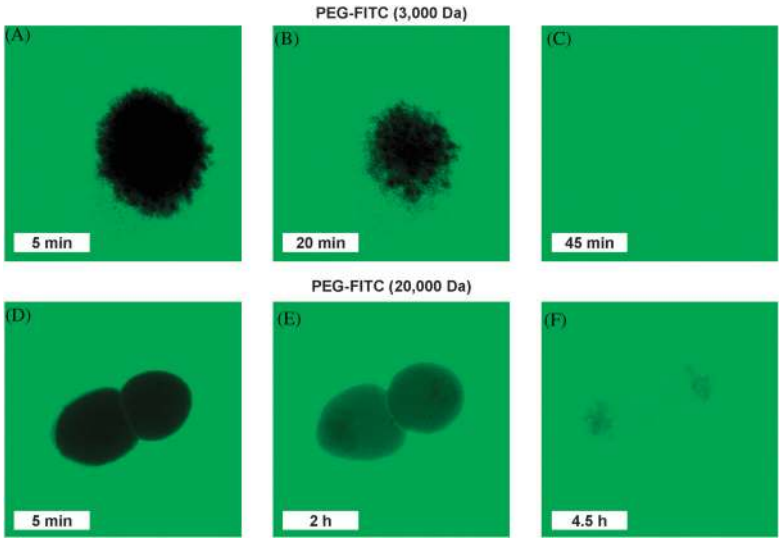


**Fig. 8** Cellular internalization of PEG polymers is critically dependent on the molecular mass of the PEGylated polymer. Images of FITC-labelled PEG-polymers (low and high-molecular mass polymers) internalized in living cells at 37 °C at different time points after the exposure. Reprinted with permission from ref. 64. Copyright 2007 American Chemical Society.

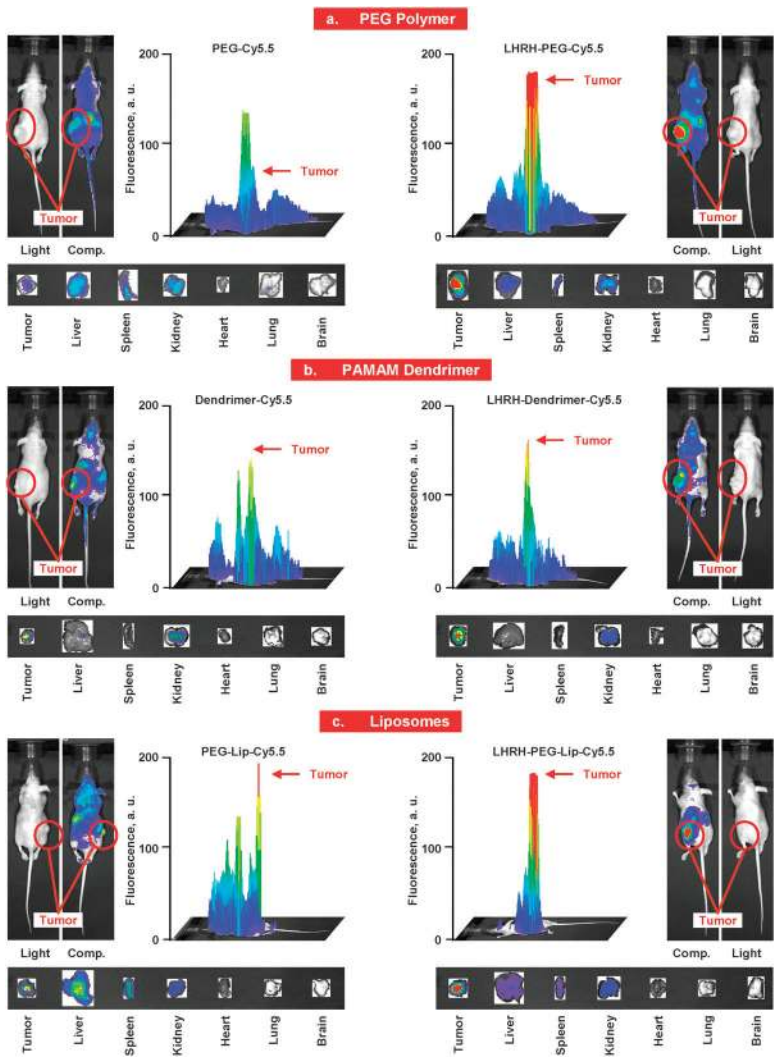


**Fig. 9** LHRH peptide as a tumor-specific targeting moiety increased accumulation of different delivery systems in mice tumors bearing xenografts of human A549 lung carcinoma. Reprinted with permission from ref. 2. Copyright 2008 Elsevier.

 

by several other critical parameters. Recently, Saad *et al.* used an imaging tool to elucidate the effective contribution of the polymeric architecture, composition, size, and molecular mass of nanocarriers on the efficacy of imaging and targetability for tumor-specific receptors.[2] The study inducted the influence of nanocarriers using a linear PEG polymer, PAMAM dendrimer, and liposome *in vitro* and *in vivo*. The nanocarriers delivered the anticancer drug (paclitaxel) and/or imaging agent (Cy5.5) with almost a similar efficiency (Fig. 9). The studies in this direction quantitatively measured the cellular internalization of nanoconjugates, which definitely imparts new avenues in nanotherapeutics.

## 3 Application of nanomedicines in specific diseases

### 3.1 Cancer

A prime focus in nanomedicine has been to deliver anticancer drugs without the toxicity and nontargetability associated with the free form of a drug. In this regard it is important to note that, extensive research conducted over several decades has led to a better understanding of the biology of oncologic disorders. It is now well-established that the indefinite and uncontrollable tumor cell proliferation and metastasis are characterized by aberrant or hyperactive complex signaling cascades. Some of the molecular components of key signaling pathways involved in the progression and sustenance of tumors include PI3K/mTOR, VEGF, CDK, HIF-1α, PDFG, KIT, EGFR, JAK-STAT, and Ras-Raf-MEK-ERK.[76,77] Each of these molecular mediators are amenable to intervention (using small molecule inhibitors and/or monoclonal antibodies) leading to a therapeutic response. For example, NVP-BEZ235 is a PI3K/mTOR inhibitor,[78] bevacizumab is a recombinant human monoclonal antibody that targets VEGF,[79] P276 is a small molecule targeting CDK-4,[80] imatinib mesylate targets PDGFR[81] and KIT, erlotinib is a EGFR tyrosine kinase inhibitor,[82] INCB18424 targets the JAK/STAT pathway,[83] and U0126 interferes with the ERK-MEK pathway.[84]

Many of the drugs developed so far have considerably increased the survival of cancer patients. Such drugs are used as a first line of therapy for cancer patients. However, the majority of these drugs (including cancer chemotherapeutics such as DOXO, vincristine, cyclophosphamide, topotecan, and paclitaxel) are not targeted, *i.e.*, the drugs not only alter the tumor cell function but also affect normal body cells leading to a significant number of side effects.[85] This has led to the concept of selectively targeting the delivery of oncologic drugs to tumor or cancer cells. The latter could be achieved either *via* active targeting using ligand(s) or carrier(s) or by passive targeting (*e.g.*, EPR effect) of drugs. Both these approaches have been discussed earlier in this review. A variety of polymeric approaches could be utilized to obtain cancer ''nanotherapeutics''. These include polymeric conjugates, nanoparticles, polymeric micelles, and liposomes.

In this context, it is noteworthy that monoclonal antibodies or antibody fragments have been used to achieve a degree of specificity for the target tissue and a wide range of binding affinities.[86] Some antibodies such as trastuzumab [anti-ERBB2, Herceptin] or rituximab [anti-CD20 (B-cell surface receptor), Rituxan] have intrinsic cytotoxicity because they interfere with molecules that stimulate cell proliferation and differentiation.[87,88]

### 3.2 Rheumatoid arthritis

Other disorders/diseases, where there is a critical focus on drug delivery through nanomedicine, are inflammation and rheumatoid arthritis (RA). RA is a chronic, inflammatory autoimmune disease that results in progressive joint destruction and increased mortality. It is a well-established fact that the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) plays a critical role in the pathogenesis of RA by orchestrating the inflammatory/immune-response in the synovium.[89] Accordingly, inhibiting the production and/or biological activity of TNF-α is considered a promising therapeutic approach. Indeed, clinically approved therapies for treating active RA include biological TNF-α inhibitors such as etanercept (Enbrel), infliximAb (Remicade), and adalimumAb (Humira).[89] More recently, a PEGylated Fab fragment of a humanized monoclonal antibody directed against TNF-α (certolizumab, a polymer–protein conjugate) has been approved for treating RA patients.[90] The pegylation leads to improved half-life thereby providing certolizumab with a greater opportunity to elicit robust and significant reductions in the pathological symptoms of RA in patients with active disease. Although these biological agents are considered as the cornerstone in the treatment of RA, their use has severe limitations (*e.g.*, parenteral route of administration, high cost of therapy).[91] As such, extensive industrial and academic research is being carried out towards finding orally active TNF-α inhibitors (and/or other anti-arthritic agents) which would have the same effect as biological agents but without the undesirable side effects.[92] The possible targeting approaches in RA have been listed in Fig. 10.

