Expert Opinion

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Drug delivery using multifunctional dendrimers and hyperbranched polymers

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Importance of the field: The review presents the design strategy and synthesis of multifunctional dendrimers and hyperbranched polymers with the objective to develop effective drug delivery systems.
Areas covered in this review: Well-characterized, commercially available dendritic polymers were subjected to functionalization for preparing drug delivery systems of low toxicity, high loading capacity, ability to target specific cells and transport through their membranes. This has been achieved by surface targeting ligands, which render the carriers specific to certain cells and polyethylene glycol groups, securing water solubility, stability and prolonged circulation. Moreover, transport agents facilitate transport through cell membranes while fluorescent probes detect their intracellular localization. A common feature of surface groups is multivalency, which considerably enhances their binding strength with complementary cell receptors. To these properties, one should also add the property of attaining high loading of active ingredients coupled with controlled and/or triggered release.
What the reader will gain: Readers will be exposed to the strategy of synthesizing multifunctional polymers, aimed at the development of effective drug delivery systems.
Take home message: Multifunctional systems upgrade the therapeutic potential of drugs and, in certain cases, may even lead to the application of new bioactive compounds that would otherwise not be feasible.

Keywords: dendrimers, dendritic polymers, drug nanocarrier, hyperbranched polymers

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1. Introduction

Dendritic polymers have tree-like structures and consist of hyperbranched polymers, dendrigrafts, dendrons and dendrimers. Each of these four classes reflects the structural features of these complex macromolecular architectures [1-8]. Dendrimers, which is the most extensively investigated class, are highly branched symmetrical macromolecules of nano-sized dimensions, have well-defined molecular mass and consist of a central core, repeating units and terminal functional groups. They are prepared under tedious experimental conditions and are, for this reason, expensive. However, because of their diverse properties, they have attracted significant scientific and technological interest. On the other hand, hyperbranched polymers are conveniently prepared and are, therefore, inexpensive but non-symmetrical and polydisperse. Both classes form nanocavities, in the interior of which various molecules, including bioactive compounds, have been encapsulated. Alternatively, bioactive compounds have also been conjugated at the dendritic scaffold, primarily on its surface. Both classes of polymers bear on their external surface a significant number of functional groups. The great number of functional groups on the surface

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Article highlights.

- Design and synthesis of multifunctional dendrimers and hyperbranched polymers for the development of drug delivery systems that can be loaded with bioactive compounds and find application as drug delivery systems.
- Selection of proper dendritic scaffolds and application of multifunctionalization strategy led to drug delivery systems that are simultaneously biodegradable and non-toxic, show stability in the biological environment, have specificity and multivalency behavior for certain cells and are able to penetrate cell membrane. Ideally, for developing effective drug delivery systems all the previously mentioned beneficial properties should be fulfilled simultaneously.
- Loading of bioactive compounds was achieved either covalently or non-covalently, and their release can be controlled and/or tuned in the biological environment, for example by changes in pH or ionic strength.
- A future challenge in this domain is the elucidation of internalization mechanisms that could assist in the synthesis of multifunctional dendritic nanoparticles that can be directed to appropriate subcellular destinations.

This box summarizes key points contained in the article.

amplifies their binding to cells, owing to the so-called multivalent effect, as more than one of the cell receptors can be accessed simultaneously by one dendritic polymer [9-13].

Taking advantage of the property of dendritic polymers to encapsulate or conjugate various bioactive compounds, they have been used as drug delivery systems [14-30]. For improving their drug delivery effectiveness, these polymers have been functionalized on their surface with a diversity of groups affording multifunctional derivatives. Each type of external group plays a specific function. Thus, as is the case with other targeted nanoparticles [31-37], specificity for certain cells has been achieved by attaching targeting ligands on the surface of dendritic polymers. In this connection, the folate targeting ligand, owing to its simple structure and convenience of attachment on the surface of the dendritic systems, is certainly the most extensively used ligand, whereas others such as cyclic Arg-Gly-Asp peptide (cRGD) peptides and herceptin (humanized monoclonal antibody) have been used to a lesser extent. The application of cell-penetrating peptides [38-43] and arginine-rich derivatives has been the basis for preparing molecular transporting dendritic nanoparticles. It is the guanidinium moiety on the surface of the dendritic polymers that interacts with the phosphate and/or carboxylate group of the cells' surface, inducing membrane transport [44-46]. Enhanced water solubility, decreased toxicity, biocompatibility, stability and protection in the biological milieu have been achieved by functionalizing the terminal groups of dendritic polymers with poly(ethylene glycol) (PEG) chains. The function of PEG chains is crucial for modifying the behavior of drugs themselves or of their drug carriers [16,47-56]. PEGylation, for example, has also been applied extensively to other nanoparticles, including the most established liposomal carriers [57-61]. Also, modification of the internal groups of dendrimers can affect their solubilizing character, rendering possible the encapsulation of a diversity of drugs. Dendritic polymers' nanocavities or conjugation of drugs on their surface have been tailored so as to trigger drug release by changes in the biological environment at the site of action [62,63]. Following this strategy, commercially available or custom-made dendritic polymers have been functionalized, affording drug delivery systems [14-30]. Schematic representations of a multifunctional dendrimer and a hyperbranched polymer are shown in **Figure 1**.

Significant progress has been made in the time period between the synthesis of the first functional dendritic polymers and the currently prepared multifunctional ones. In this review, the focus is almost exclusively on multifunctional dendrimers and hyperbranched polymers, primarily presenting the strategy of their synthesis, achieved through molecular engineering of the surface of basic and commercialy available dendritic polymers. This review is in some way complementary to the authors' previous one on 'Gene delivery using functional dendritic polymers' [64], published in 2009. In this review, the two most extensively investigated dendrimers, that is, diaminobutane poly(propylene imine) (PPI) (**Figure 1**) and poly(amidoamine) (PAMAM) (**Figure 1**), are used as scaffolds for multifunctionalization. It should be noted that PAMAM-based polymers have been extensively subjected to multifunctionalization. In addition, the hyperbranched polyglycerol (PG) (**Figure 1**) and also the recently introduced and very promising biodegradable hyperbranched aliphatic polyester Boltorn^{TM} (Perstorp AB, Sweden) (**Figure 1**) [65-67] are used as basic dendritic polymers. The review is not intended to be exhaustive, but rather to highlight dendritic drug delivery systems' design and methodologies.

As discussed later, many *in vitro* studies demonstrate the great potential of dendritic polymers as drug delivery systems, and, more recently, research has been initiated including *in vivo* experiments, which were presented in a recent review [68].

2. Multifunctional dendrimers as drug delivery systems

A model system of multifunctional dendrimeric nanocarrier was prepared [62] using diaminobutane poly(propylene imine) with 64 amino endgroups. The designed and prepared carrier (**Figure 2**) was intended to address simultaneously stability and prolonged circulation in biological milieu, enhanced water solubility, strong binding to cell membrane and transport through it and also pH-triggered release. Thus, in addition to surface protective PEG chains, guanidinium moieties were introduced for interacting with phosphate groups or other anionic groups on the cell surface, the binding of which is amplified owing to multivalency effects. Simultaneously, the accumulation of guanidinium groups on the surface of