A large number of studies have investigated the role of various signal transduction pathways in the induced production of TNF-α. These studies led to the identification of p38 MAP kinase and PDE4D as attractive therapeutic targets for alleviating RA by inhibiting TNF-α production. The promise of p38 MAP kinase inhibitors and PDE4D inhibitors has been evaluated in multiple clinical trials. Much to the disappointment of industry these clinical trials have failed.[93] In order to attain ''effective'' drug concentrations in affected joint tissues, high doses of aforementioned inhibitors were administered to patients which led to significant adverse effects (hepatotoxicity with p38 MAPK inhibitors)[93] and nausea and emesis with PDE4D inhibitors.[94] With the objective of reducing the side effects, the dose of PDE4D inhibitors was reduced but, in these studies, lower doses of PDE4D inhibitors failed to elicit a therapeutic response.[94] Given this, polymeric chemistry approaches, which specifically target these agents to affected joints, can be investigated with the hope of realizing the potential of these small molecule inhibitors. An attractive strategy to target these inhibitors to the rheumatic joint is to exploit the acidic microenvironment of the synovial joint. The pH of the synovial fluid from joints of RA patients, which is reported to be as low as 6.0, correlates inversely with the disease severity.[95]

Wang *et al.* have developed a novel water-soluble, *N*-(2-hydroxypropyl) methacrylamide (HPMA) based polymeric delivery system that selectively delivers dexamethasone (in a pH sensitive manner) to inflamed joints of arthritic rats.[92] In an experimental model of arthritis, the polymeric dexamethasone–HPMA conjugate afforded superior and longer lasting anti-inflammatory effects, including greater bone and cartilage

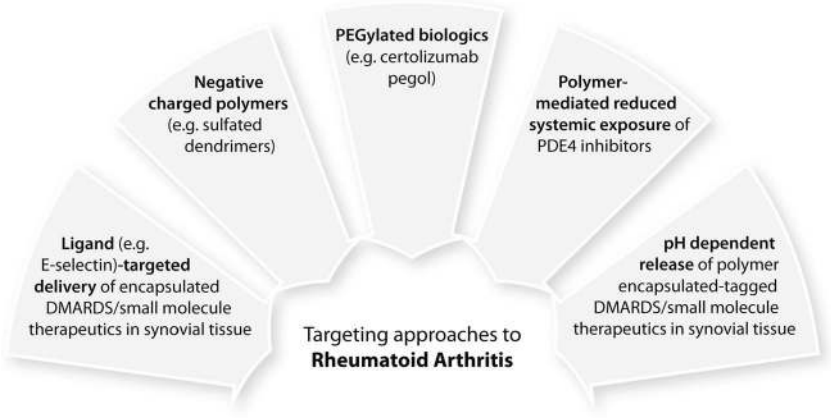


**Fig. 10** Possible polymeric targeting approaches in rheumatoid arthritis.

preservation, compared to free dexamethasone.[92] The proof-of-principle "polymer-chemistry" studies for p38 MAPK inhibitors have been performed in the settings of acute myocardial infarction.[96] Sy *et al.* have demonstrated that microspheres formulated from the polymer, poly(cyclohexane-1,4-diyl acetone dimethylene ketal) (PCADK), which encapsulate the p38 MAPK inhibitor SB239063, can markedly improve the treatment of acute myocardial infarction.[96] Analogous polymeric-drug conjugate delivery systems could be adopted with promising p38 MAPK inhibitors and/or PDE4D inhibitors to achieve a sustained therapeutic response in RA. Given that an acid azabisphosphonic-capped, phosphorus-containing dendrimer elicits immunosuppressive responses on monocytes (a cardinal immune cell in pathology of RA), a "dual-therapeutic response" strategy would be to tag these promising p38 MAPK/PDE4D inhibitors onto immunosuppression eliciting dendrimers.[97] Furthermore, as shown by Chandrasekar *et al.* PEG conjugates of anionic poly(amidoamine) dendrimers can be targeted using folate receptor (which is overexpressed on the activated—but not quiescent—macrophages in both animals model and human patients with naturally occurring RA) as an active targeting moiety.[98] In the context of harnessing the potential of PDE4D inhibitors, it is noteworthy that "dual-complementary" polymeric nanocarrier approaches could be adopted which not only target their delivery to the affected rheumatic joint but also reduce their systemic concentrations (which would lead to diminished side effects).

Besides TNF-α inhibitors, the standard therapy for RA consists of disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate. An important limitation of methotrexate (MTX) therapy is that a meaningful protective response is observed only after several weeks of therapy. In one such study by Homma *et al.* MTX was conjugated to hyaluronic acid (HA) using a PEG13 linker.[99] This MTX-HA conjugate consisted of MTX bonded through α- or γ-carboxylic acid, cleavable by enzymes; a peptide chain recognized and cleaved by intracellular enzymes; a pegylated linker to avoid the steric hindrance of HA against the approach of enzymes and HA modified through its carboxylic acid.[99] This MTX-HA conjugate showed anti-proliferative effects on human synovial cells stimulated by using TNF-α. Moreover, it inhibited knee swelling in an antigen-induced monoarthritis rat model.[99]

Besides the aforementioned therapeutic strategies, an alternative approach to achieve a therapeutic response in RA is to modulate the aberrant leukocyte-synovial microvascular endothelial cell adhesion.[100] The leukocyte-endothelial cell adhesion is mediated by specific cell adhesion molecules expressed on the endothelium (*e.g.*, E-selectin, P-selectin, VCAM-1) and their cognate ligands on the leukocytes (*e.g.*, sLeX, PSGL-1, L-selectin, VLA-4). It is anticipated that therapeutics, which interfere with the expression and/or functionality of these cell-adhesion molecules, will attenuate the inflammatory cellular infiltrate in the synovium thereby providing a therapeutic response. Polymeric chemistry methodologies have been employed to exploit the promise of this alternative approach. For example, Ali *et al.* have demonstrated that multivalent presentation of sLeX-mimetics on a polylysine backbone imparts an ~30-fold improvement in inhibition of *in vivo* E-selectin dependent leukocyte rolling.[101] Haag *et al.* have synthesized dendritic polyglycerol sulfates (dPGS) that simultaneously antagonize L-selectin on leukocytes and P-selectin on inflamed vascular endothelium thereby reducing leukocyte extravasation.[102] The extent of L-selectin inhibition is dependent on the core size and degree of sulfation of dPGS core.[102] Most importantly, imaging studies have revealed that dPGS targets and accumulates at the site of inflammation (see below). Clearly, future studies aimed towards evaluating the potential of the polymeric scaffolds ameliorating experimental arthritis are warranted. In this context, it is noteworthy that Chauhan *et al.* have demonstrated anti-inflammatory efficacy of naked, unmodified poly(amidoamine) (PAMAM) dendrimers bearing simple surface functionality (*e.g.*, $-NH_2$, –OH, *etc.*) in rat adjuvant-induced arthritis.[103]

The heightened expression of adhesion molecules on synovial microvascular endothelium provides an opportunity for site specific delivery of anti-rheumatic drugs. For example, a targeted drug delivery scheme could be employed wherein anti-rheumatic drugs are incorporated into drug carriers that bear a ligand for a selectively expressed endothelial cell adhesion molecule (*e.g.*, E-selectin or VCAM-1). Ideally, once administered, the carriers would selectively bind to endothelium within inflamed synovial tissue *via* the ligand-ECAM chemistry and not bind to other segments of the endothelium or other tissue. In separate studies, it has been demonstrated that HPMA copolymer-doxorubicin conjugated displaying a high-affinity E-selectin binding peptide[104] or PAMAM dendrimers conjugated with an E-selectin antibody[105] can be targeted to human endothelial cells. Analogous polymer-encapsulated drug (*e.g.*, PEG-PLA biodegradable

 

particles encapsulating MTX) or polymer–drug conjugate approaches (*e.g.*, HPMA–copolymer conjugated with methotrexate instead of doxorubicin) could be adopted to achieve the "synovium-specific" delivery of anti-rheumatic drugs with the objective of achieving a meaningful therapeutic response with diminished side effects.

## 4 Structural aspects of nanoconjugates

Nanoforms of polymeric prodrugs have been extensively used to deliver drugs and other biomolecules. The process involves synthetically coupling the biological active component with a polymer carrier to form a unique molecule which possesses collective property or its individual characteristic. Jatzkewitz utilized a prodrug of peptamin-polyvinylpyrrolidone (PVP) as a means of improving the efficacy of the drug as early as 1955.[106] Unfortunately, for the next couple of decades the implications of prodrugs in therapeutics were not significantly noted. Since Ringsdorf was the first to propose a rational model for pharmacologically active polymers, he has been often considered the pioneer of prodrug research.[20] The prodrug model typically consists of multiple components including (i) a polymer as a carrier, (ii) a drug, peptide or protein as a biological active component, (iii) a spacer molecule for conjugation chemistry, and achieving cellular hydrolysis, and (iv) an optional imaging or targeting moiety.

Most of the state-of-the-art nanoconjugates possess a degradable bond to release the drug at the site of action per the pH gradient.[107,108] The selection of the pro-drug components is very crucial for designing the conjugates that can achieve molecular targeting. The following properties of drug candidates make them suitable for translation into a polymeric prodrug from (i) chemical functionality for conjugation, (ii) low aqueous solubility, (iii) instability at varied physiological pHs, (iv) higher systemic toxicity, and (v) lower cellular entry.

Linking of a polymer to the drug is a first key step in regulating the biological activity of drug and for supporting a trigger for activation of the macromolecular prodrug.[20] Prodrug conjugates have varied chemical linkages which are susceptible to neutral pH (*e.g.* ester, hydrazone linkages), acid catalyzed cleavage (*e.g. cis* aconityl groups), and their tendency to cleave by exposure to the targeted enzyme such as cathepsins or esterase. Several PEGylated enzymes (adenosine deaminase, L-asparaginase) and cytokines (including interferon α and G-CSF) are routinely used in therapeutics. PEG-modified adenosine deaminase (ADAGEN®) and PEG-L-asparaginase (ONCASPAR®) were the first PEG modified enzymes to be on the market in early 1990s. In general multifunctional biocompatible polymers are required as efficient nanocarriers that fulfil the above mentioned characteristics.

### 4.1 Multicomponent PEG conjugates for cancer targeting

PEGylation methodology can be utilized to maximize the 'tumor cell-death effect' of oncologic drugs. In this approach, the PEGylated oncologic drugs are targeted to cancer cells at specific tumor sites by using targeting moieties (*e.g.*, folic acid, LHRH, antibodies).[48,49] This multitargeting approach was demonstrated by Dharap *et al.* by using a combination of PEGylated camptothecin (CPT) and two different targeting agents LHRH and BCL2 homology 3 (BH3) peptide.[69] The authors utilized the LHRH peptide as a targeting moiety to recognize extra-cellular LHRH receptors, which are distinctly over-expressed in several cancer cells. Similarly, the BH3 peptide was utilized to target the intracellular machinery critically controlling apoptosis to enhance the anticancer activity of CPT.[69] CPT was chemically conjugated to Boc-Cys (Trt) amino acid to form a biodegradable ester bond with the hydroxyl group. The resultant prodrug conjugate (CPT-cysteine ester) had two potential, orthogonal conjugation sites: the amino group and the thiol group (Fig. 11).