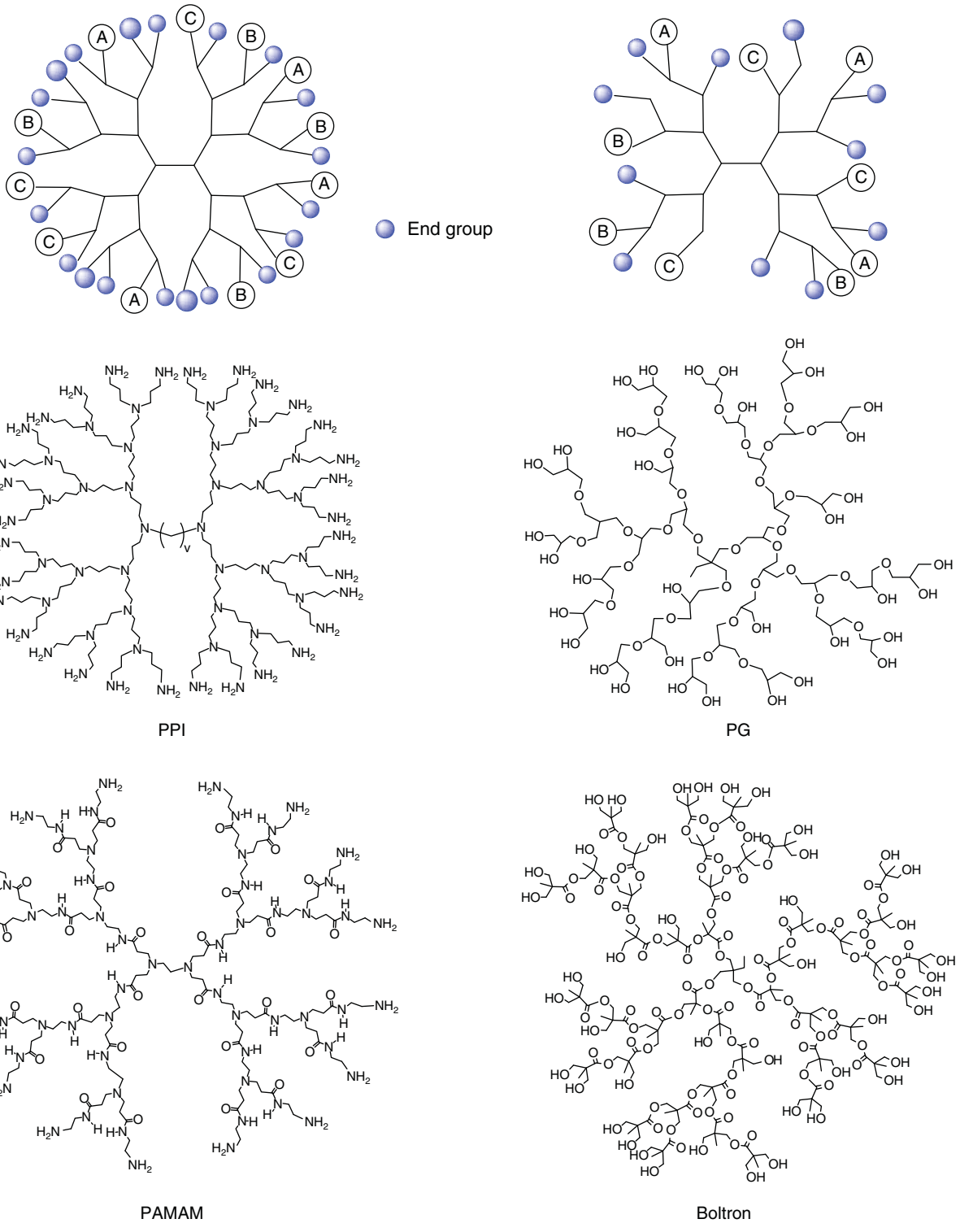

Figure 1. Schematic representation of a multifunctional dendrimer (left) and hyperbranched polymer (right) together with dendrimeric (left column) and hyperbranched polymers (right column) used for multifunctionalization. Endgroups **A, B** and **C** depict the presence of targeting ligands, protective coating, transport agents, fluorescent probes, or in some cases a drug conjugated on the surface of the dendritic carrier.

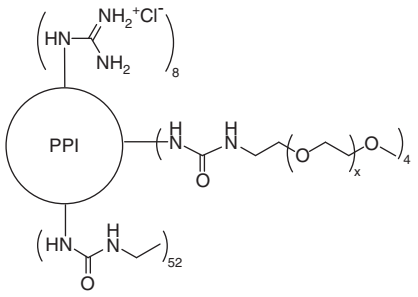

Figure 2. A multifunctional dendrimeric derivative based on diaminobutane poly(propylene imine) dendrimer with 64 endgroups.

the dendrimer can also facilitate its transport through cell membrane in a manner analogous to oligoarginine peptides [40]. In addition, owing to the polyamine character of the nanocavities, it is possible to tune the release of the encapsulated ingredient by pH changes [62]. Finally, toxicity decrease is anticipated by the reduction in the number of toxic amino groups [69,70].

Encapsulation and release properties of this multifunctional dendrimer were investigated with betamethasone valerate (BV), a bioactive compound, and pyrene (Py), a well-established fluorescent probe. The multifunctional dendrimer encapsulated significantly higher concentrations of the above compounds compared with the parent dendrimer. This is particularly useful for the hydrophobic betamethasone valerate, of which seven molecules are solubilized per dendrimeric molecule. Specifically, betamethasone valerate loading capacity is 11 wt% inside the multifunctional dendrimer, that is, almost double compared with the loading capacity of the simply PEGylated dendrimer (6 wt%) [62], and more than five times compared with the loading capacity of the parent dendrimeric solution (1.7 wt%) [71].

Although the above multifunctional dendrimer is pH-responsive, owing to the presence of tertiary amino groups in the interior of its nanocavities, a two-step triggered process was required for the release of the lipophilic drug BV. Release of the drug with hydrochloric acid has not been achieved because BV remained solubilized within the dendrimeric environment and preferably within the PEG chains. However, BV encapsulated in this dendrimeric derivative was completely released on addition of sodium chloride. It is interesting to note that within the concentration range of sodium cation in extracellular fluids, that is, 0.142 M [72], BV was released at a relatively low percentage. Sodium chloride found in extracellular fluids can interact with PEG chains, affecting the overall release profile of the drug. Thus, the possibility of triggering premature drug release in the extracellular fluid, that is, before endocytosis to the target cells, should be taken into account when designing a targeted PEGylated drug delivery system. It is therefore advisable to follow the release of drugs from PEGylated dendrimers with simulated experiments before proceeding to *in vitro* and *in vivo* tests.

In the previous example the framework of multifunctional systems design was set forward while work continued using primarily the biodegradable PAMAM dendrimers [73]. Thus, stepwise modification of PAMAM dendrimers afforded multifunctional polymers [74] in which the drug was covalently attached on the dendrimeric scaffold. Thus, partial acetylation of the primary amino groups of fifth-generation (G5) PAMAM dendrimer prevented nonspecific interactions during drug delivery both *in vitro* [75] and *in vivo* [76]. The remaining primary amino groups were used for attaching functional moieties, for example fluorescein isothiocyanate (FITC), which is an imaging agent, folic acid (FA), which targets folate receptors on specific cancer cells, and methotrexate (MTX), an anticancer drug (**Figure 3A**). It should be noted that the folate moiety, which has frequently been attached on nanocarriers, is overexpressed in a broad variety of human cancers and on activated macrophages [77], and therefore folate-mediated targeting [78,79] has been widely applied to a diversity of carriers, such as liposomes [80,81], dendrimers [82-84], hyperbranched polymers [85,86], and other types of nanoparticles [87-94]. In this manner, targeted delivery of chemotherapeutic and imaging agents to specific cancer cells is achieved.

By a strategy analogous to the one described above, another multifunctional system [95] was prepared (**Figure 3B**) based on fifth-generation PAMAM scaffold that had covalently attached the anticancer drug paclitaxel (Taxol® Bristol-Myers Squibb, New York, USA). Experiments *in vitro* have shown efficient cell uptake of this multifunctional dendrimeric system and that it is completely non-toxic to KB cells at a concentration of 200 nM.

Following preliminary reports [96,97] in which an Arg-Gly-Asp (RGD) peptide was conjugated to a fifth-generation PAMAM for *in vitro* targeting to $\alpha_V\beta_3$ integrin receptor expressing cells, in a recent work the same dendrimer was subjected to multifunctionalization, affording the nanocarrier shown in **Figure 3C** [98]. This carrier bears a cRGD peptide attached to the dendrimeric surface and biotin groups that amplify detection of the carrier by anti-biotin antibody or avidin linked to horseradish peroxidase. This drug delivery system shows selective targeting of $\alpha_V\beta_3$ integrins when compared either with the same, free cRGD peptide or with the biotinylated nanocarrier without any covalently attached peptide.

The synthesis of the nanocarrier was achieved in stages, that is, surface amino groups of PAMAM were first partially acetylated and then biotinylated, while the remaining primary amino groups were converted to succinamic acid groups, some of which were conjugated with cRGD peptide residues through the amino group of the lysine side chain. Cytotoxicity was investigated using B16F10 melanoma cell cultures using the XTT colorimetric assay for cellular viability. Binding of the nanocarriers to the target was determined using plates coated with human $\alpha_V\beta_3$ integrin and $\alpha_V\beta_3$ receptor expressing human dermal microvascular endothelial cells. The nanocarrier is non-toxic within physiologic concentration