Facile chemical routes have been adapted to conjugate the anticancer drugs with bifunctional PEG polymers. Furthermore, the addition of other bioactive agents is critical for delivering targeted multicomponent systems (Fig. 11). The efficacy of the prodrug conjugates was studied by cytotoxicity, gene expression analysis, and apoptosis induction in human ovarian cancer cells.[69] PEG-CPT nanoconjugates elicited a higher cytotoxicity and apoptosis induction than free CPT. Furthermore, targeting using the BH3 peptide or LHRH peptide resulted in a better efficacy of non-targeted CPT-PEG conjugate.[69] These findings demonstrated that the simultaneous targeting and suppression of

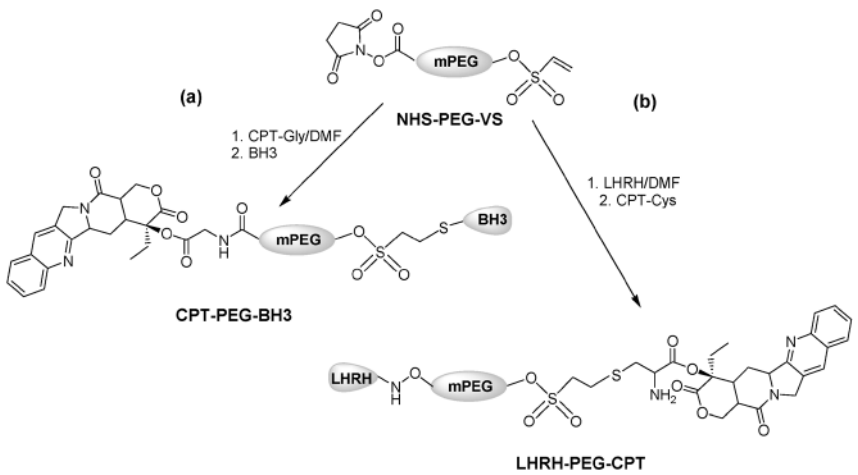


**Fig. 11** (a) Synthetic scheme for CPT-PEG-BH3 nanoconjugate. CPT was first coupled to an amino acid *via* a biodegradable ester bond to the hydroxyl group, using Boc-Cys (Trt) amino acid. (b) Synthetic scheme for LHRH-PEG-CPT conjugate.[69]

cellular anti-apoptotic defense substantially increased the anti-cancer activity of CPT. However, a major drawback of bifunctional PEGs is the poor drug loading, therefore multifunctional polymers, especially dendritic nanocarriers, are of great interest as polymeric scaffolds.

### 4.2 Dendritic polymers as nanocarriers

In the early 1980s, polymer science research introduced versatile nanosized dendritic polymers, named after the Greek word 'dendron' for tree, which are macromolecules that can be chemically designed and synthesized to possess precise structural characteristics including a branch on branch, tree-like architecture.[109,110] Dendritic polymers can be classified as: (a) perfect dendrimers, (b) dendrons, (c) dendronized polymers, and (d) hyperbranched polymers. Perfect dendrons and dendrimers are unique nanosystems because they can be expected to achieve monodispersity (PDI $\approx$ 1.0), nanometre dimensions (1–10 nm), low viscosity, multiple functionality at the terminal groups, high solubility, and biocompatibility. Hyperbranched and dendronized polymers have broadened the nanosize range of dimensions up to micrometre scale (PDI $\geq$ 1.1), with the concomitant increase in the field of applications.

In last 10 years extensive work has been published on dendritic nanostructures and their conjugation strategies to deliver active agents *e.g.* MTX, CPT, paclitaxel, cisplatin, *etc.*[111,112] Additionally, they have been used to evaluate their potential in tumor cell specificity and targetability using folate residues, antibodies, and hormones.[7,113,114] These versatile polymers are synthesized from monomeric units with new branches being added in steps until a uniform tree-like structure is formed. A comparison of dendrimer and linear polymer features shows that the dendritic polymer architecture is advantageous for many delivery applications.[115–121] For example, the defined multivalency of dendrimers can be used to encapsulate or conjugate similar or different drug molecules while adding on targeting, imaging probes, and/or solubilizing modalities on the same construct in a controlled fashion. In addition, their low polydispersity should provide a more reproducible pharmacokinetic behavior than in linear polymers. Furthermore, the more globular shape of dendrimers, as opposed to the random coil structure of most linear polymers, could affect their biological properties and thus lead to the discovery of interesting effects due to their macromolecular architecture.[122]

In particular, the synergy between their multifunctionality and size on the nanoscale enables a chemical "smartness" along their molecular scaffold that achieves environmentally sensitive modalities. Therefore these functional materials can be expected to revolutionize the existing therapeutic practice. Dendritic molecules (Fig. 12), such as polyamidoamine (PAMAM),[123–125] poly(propylene imine),[126] polyaryl ethers,[127] polylysine,[128] polyester,[129,130] polyamide[131] polyglycerol (PG),[132,133] and triazine dendrimers,[134] have been introduced for biomedical applications to amplify or multiply molecularly pathopharmacological effects and have already shown a promising higher efficacy in the polymer therapeutics field.[135]

### 4.3 Dendritic nanostructures as targeted drug delivery systems

Among the many special structural features of dendritic nanostructures, the high density of functional groups is particularly interesting for the design of targeted drug–polymer conjugates. The surface decoration of dendritic nanostructures with solubilizing agents and targeting moieties together with the inherent charge profile of the dendritic polymer, confer structural benefits with consequences in a faster cellular entry, reduced macrophage uptake, targeting, and easier passage across biological barriers by trancytosis.[112]

In addition, their branched nature provides a better *in vivo* application profile than their linear polymeric analogs. For instance, for polymers with similar MW and chemistry, increasing the number of branches or arms increases the blood circulation half-life. A systematic study with a library of PEGylated polyester "bow-tie" dendrimers (Fig. 13) established the relationship between branching and blood circulation time.[136,137] For a series

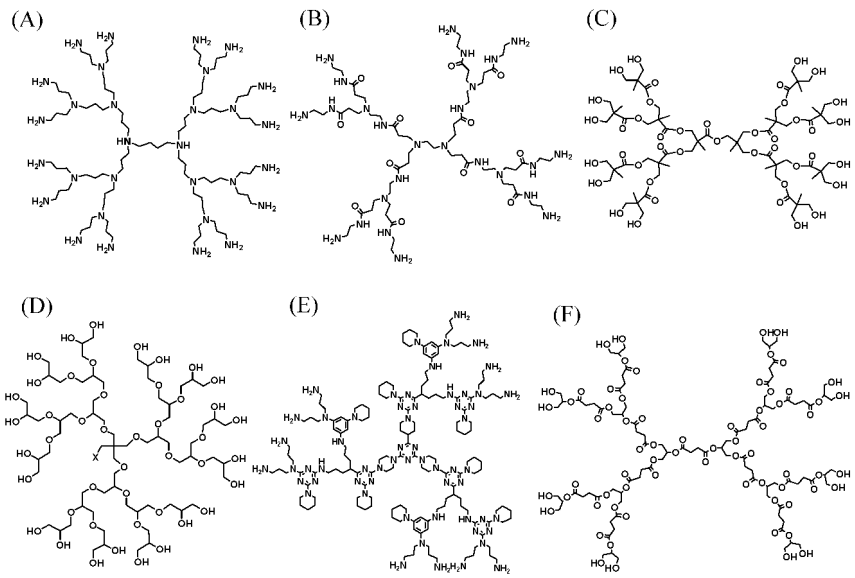


**Fig. 12** Examples of dendritic scaffolds commonly used in drug delivery applications. (A) poly(propylene imine), (B) polyamidoamine, (C) polyester, (D) polyglycerol dendrimer, (E) triazine-based dendrimer, and (F) poly(glycerol-succinic acid) dendrimer.[133]

 

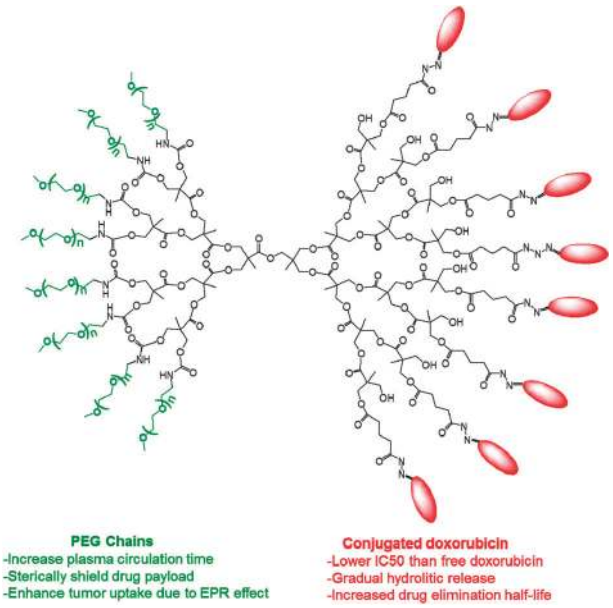


**Fig. 13** Doxorubicin loaded on the bow-tie dendrimer through a hydrazone linker.[136,137]

of bow-ties with equivalent MW (~40 kDa), there was an increase in $t_{1/2}$, from 1.4 ± 0.4 h for the two-arm dendrimer, essentially a linear polymer, to 26 ± 6 h for the four-arm dendrimer, and finally to 31 ± 2 h for the eight-arm dendrimer. Corresponding biodistribution studies in healthy mice showed no significant variation in tissue uptake among the three polymers and decreased polymer excreted in the urine with increased branching. This polymer drug carrier studied in C26 colon tumor mice showed long blood circulation times and remarkable efficacy in delivering the chemotherapeutic drug doxorubicin to tumors.[136,137]

PAMAM dendrimers, which are commercially available, are being highly investigated for their biomedical applicability. The practicability of PAMAM dendrimers for cancer treatment is under critical investigation, as these nanocarriers serve in the delivery of targeted drug components, therapeutic agents, and imaging agents.[138,139] Their characteristic non-toxicity in biological systems makes them much more biocompatible than many other materials currently researched for use as controlled, chemotherapeutic drug delivery systems.[125] However, the possibility of retro-Michael addition limits their shelf-life under ambient conditions.