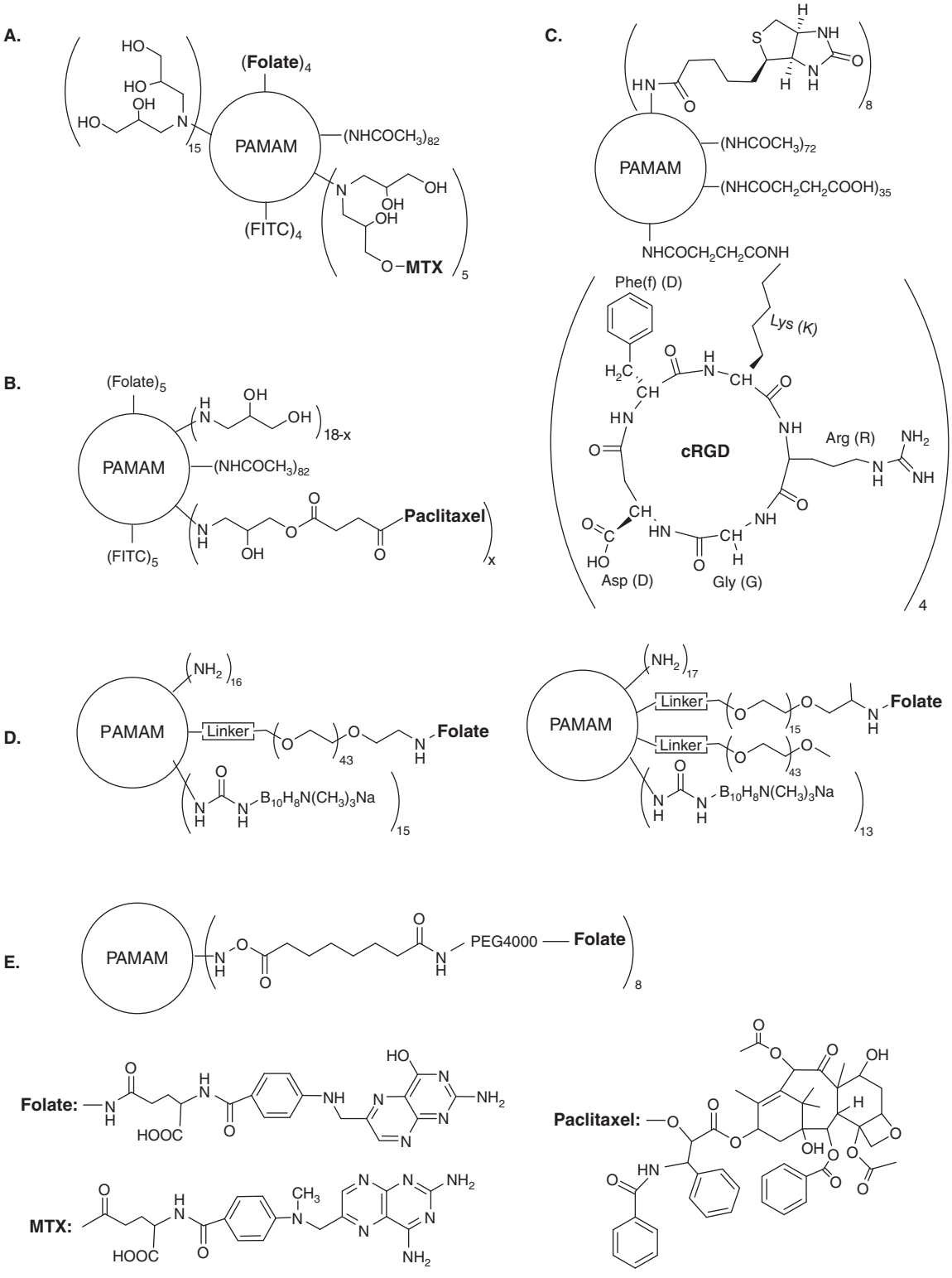

Figure 3. Multifunctional PAMAM dendrimeric derivative with covalently bound MTX (A) or paclitaxel (B), a PAMAM nanodevice with cyclic RGD and biotin moieties (C), boronated third-generation PAMAM dendrimers functionalized with PEG and PEG-folate moieties (D), and a fourth-generation PAMAM dendrimer conjugated, through a spacer, to a PEG-folate moiety (E).

ranges and specifically binds to the $\alpha_V\beta_3$ integrins much more strongly than the cRGD peptide itself.

As mentioned in previous paragraphs, folate receptor expresses in a wide variety of cancers. For designing and preparing an effective dendrimeric drug delivery system, therefore, a folate moiety together with other appropriate functional groups were introduced on the surface of a third-generation PAMAM dendrimer for the synthesis of multifunctional dendrimeric carriers [99]. These nanocarriers, in addition to bearing the protective PEG coating, are also functionalized with the folate targeting ligand at the end of the PEG chain for inducing endocytosis into folate receptor-bearing cells. In addition to the previously functionalized dendrimer, 12 – 15 decaborate clusters were covalently attached, which can be used for the treatment of cancer in boron neutron capture therapy (BNCT) requiring the selective delivery of 10B to cancerous cells within a tumor. By using various numbers of PEG chains that differ in chain length, it is possible to reduce hepatic uptake of these boronated dendrimers. Among the prepared combinations, boronated dendrimers with 1 – 1.5 PEG_{2000} units showed the lowest hepatic uptake in C57BL/6 mice (7.2 – 7.7% injected dose (ID)/g liver).

The structures of folate receptor-targeted and boronated third-generation poly(amidoamine) dendrimers are shown in **Figure 3D**. Specifically, one multifunctional system bears ~ 15 decaborate clusters and ~ 1 PEG_{2000} unit with a folate moiety attached to the distal end, whereas the other bears ~ 13 decaborate clusters, ~ 1 PEG_{2000} unit and ~ 1 PEG_{800} unit and the folate moiety attached to its distal end. *In vitro* studies using folate receptor-positive KB cells have shown receptor-mediated uptake of the second derivative functionalized with the folate moiety. Biodistribution studies with this derivative in C57BL/6 mice bearing folate receptor-positive murine 24JK-FBP sarcomas resulted in selective tumor uptake (6.0% ID/g tumor), but also high hepatic (38.8% ID/g) and renal (62.8% ID/g) uptake. This indicates that attachment of a second PEG chain and/or folate moiety may adversely affect the pharmacodynamics of this nanocarrier. It can, therefore, be deduced that the optimal modification of boronated dendrimers and in general of dendrimers functionalized with PEG chains for reducing reticuloendothelial system affinity appears to be a highly complex process that is dependent on a variety of factors requiring extensive evaluation.

In a recent investigation [100] folate moiety was conjugated to a fourth-generation PAMAM dendrimer either directly or indirectly through a PEG_{4000} chain, as shown in **Figure 3E**. The anticancer drug 5-fluorouracil was encapsulated in both dendrimeric derivatives and investigated in *in vitro* and *in vivo* experiments. Specifically, the most promising folate-PEG-dendrimeric derivative was significantly safer and more effective in tumor targeting compared with the non-PEGylated carrier. Functionalization with the PEG-folate moiety reduced hemolytic toxicity, which secured sustained drug release as well as higher accumulation to the tumor site.

In a recent report [101], instead of folate another targeting ligand was used, and specifically herceptin, which is a humanized monoclonal antibody binding to human growth factor receptor-2 (HER2). It was covalently attached to a fifth-generation PAMAM in which methotrexate anticancer drug was also conjugated [101]. The specificity of this FITC-labeled nanocarrier and its internalization were demonstrated in cell lines overexpressing HER2 by flow cytometry as well as confocal microscopy. In addition, binding and uptake of these antibody-conjugated dendrimers was completely blocked by excess non-conjugated herceptin. Colocalization experiments with lysotracker red indicate that the antibody, although internalized efficiently into cells, has an unusually long residence time in the lysosomes. Reduced cytotoxicity of the conjugate in comparison with free methotrexate was attributed to the slow release of methotrexate from the carrier and its long retention in the lysosomes. Although the conjugate was less toxic to cells than methotrexate alone *in vitro*, it is possible that this carrier might be superior for *in vivo* experiments owing to its reduced toxicity for HER2-overexpressing breast cancers as a result of its specificity to target tumor cells.

3. Multifunctional hyperbranched polymers as drug delivery systems

Hyperbranched PGs [102,103] have been widely used and show low toxicity and biocompatibility. They have been functionalized by an analogous strategy to dendrimers leading to multifunctional drug delivery systems. For this purpose, PEGylated (PG-PEG) and PEGylated-folate (PG-PEG-folate) functional derivatives of polyglycerol were prepared (**Figure 4**) and investigated as prospective drug carrier systems [104]. Encapsulation properties of these model dendritic derivatives were assessed by using pyrene probe and tamoxifen (TAM), a hydrophobic anticancer, non-steroidal, antiestrogen drug that is widely used in the treatment and prevention of breast cancer [105,106]. Its encapsulation and simulated release properties were comparatively investigated for the parent polyglycerol, the PEGylated derivative, PG-PEG, and the multifunctional derivative, PG-PEG-folate. The solubility of TAM in water is 1.9×10^{-6} M, which increases by a factor of 5 when solubilized in 1 mM PG solution. The solubility of TAM is considerably enhanced by a factor of 65 in the presence of PG-PEG. This significant increase indicates that TAM is encapsulated not only inside the hyperbranched interior, but also within PEG coating. This behavior is analogous with previous results, that is, when PEGylated dendrimeric derivatives [62,71] were used. Therefore, the introduction of the PEG chains enhances, in general, the solubilization efficiency of dendritic polymers. It should be noted that for PG-PEG-folate an ~ 1300-fold increase of TAM solubility was observed.