Targeted dendritic anticancer prodrug conjugates of PAMAM dendrimers have been successfully synthesized by using either a multistep or one-pot approach, and targeting modalities like antibodies, folic acid, biotin, peptides (RGD, LHRH, *etc.*), *etc.* The internalization of dendritic nanocarriers by cancer cells is much more efficient than the penetration of free low molecular weight drugs or non-targeted drug delivery system (DDS) by a simple diffusion or endocytosis, respectively. Switching these mechanisms substantially enhances intracellular internalization and the anticancer efficacy of the delivered drug and other active components of DDS.[140] This was demonstrated by using the lutenizing hormone-releasing hormone (LHRH) peptide as a tumor targeting moiety to receptors that are overexpressed in the plasma membrane of many types of cancer cells. This is advantageous for two reasons. First, in contrast to non-targeted dendrimer-based DDS that accumulates almost equally in a tumor, liver, and kidney, peptide used as a targeting ligand are able to subject the entire nanocarrier system specifically to the tumor and thus simultaneously prevent its accumulation in healthy tissues. Secondly, the LHRH peptide enforced the internalization of DDS by cancer cells.

Majoros *et al.* reported conjugation of fluorescein isothiocyanate (FITC), folic acid (FA), and methotrexate (MTX) to G5 PAMAM dendrimer.[116] Typically, FA belongs to the vitamin B family and is important in cell division, since it participates in the biosynthesis of nucleotide bases. While folic acid receptors (FR) are membrane bound receptors and could be targeted using FA as a ligand, it has been noted that the expression of FR in normal tissues is low and restricted to various epithelial cells, such as placenta, choroid plexus, lungs, thyroid, and kidneys.[141,142] Moreover, the FRs are overexpressed in many epithelial cancer cells, such as breast, ovary, lung, kidney, head and neck, brain, and myeloid cancers.[143,144] The dendritic device synthesized was targeted to overexpressed membrane associated folate receptors with FA which induced cellular cytotoxicity.

A novel "one pot" method for targeted delivery consisting of generation 5 (G5) polyamidoamine (PAMAM) dendrimer, folic acid (FA), and methotrexate (MTX) was reported.[59] The ratio of FA *versus* MTX conjugated to the dendrimer was tuned to achieve the desired therapeutic effect. *In vitro* studies performed on FA receptor-expressing KB cells showed that the conjugate has a similar affinity and cytotoxic potency to G5-FA-MTX synthesized using the traditional multiple-step approach.

A partially acetylated generation 5 (G5) polyamidoamine (PAMAM) dendrimer was conjugated with the targeting moiety biotin and the imaging moiety fluoresceinisothiocyanate (FITC). The bifunctional conjugate (dendrimer–biotin–FITC) exhibited much higher cellular uptake into HeLa cells than the conjugate without biotin. The results indicated that the biocompatible biotin–dendrimer conjugate might be a promising nanoplatform for cancer therapy and cancer diagnosis.

The Baker group has reported the use of Fibroblast Growth Factor Receptor (FGFR), which is overexpressed in a wide variety of tumors, as an active targeting fragment to be used in cancer, wound healing, and in angiogenesis. Purified recombinant FGF-1 was coupled to a G5 PAMAM dendrimer. The specific binding and internalization of this conjugate labelled with FITC was investigated by flow cytometry and confocal microscopic analysis in cell lines expressing FGFR. While the binding and uptake of FGF-conjugated dendrimers was completely blocked by excess nonconjugated FGF-1, confocal microscopic analysis showed cytosolic as well as nuclear localization.[145]

It should be noted that the ratio of drugs conjugated per nanocarrier has largely not been realistically achieved with a high payload of drug or targeting moieties. This is particularly true for dendritic architectures for the following reasons: (a) nanosized radius of gyration ($R_h$), (b) higher steric hindrance exhibited by the biomolecule as well as at the peripheral functional groups of the dendrimer, (c) low reactivity of terminal functional groups for chemical conjugation with biocomponent, and (d) crowding effect of reactive end groups in dendrimers. In one attempt to overcome these issues, a high payload, averaging 50 ibuprofen molecules

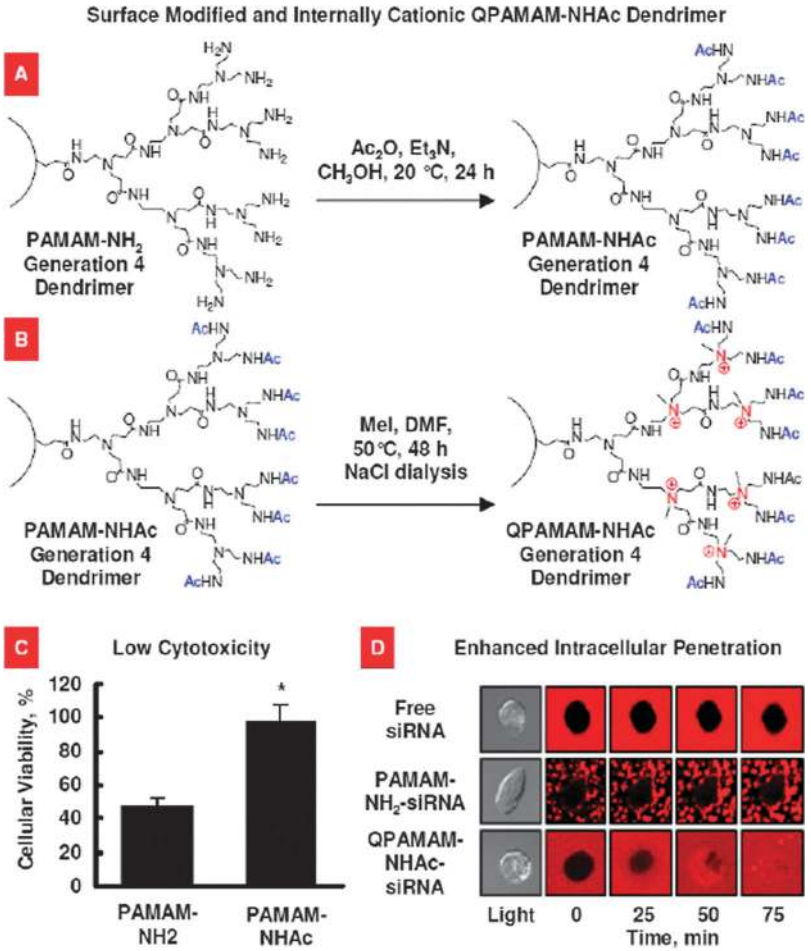


**Fig. 14** Synthesis and evaluation of surface modified (A) and surface modified and internally quaternized (B) dendrimers. (C) Cytotoxicity of a traditional (PAMAM–$NH_2$) and novel surface modified (PAMAM–NHAc) dendrimers. (D) Cellular internalization of free siRNA, and dendrimer–siRNA complexes with a traditional (PAMAM–$NH_2$) and internally cationic and surface modified (QPAMAM-NHAc) dendrimers *via* receptor-mediated endocytosis (Fig. 14C and D). Adapted with permission from ref. 140. Copyright 2010, Springer Science + Business Media.

were conjugated per mole of poly(amidoamine) PAMAM G4 hydroxyl-terminal dendrimers.[146] By decreasing the steric hindrance and increasing the reactivity of a drug, as many as 12 methylprednisolone (MP) molecules were conjugated to a PAMAM G4 hydroxyl-terminated dendrimer. The spacer molecule glutaric acid (GA) was coupled to MP to enhance the reactivity of the drug. The resulting MP–GA–COOH moiety was further conjugated with a hydroxylterminal dendrimer using DCC as a coupling agent.[146] The conjugate demonstrated comparable therapeutic activity to the free drug, even over short intervals of time.

In contrast, other forms of dendrimers could be efficiently used to deliver siRNA and nuclear components. It was effectively demonstrated that non-quaternized dendrimers often form microtubule like structures with non-covered and non-protected siRNA (Fig. 14A and B).[140] Secondly, surface modification with charge neutral groups (acetylation or hydroxylation) leads to the low cytotoxicity of empty dendrimers and enhanced internalization of the entire DDS by cancer cells (Fig. 14C and D). The synthesis of several DDS based on such dendrimers provides experimental data that support the advantages of this approach to complex multifunctional tumor targeted pro-apoptotic delivery systems.

In response to the need of nanodelivery vehicles, a future perspective is to control the properties and function of polymeric carriers by designing well-defined molecular architectures, to impart biocompatibility, and to develop chemical versatility. Dendrimers are an efficient mean for the delivery of such traits to multiple drugs, to ligands, at the sites of contact using a single molecule. However, there is further need to design and evaluate these 'dendritic nanocarriers' for their *in vivo* toxicity, biodegradability, cellular uptake, release, hemolytic effect, and bio-interactions. In particular, issues related to the conjugation of hydrophobic agents on the surface of dendrimers (loss of homogeneity, steric hindrance, conformational changes, high surface charge, *etc.*) should be overcome. The development of a targeted nanocarrier system for optimum efficacy remains elusive and therefore needs to be designed experimentally on a case to case basis.

## 5 Dendritic polyglycerol as a new platform for nanomedicine

Dendritic polyglycerols (dPGs) are characterized by tunable end group functionalities, defined topological 3D architecture, and inertness to non-specific interactions with biological

 

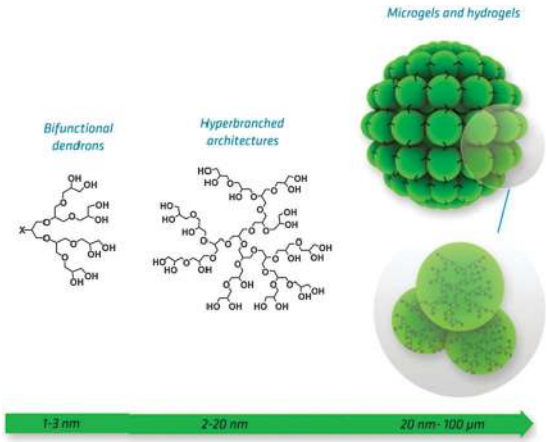


**Fig. 15** Synthetic evolution of dendritic PGs: from dendrons to megamers. Adapted with permission from ref. 1. Copyright 2010 John Wiley & Sons, Inc.

environments. dPGs present a novel platform for next generation biomaterials.[1,7,147] Multiple approaches to design different PG architectures have been reported, which offer a great variety in the degree of branching, size, surface topology, and chemical properties in general. Along with the synthesis of hyperbranched PG, fabrication routes to perfect dendrimers, dendrons, microgels, and nanogels have also been reported over the last decade (Fig. 15). A systematic library of PG architectures with varying properties was synthesized using a careful selection of starting materials through an economic synthetic route that provided an option to perform post modification on the PG scaffold.[148]

Since dPGs are synthesized in a controlled manner to obtain definite molecular weight and narrow molecular polydispersity, they have been extensively evaluated for a variety of biomedical applications.[1,115,149] Several studies have demonstrated the biocompatibility of dendritic PGs and a potentially safe profile for *in vitro* and *in vivo* applications. In preliminary cell culture experiments, hyperbranched PG with a molecular weight of 5 kDa showed absolutely no toxicity on the cellular level.[150] Brooks *et al.* reported several studies including a comprehensive analysis of PGs in a broad MW distribution and with different compositions.[151–154] Both linear and hyperbranched PGs were reported to have a similar or even better biocompatibility profile than PEG with MW ranging from 4.2 kDa to 670 kDa. *In vivo* studies conducted on mice revealed no sign of toxicity after i.v. injection of the dose up to 1 g $kg^{-1}$. Although the biocompatibility of polymers in general is a function of molecular weight, no MW dependent toxicity was found up to 540 kDa for dendritic PG architectures. Dendritic PGs exhibit a plasma half-life of 32 hours (106 kDa) in mice for lower molecular weight polymer, whereas it can be as high as ~57 hours for high molecular weight PGs (540 kDa).[154] Currently, hyperbranched PGs are considered as new delivery enhancers for many bio-actives which could substantially increase the internalization of active components specifically into targeted cells to enhance the specific activity of the whole drug delivery system and thereby decrease the adverse side effects.[155,156] Recently we demonstrated that chemically post-modified hyperbranched polyglycerol presented sufficiently low zeta potentials, lower interactions with serum albumin, enhanced cellular uptake, and high cellular viability on human hematopoietic cell line U-937.[17] Hyperbranched polyglycerol scaffolds with average MW between 10 kDa and 20 kDa, and surface functionalities suitable for further drug encapsulation or conjugation were assayed for biocompatibility compared to linear PEG polymer or dextran, indicating the suitability of dPG derivatives in delivering therapeutic agents systemically.[7]

In this way, dPG presents an ideal platform for nanomedicine because it possesses a combination of the following properties: (a) unique architectural and chemical surface tunability, (b) significantly tolerable surface charge for cell uptake, (c) moderate affinity and low interactions with plasma proteins, (d) variability in the size and architecture toward optimization of cellular internalization and passive accumulation in damage tissues.

### 5.1 Designing functional architectures based on PG

The linear monohydroxy and terminal dihydroxy functionalities of dPG scaffolds can easily be modified or functionalized following classical hydroxyl group chemistry to render a broad spectrum of products. High loading capacity, water solubility, and ease of purification of the product make dPGs attractive architectures for carrying out post-polymerization modifications. A substantial amount of research has been directed to design different architectures by modification of dPG hydroxyl groups into different functionalities. Fig. 16 summarizes different group functionalities that have been used for the further functionalization of the dPG toward different biomedical applications.[1]

These architectures have already demonstrated their usefulness in therapeutic approaches related to multivalency, given by the synergy between the nanosized dimensions combined with the high density of functional groups. A challenging approach to the application of multivalent interactions is the mimicry of functional biomacromolecules with therapeutic relevance. Several attempts have been made to mimic specific proteins, *e.g.*, histones or polysaccharides like heparin. In these cases, mimicry is mostly based on the surface charge of the polymer molecules. In the particular case of dPGs, (1) the neutral species with hydroxyl end groups represents a good analog of polysaccharides, (2) polyanionic derivatives present similar activities to negatively charged polysaccharides,

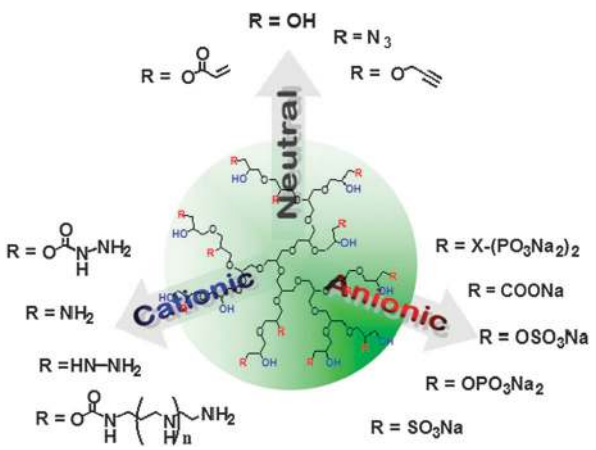


**Fig. 16** Schematic representation of dPG derivatives used for the development of functional materials in the field of biomedicine. The depicted polymer structure represents only one possible isomer and a small part of the polyglycerol scaffold.[17]

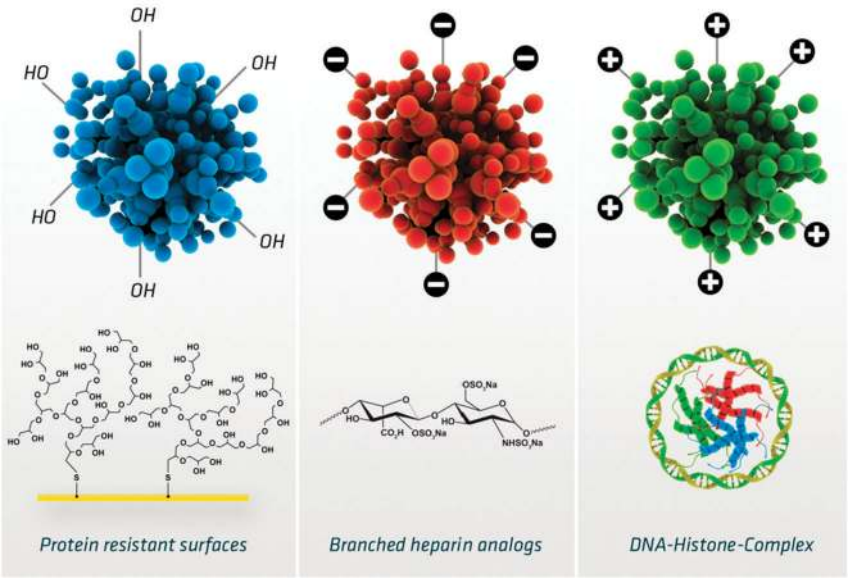


**Fig. 17** Mimicry of biologically active macromolecules. Adapted with permission from ref. 1. Copyright 2010, John Wiley & Sons, Inc.

*e.g.*, heparin, polysialic acid, and (3) the amine terminated PGs can act in a similar fashion as histones binding and compacting DNA (Fig. 17). Applications range from protein resistant coatings (neutral species) to DNA-transfection agents (polycationic systems), anticoagulating and anti-inflammatory drugs (polyanionic systems).[1]

### 5.2 Multifunctional PG–drug conjugates for tumor targeting

In a recent communication, we reported the use of the hyperbranched PG scaffold for conjugation to maleimide-bearing prodrugs of doxorubicin or methotrexate which incorporate either a self-immolative *para*-aminobenzyloxycarbonyl (PABC) spacer coupled to the dipeptide Phe–Lys or the tripeptide D-Ala-Phe-Lys as the protease substrate. Both prodrugs were cleaved by cathepsin B, an enzyme over-expressed by several solid tumors, to release doxorubicin or a methotrexate lysine derivative (Fig. 18). Cytotoxicity of the conjugates against human tumor cell lines showed that the activity of the drugs was primarily retained, which confirmed the macromolecular prodrug concept.[157]

Strategically, thiolated hyperbranched polyglycerols have been designed to couple either diagnostics or therapeutic agents. The synthetic protocol consists of four steps. The first three steps for the synthesis of polyglycerolamine with average molecular weights between 10 and 500 kDa, and up to 20% of total hydroxyl groups functionalized to amine groups.[158]

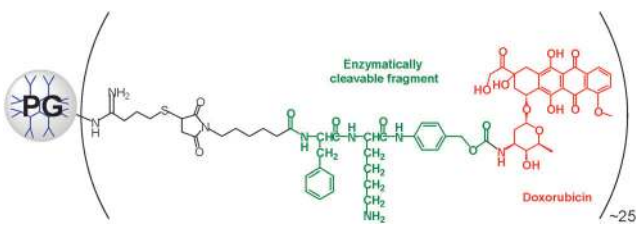


**Fig. 18** Enzymatic cleavable prodrug derived from dendritic polyglycerol.[156]

For the synthesis of the thiolated derivatives, three different pathways were studied using 3-(tritylthio)propionic acid, 2-iminothiolane, or acetyl-thiopropionic acid. Among all the thiolation reactions studied, the 2-iminothiolane pathway was the most reproducible for the *in situ* Michael reaction with maleimide derivatives as the following step.