The release of TAM that was encapsulated in PG and its derivatives was triggered by the addition of NaCl in the solution. Molecules encapsulated in PEG coating can be replaced by metal ions. It is, therefore, necessary to investigate whether

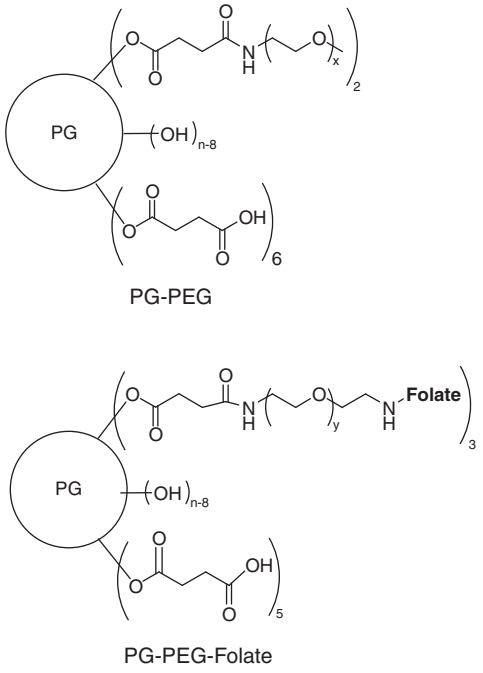

Figure 4. PEGylated (PG-PEG) and PEGylated-folate (PG-PEG-folate) functional derivatives of hyperbranched polyglycerol.

sodium cation complexation can cause premature release of the drug in the extracellular fluids, that is, before the drug-loaded nanocarrier reaches the target cell. By the addition of 0.142 M NaCl solution, 39 and 24% of the solubilized TAM in PG and PG-PEG were released in aqueous media, respectively. Under the same conditions, in the presence of PG-PEG-folate, only 6% of the solubilized TAM was released. It is interesting, therefore, to note that using the multifunctional derivative as nanocarrier, most of the TAM remained encapsulated inside the polymer, and it is not released in the extracellular fluid at a concentration of 0.142 M NaCl solution. Hence, it can be assumed that this nanocarrier, when used in *in vivo* experiments, will reach, in principle, target cells appreciably loaded with TAM.

Biodegradable dendritic hyperbranched polyesters have recently been prepared [65] based on 2,2-bis(hydroxymethyl) propanoic acid as an AB_2 monomer and 2,2-bis(hydroxymethyl)-1,3-propanediol as central core. These dendritic polymers are supplied in various molecular masses, bearing a different number of primary hydroxyl groups, that is, 16, 32 and 64 for Boltorn H20, H30 and H40, respectively. Two glycodendritic polymers [107] were initially prepared with 16 and 32 mannose moieties based on Boltorn H20 and H30 hyperbranched polymers, which were used as controls. These glycodendritic polymers are water-soluble, have low toxicity and have the capability to interact with lectin receptors. They can, therefore, be considered as promising candidates for drug delivery.

In another investigation, Boltorn H40 was functionalized with PEG chains, affording a water-soluble PEGylated dendritic derivative, BH40-PEG (**Figure 5A**) [108]. In this case, PEGylation of the dendritic scaffold is indispensable not only for protecting the drug carrier when found in biological milieu but also for rendering it water-soluble. The solubility of paclitaxel is increased by a factor of 65, 110, 210 and 350, in 1, 3, 6 and 9% w/v BH40-PEG solutions, respectively, compared with aqueous solubility. The release of paclitaxel was determined by the dialysis method and showed that ~ 60% of the encapsulated drug was released in the aqueous phase during the first 6 h, and its release was almost completed in 12 h. The cytotoxicity was assessed *in vitro* with A549 human lung carcinoma cells. The dendrimer was found to be non-toxic for 3 h incubation at concentrations ≤ 50 μM, whereas LD_{50} was 100 μM.