This modular approach proved to be flexible for coupling different drugs, solubilising agents, as well as imaging and targeting moieties.[17,18] In a recent example we explored this methodology to prepare PG doxorubicin prodrugs that were flexible for drug loading by using an acid-sensitive hydrazone linker and further post-modification with poly(ethylene glycol) shell (Fig. 19). The resulting drug polymer conjugates showed optimal properties for *in vitro* and *in vivo* applications because of their high water solubility, an appropriate size for passive tumor toxicity, a high stability at physiological conditions, pronounced acid-sensitive properties, cellular internalization, and a favorable toxicity profile. Doxorubicin polyglycerol conjugates with a high drug loading ratio showed clearly improved antitumor efficacy over doxorubicin in an ovarian xenograft tumor model (A2780). This induced transient complete remissions and thus demonstrated its potential for development of an efficient multifunctional dendritic drug delivery using our modular approach.[159–163]

### 5.3 Applications of polycationic derivatives of PG

Several amine functionalized hyperbranched PGs have been reported to be potential gene delivery systems after a proper surface group functionalization.[164–169] In comparison to other dendritic structures, these scaffolds have the added advantage of being open, flexible, and possessing a polyether backbone which keeps the toxicity profile low. Different systems have been studied by post-modification of the hydroxyl groups from the polyglycerol structure with amine bearing compounds. The post-modification approach for the preparation of hyperbranched polyglycerols based on core–shell architectures allowed

 

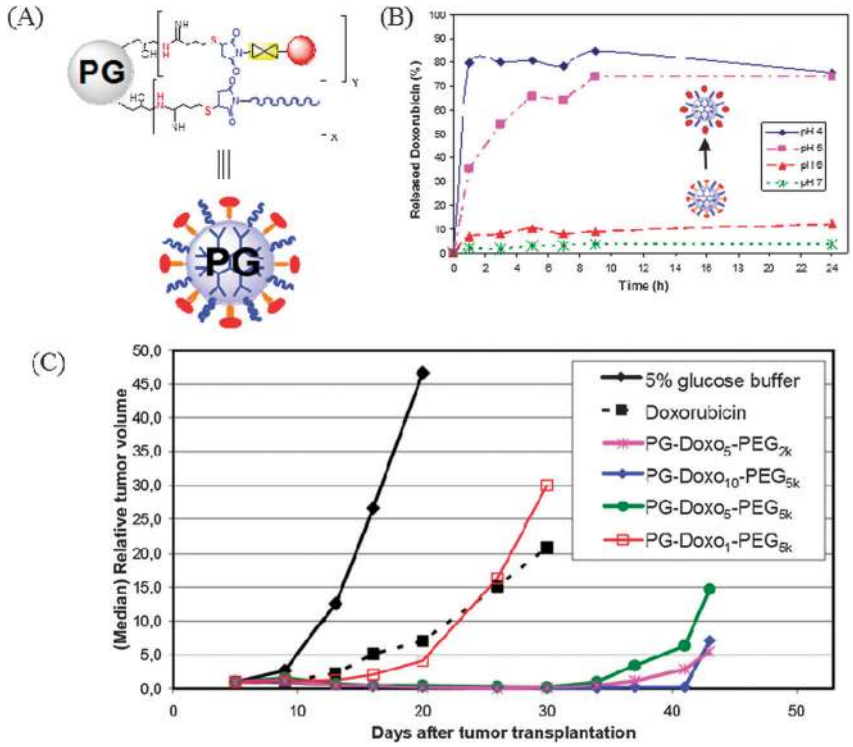


**Fig. 19** (A) Schematic representation of the PG doxorubicin prodrugs. (B) Representative release profile of PG-Doxo$_5$-PEG$_{5k}$ incubated at pH 4, 5, 6, and 7 at 37 °C. (C) Curves depicting tumor growth inhibition of subcutaneously growing A2780 xenografts under therapy with doxorubicin and the conjugates. Adapted with permission from ref. 163. Copyright 2011, Elsevier.

an easy control of the transfection/toxicity ratio by tuning the surface chemistry. It was proved that it is possible, by fine-tuning the nitrogen containing shell, to obtain better transfection/toxicity ratios *in vitro*.[169]

Our group has recently reported the synthesis of hyperbranched polyglycerolamine (PG–$NH_2$) with average MW of 10 kDa in an attempt to explore the effects of post-modification of hyperbranched polyglycerol with primary amines in the favourable 1,2-orientation.[169] In a previous study, the polyglycerolamine architecture, which consists of primary amine groups spread all around the polyglycerol structure, has shown promising properties as a prospective system for gene delivery, namely because of its high charge with a relative low cytotoxicity and an optimal charge/pH behavior so far as the buffering capacity is concerned.[17] Of all the polyglycerol systems analyzed for gene transfection,[169] the hyperbranched polyglycerolamine showed the highest affinity towards DNA fragments, according to an ethidium bromide displacement assay. The polymer was able to complex siRNA yielding slightly positive charged globular polyplexes. The knockdown efficiency of the siRNA-polyplex was comparable to HiPerFect for the proteins Lamin, CDC2, and MAPK2 in HeLAS3 cells. In a comparison of silencing efficiency and cytotoxicity with poly(ethylene imine), (PEI) derivatives, the polyglycerolamine architecture showed a better toxicity profile at concentrations relevant for its activity. It was found that the siRNA polyplex was internalized into glioblastoma cells within 24 hours by endosome–lysosome mediated system (Fig. 20A). More interestingly, siRNA–PG–$NH_2$ polyplex was administered intratumorally or intravenously to tumor-bearing mice, resulting in a major silencing effect and no apparent toxicity (Fig. 20B). High levels of fluorescently labelled siRNA were detected in the tumor but not in other healthy organs examined, which probed the passively targeted delivery of siRNA through EPR effect mediated by the polyglycerolamine species.

More recently, PG–$NH_2$ has been successfully used in xenografted nude mice to deliver siRNA that down-regulate the mRNA expression of ferrochelatase (FECH).[170] FECH is an enzyme that is responsible for the last step of the heme-synthesis, the incorporation of iron into protoporphyrin IX (PpIX). The *in vivo* studies demonstrated that siRNA-based inhibition of FECH results in a blockage of heme-synthesis, allowing the detection of xenotransplanted human tumor due to endogenous accumulation of PpIX. The fluorescence imaging results on animals with xenografted tumors demonstrated that PG–$NH_2$ improved the local bioavailability of siRNA within the tumor tissue and facilitate the transfer of siRNA across the cell membrane. Moreover, siRNA transfected in this way reached the cytoplasm and was effective in silencing its target FECH as proven by the high time dependent fluorescence emission of PpIX.

### 5.4 Applications of negatively charged PG derivatives

Lectins are multivalent carbohydrate-binding proteins which specifically bind different sugar structures. They have received considerable attention recently due to their importance in cell surface interaction and biological recognition. Multivalency in lectins has been discussed in detail and is considered to be a good model for studying multivalency of dendritic polymer derivatives.[1] Our group recently explored the use of multivalent

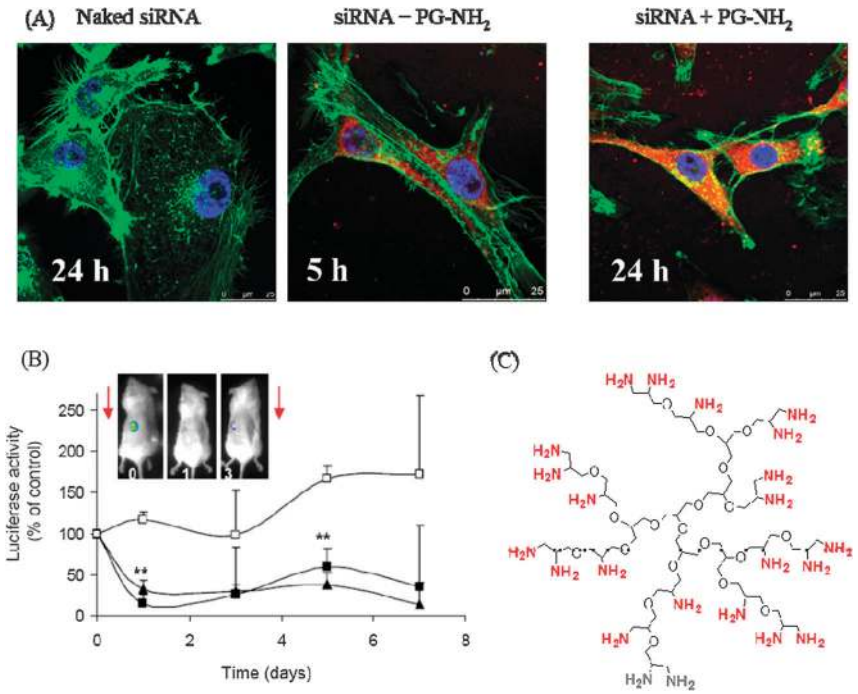


**Fig. 20** (A) siRNA–PG–amine polyplex intracellular uptake. U87-Luc cells were incubated with TRITC-labeled siRNA (red) either alone (naked-siRNA) or complexed with PG–amine (siRNA + PG–$NH_2$). Actin filaments were stained with phalloidin (green). Scale bar represents 25 mm. (B) SCID mice bearing U87-Luc tumors treated with 10 mg $kg^{-1}$ PG–$NH_2$, complexed with 2.5 mg $kg^{-1}$ luciferase siRNA (■), 20 mg $kg^{-1}$ PG–$NH_2$, complexed with 5 mg $kg^{-1}$ luciferase siRNA (▲), or saline as control (□). (C) Idealized fragment of polyglycerolamine. Adapted with permission from ref. 170. Copyright 2010, John Wiley & Sons, Inc.

glycoarchitectures based on PG for the inhibition of L- and P-selectins, a general class of receptors which displays a selective adhesion and includes a lectin-like domain.[171] Two structures were compared, namely, free PG-galactose and PG-sulfated galactose. Selectin inhibition studies carried out with surface plasmon resonance (SPR) measurements indicated a clearly enhanced effect due to multivalency. Using L-selectin, the nanomolar binding affinity of PG-galactose was observed. Notably, sulfated dendritic galactose showed a further enhancement with an $IC_{50}$ of 1 nM. This indicates the importance of negatively charged sulfate groups on the surface of these polysaccharide analogs. However, it was speculated that the negative charge of the sulfate groups alone could be responsible for the strong interaction with L- and P-selectins.

In an ongoing project by the Haag group, a similar study was performed using dendritic polyglycerol sulfates (dPGS, Fig. 21), initially reported as new heparin analogues.[172–174] These structures were found to prolong the time of activated partial thromboplastin as well as thrombin and to inhibit both the classical and alternative complement activation more effectively than heparin itself.[166,167] The biocompatible and well-tolerated PG sulfate acts as multivalent selectin ligand mimetics and efficiently blocks leukocyte migration. L- and P-selectin binding to immobilized ligands was drastically reduced by the PG sulfates *in vitro* and gave $IC_{50}$ values in the low nanomolar range. The inhibition was strongly dependent on the core size and degree of sulfation for different derivatives.[175] Furthermore, only the sulfate groups showed a nanomolar binding to L-selectines, whereas all other tested polyanions (carboxylates, sulfonate, phosphonate, phosphate) resulted in a much weaker binding (~1000 fold).[176] In an *in vivo* model it was observed that the administration of dendritic PG sulfates in a contact dermatitis model dampened leukocyte extravasation as effectively as did

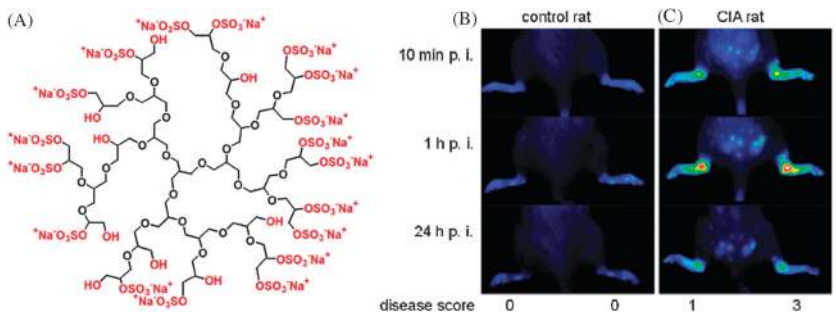


**Fig. 21** (A) Schematic representation of dendritic polyglycerol (dPGS). (B) Comparison of fluorescence images in false colors (normalized to a fluorescence reference cube) of a control rat and rats with collagen-induced rheumatoid arthritis (different clinical scores are indicated) after 10 min, 1 h and 24 h post injection of 6 (4 mg $kg^{-1}$ b.w.). One representative example of at least $n = 5$.[174,177]

 

glucocorticoids, and edema formation was significantly reduced. In addition, dPGS interacted with complement factors C3 and C5 as was shown *in vitro* and reduced C5a levels in a mouse model of complement activation.[165] In order to investigate whether dPGS addresses inflamed tissue, imaging studies were performed using dPGS labeled with the near infrared (NIR) dye indocyanine green (ICG) in an animal model of rheumatoid arthritis. The *in vivo* accumulation results demonstrated a fast and selective uptake which enabled the differentiation of disease scores and allowed identification of joints with early signs of inflammation. Localization in tissues using fluorescence histology showed that the conjugates are mainly deposited in the inflammatory infiltrate in the synovial membrane, whereas non-sulfated control was not detected in association with disease (Fig. 21).[175,177]

In conclusion, dendritic polyglycerols are defined 3D architectures with tunable functionality and inertness to nonspecific interactions with biological systems. Therefore PG represents a novel platform for next generation biomaterials in nanomedicine. The toxicity profiles are well below the limits for *in vivo* applications and suited for targeting the disease like cancer and inflammation.

## 6 Polyglycerol nanogels and their biomedical applications

Many of the 'proof of concept' examples previously described for dendritic polyglycerol architectures were performed for particles with sizes between 2 and 15 nm. The continuous need of new polymeric entities which may be applied in the biomedical field, calls for careful investigation of dimensional aspects of polymers, along with their topological features. In this context, several methodologies have been reported in the past for the synthesis of PG hydrogels with dimensions in the micrometre scale.[178–180] In order to address different length scales in biology (proteins, viruses, bacteria and cells), multifunctional micro- and nanogel have been reported by Haag *et al*. In the following sections the synthetic methodologies, as well as the potential applications of such promising polyglycerol nanogel particles are highlighted.

### 6.1. Synthetic methodologies

In the pioneer work of Sisson *et al*. hyperbranched PG monomers were converted to their high-molecular weight variant using the nanoreactor template, whereas cross-linking was achieved by an easy "click" type Huisgen alkyne/azide cycloaddition reaction.[181] It is noteworthy that due to the confinement of space, no copper was needed for this thermal [2 + 3] cycloaddition at only 80 °C. Both hydrophilic and hydrophobic nanoparticles could therefore be prepared by the direct and inverse miniemulsion process, yielding nanogels with particle sizes between 25 and 90 nm.[182]

More recently, a new concept was developed by our group in which functional PG nanogels were synthesized by an acid catalyzed polyaddition of glycerol to trisglycidyl glycerol ether utilizing the inverse miniemulsion technique where the polar reactants were dispersed in non-polar cyclohexane (Fig. 22).[44,183] A poly(ethylene-co-butylene)-block-poly(ethyleneoxide) surfactant was used as a stabilizer and a small amount of DMSO was used to prevent Ostwald ripening. Alternatively, multifunctional alcohols were

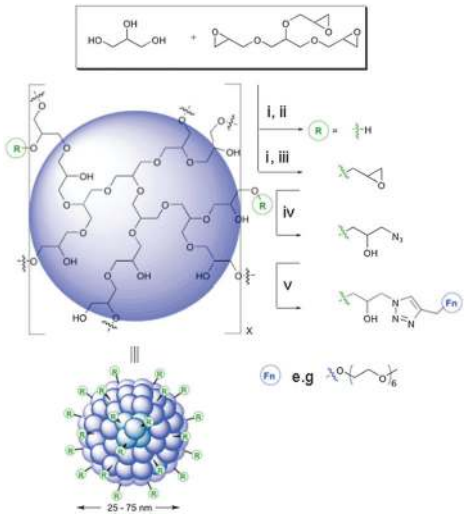


**Fig. 22** Synthetic pathways towards pure PG-μ-gel and surface functionalized PG-μ-gel particles: (i) cyclohexane/DMSO/block copolymer surfactant, sonic tip miniemulsification 4 × 1 min; (ii) *p*-TSA (cat.), 115 °C, 16 h; (iii) *p*-TSA (cat.), 115 °C, varied time; (iv) $NaN_3$, DMF, 60 °C, 24 h; (v) propargyl derivative, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, $H_2O$, 24 h. Adapted with permission from ref. 1. Copyright 2010 John Wiley & Sons, Inc.

used as monomers and di- and triepoxides as crosslinking agents.[184] The properties of these nanogels, *i.e*., size, degree of branching, viscosity, and swelling behavior, could be controlled by varying the functionalities of the monomers and cross-linkers.

### 6.2. Polyglycerol nanogels in biomedical applications

Nanogel technology has already established itself as a robust platform for creation of functional materials with optimal size and multifunctionality for different fields of applications. The inherent properties of the polyglycerol gels, related to the high hydrophilicity, the high biocompatibility, and the controllable size/architecture in between 20 nm and several micrometres, enabled their application in several biomedical scenarios. In particular, the easily functionalizable surface equates to nanoscale multivalent substrates which could have enhanced recognition properties toward biological surfaces.[185] In addition, such systems have been postulated for their potential in the field of tissue engineering, since PG gels might biomimic extracellular matrix (EMC) component proteins.

The interest in PG nanogels spearheads from their non-trivial synthesis into their biological implications. For example, nanogels with sizes between 25 and 350 nm have been shown to rapidly internalize into the cell, with a preferred localization in the perinuclear region. As shown in Fig. 23, there is evidence for a size dependent endocytotic mechanism of cell entry. In addition, such PG gels architectures afforded a safe cytotoxicity profile in the mg $mL^{-1}$ range.[44,183]

For the design of a smart system, biodegradable PG nanogels and hydrogels were prepared *via* an acid catalyzed ring-opening polyaddition of disulfide containing polyols and polyepoxides (Fig. 24).[186,187] Varying conditions allowed tuning of the particles and the disulfide content within the polymer network, yielding particles with narrow polydispersities and diameters in the range from 25 to 350 nm. Interestingly, the disulfide containing

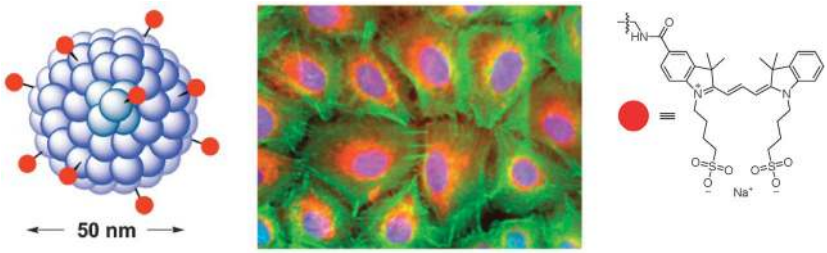


**Fig. 23** Fluorescence microscopy shows clear evidence for cellular uptake of fluorescently labeled PG microgels *via* an endocytotic pathway. Adapted with permission from ref. 1. Copyright 2010 John Wiley & Sons, Inc.

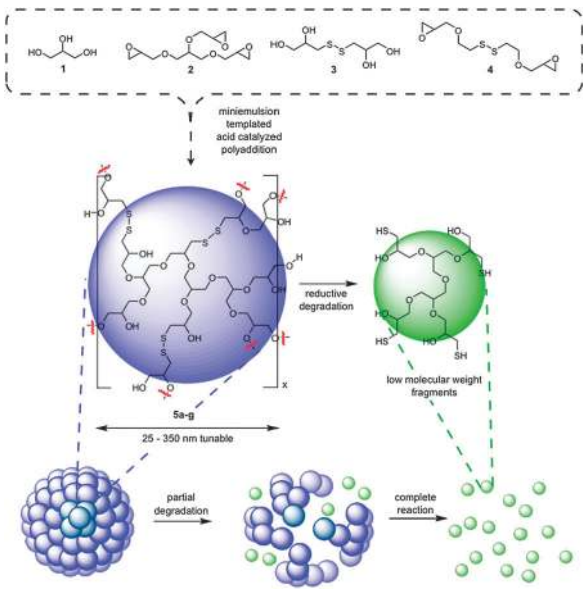


**Fig. 24** Synthetic route to biodegradable polyglycerol nanogels, showing a generalized depiction of a nanogel and degradation fragment. Adapted with permission from ref. 186. Copyright 2011, John Wiley & Sons, Inc.

polyglycerol nanogels were found to be highly biocompatible and to degrade into small oligomeric subunits in reducing environments. Additionally, a near infrared fluorescent dye was encapsulated in the hydrogel network that showed complete degradation in reducing media and a controlled release of the fluorescence dye.

Similarly, Groll *et al*. reported an approach for the preparation of degradable and biocompatible nanogels from thiol-functional macromers.[188] Linear polyglycerol and star-shaped poly(ethylene oxide-*stat*-propylene oxide) were functionalized with thiol groups by a polymer-analogous reaction and were cross-linked in inverse miniemulsion conditions. The disulfide crosslinked particles showed to degrade upon addition of aqueous glutathione solution that resembles cytosolic conditions.

The important role of the nanogel dimension in biological interactions was recently highlighted in a systematic analysis of multivalent glycoarchitectures based on PG nanogels in the inhibition of the influenza virus.[189] In this study, particle sizes were varied along with the degree of functionalization to match the corresponding virus size and receptor multiplicity in order to achieve maximum binding efficiency. It was shown that the inhibitory activities of the polymeric glycoconjugates drastically increased with the nanoparticle size. Comparing the inhibition of binding and fusion to influenza virus, PG nanogels with 50 nm of diameter was $7 \times 10^3$ folds more effective than hyperbranched PG with diameter of 3 nm at comparable sugar concentrations. Moreover, it was demonstrated that the nanogel reduced viral activity by up to 80%. This emphasizes the importance of matching sizes and multiplicity for biological surface interactions, which is achieved by the particles dimensionality of the PG nanogels (Fig. 25).

The fabrication of thermo-responsive PG nanogels was recently developed by Calderón *et al*., in an attempt to develop stimuli-responsive materials based on dendritic polyglycerols.[190] In this work, a precipitation polymerization method was used to cross-link *N*-isopropylacrylamide (NIPAm) and hyperbranched PG to yield nanogels with sizes between 50 and 200 nm. The incorporation of PG as crosslinking agent enhanced the water solubility of the nanogels, improved their biocompatible profile, and allowed a fine tuning of the thermo-responsive profile regarding the size of the nanogels in solutions (Fig. 26).

## 7 Opportunities

Nanotechnology revolves around the design and control of materials at length scales between 1–1000 nm which implicates its usefulness for applications in nanomedicine. In the next 10–15 years, many specialized medical applications on the nanoscale will arise especially in the areas of pharmaceutical products, drug delivery systems, and health-monitoring devices.[191] The need to design and evaluate new polymeric biomaterials for individual biological applications is larger than ever. Recently, a variety of new macromolecular architectures have been synthesized based on properties and functions of delivery components. In addition, such nanotherapeutics must reduce accumulation in normal tissues, thereby decreasing drug related adverse side effects. Furthermore, the polymer and its bioconjugate should enhance the aqueous solubility and stability of the bioactives.

One of the key challenges in creating an effective and safer nanoplatform is to achieve targetability to the appropriate tissues and cells. Although biological targeting using aptamers or antibodies on the surface of nanoparticles is one popular option, researchers are beginning to explore the physical characteristics of the nanoforms to guide them to desired locations. The size, shape, physical properties, density, and charge all affect how nanomedicines will travel through the body, and whether or not they will cross biological membranes. Enormous efforts have been made to combine pH and temperature responsive modalities in nanocarriers to develop a smart delivery system based on dendritic polyglycerols.[1] The resulting nanosystems have many biomedical applications, since the

 

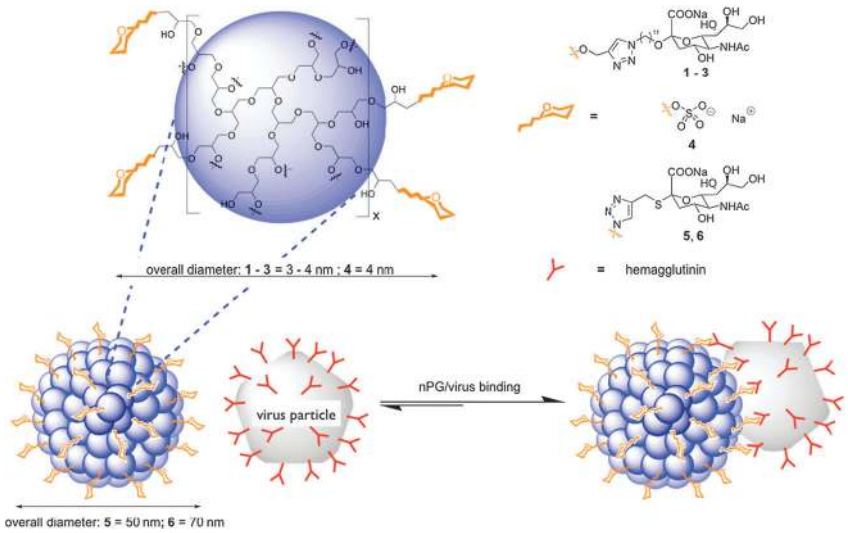


**Fig. 25** Schematic representation of sialic acid-conjugated PG-based nanoparticles that match influenza virus size and receptor multiplicity. Adapted with permission from ref. 189. Copyright 2011, John Wiley & Sons, Inc.

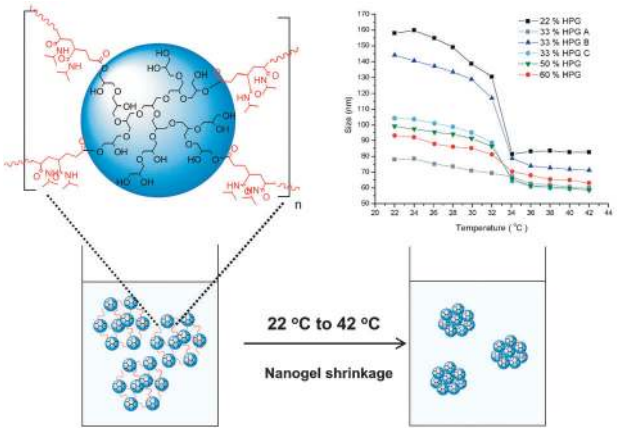


**Fig. 26** Thermo-responsive polyglycerol based nanogels synthesized through precipitation polymerization. The nanogels showed a tendency to shrink with increasing the solution temperature as shown by DLS measurements.

temperature and pH of the target sites could be modulating factors for triggering activity of biomolecules.

The choice of appropriate nanocarriers is not obvious, and comparative studies are difficult to interpret because many factors simultaneously affect the biocompatibility, biodistribution, and targeting. However, the improved therapeutic efficacy of targeted nanocarriers has been established in multiple animal models of cancer, and currently more than 120 clinical trials are underway with various combinations of nanoformulations.[192] And yet, many challenges still remain such as the synthesis of a highly biocompatible, biodegradable, intelligent releasing nanocarrier system which can be cleared up by kidneys, to achieve the desired biological availability and biodistribution.

## 8 Conclusions

Nanomedicine is driven by the success in the creation of innovative and safe 'nanosize materials' through the control of synthesis on the nanometre scale. The definable architectures, synthetic tunability, and chemical versatility of nanocarriers have increased the perspectives in the field of nanotherapeutics and nanodiagnostics. Also, they have created a new field of therapeutics by combining both modalities. The inherent nanoscale functions of dendritic polymers seem to be inevitable in numerous applications in life sciences and offer extraordinary, paradigm-changing opportunities with significant advances in cancer diagnosis and treatments. Especially, muli-functional PEG-like polymers, such as dendritic polyglycerols, provide a new platform for bridging the gap between molecular sciences, functional materials, and polymer therapeutics. Polyglycerols can be produced by straightforward syntheses, various architectures ranging from perfect dendrons to well-defined hyperbranched polymers, micro and nanogels, and multicomponent nanoconjugates. Although some first promising results are available for dendritic polyglycerols, maximized biocompatibility on the cellular and systemic levels, metabolism, PKPD profile, and maximum tolerated dose of specific drug conjugates still need to be studied in greater detail. Nanomedicines derived from dendritic polymers already play an important role in the treatment of cancer and inflammation in two broad areas: the development of nanovectors, such as nanodepots and nanoconjugates, with drugs or imaging agents and for targeting the tumors and inflammation sites. Combined, such technologies could lead to an earlier diagnosis and better treatment of patients with chronic inflammation and cancer. The prime focus is now initiated in designing more versatile biomaterials to be implied in nanomedicines, considering biophysicochemical interactions at the nano–bio interface. More importantly, nanomedicines have to pass through very stringent regulations and approval protocols before entering into phase I clinical trials, thereby delaying overall timelines.

Conclusively, the design of polymeric architectures, their structure–activity relationship and translation into multicomponent nanosystems for biological applications and targetability is still in its infancy. The controlled synthesis under GMP-like protocols and vigorous biological evaluation of

polymeric nanocarriers are absolutely necessary for the translation of nanomedicines from laboratory into the clinic.

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