Furthermore, a folate-conjugated amphiphilic hyperbranched block copolymer (H40–PLA-*b*-MPEG/PEG–FA) based on Boltorn H40 was synthesized (**Figure 5B**) [85]. For its synthesis, a hydrophobic poly(L-lactide) (PLA) inner shell, a hydrophilic methoxy poly(ethylene glycol) (MPEG) and a folate moiety that was attached on PEG chain terminal group (PEG–FA) were introduced on Bolton H40 scaffold. Owing to its core–shell structure, this block copolymer forms unimolecular micelles in aqueous solutions. The micellar properties of this block copolymer were established by dynamic light scattering, fluorescence spectroscopy and transmission electron microscopy. Encapsulation of the anticancer drug doxorubicin (DOX) in its base form afforded a micellar drug delivery system, H40–PLA-*b*-MPEG/PEG–FA. Studying drug release of these DOX-loaded micelles, an initial 'burst release' (up to 4 h) was observed, which was followed by a sustained release of the entrapped DOX over a period of ~ 40 h. Cellular uptake of the DOX-loaded H40–PLA-*b*-MPEG/PEG–FA micelles was found to be higher than that of the DOX-loaded H40–PLA-*b*-MPEG micelles because of the folate receptor-mediated endocytosis. They show, therefore, higher cytotoxicity against the 4T1 mouse mammary carcinoma cell line. Degradation *in vitro* studies revealed that H40–PLA-*b*-MPEG/PEG–FA block copolymer is hydrolytically degraded into polymer fragments within 6 weeks. These results have indicated that the micelles prepared from this block copolymer have great potential as tumor-targeted drug delivery nanocarriers.

In an analogous manner, amphiphilic core–shell multifunctional dendritic hyperbranched polymers bearing folate group as targeting ligand were synthesized (**Figure 5C**) [109]. The core of these polymers was the aliphatic polyester Boltorn H40 scaffold. The inner part of the amphiphilic polymers was hydrophobic poly(epsilon-caprolactone) chains, whereas the outer shell was hydrophilic PEG chains. Tumor cell targeting was achieved by folate moiety attached at the terminal hydroxyl group of the PEG chain. Two anticancer drugs, 5-fluorouracil and paclitaxel, were encapsulated into these dendritic nanocarriers. Drug release and targeting properties of these drug-loaded nanoparticles to different cell lines were

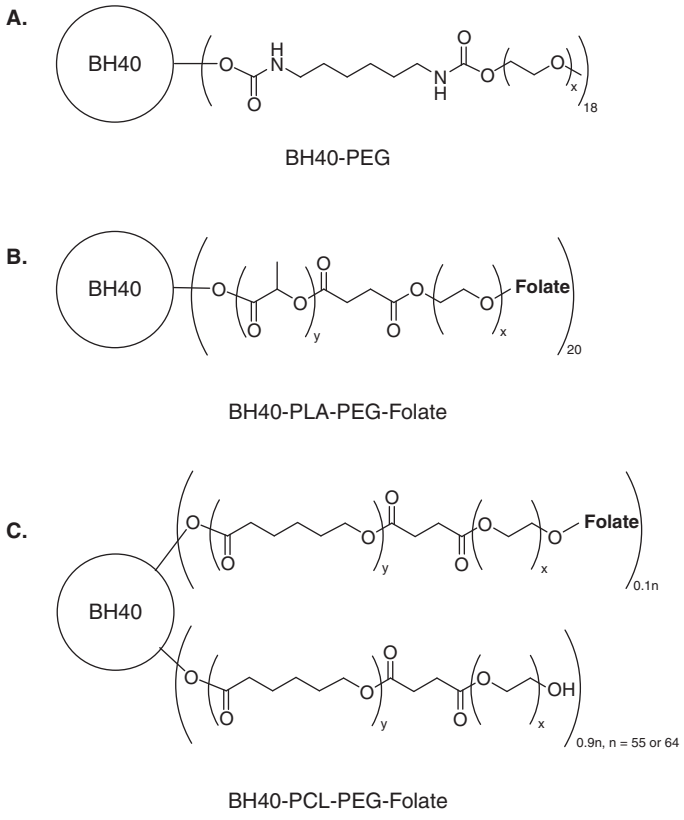

Figure 5. Chemical structures of a Boltorn H40 PEGylated dendritic derivative (A), a Boltorn H40 poly(l-lactide)–PEGylated-folate derivative (B) and a poly(epsilon-caprolactone)–PEGylated-folate dendritic derivative (C).

investigated. The *in vitro* experiments showed that drug-loaded nanoparticles showed enhanced cell growth inhibition attributed to folate targeting increasing cytotoxicity of drug-loaded nanoparticles against folate receptor expressing tumor cells.

4. Conclusion

Appropriate surface multifunctionalization of dendrimers and hyperbranched polymers leads to the development of nanocarriers of low toxicity, significant encapsulating capacity, specificity to certain biological cells and transport ability through their membranes. This drug delivery effectiveness, attributed primarily to molecular engineering of dendritic surface, is also affected by covalent or non-covalent loading of selected bioactive ingredients, the release of which can be triggered by changes in the biological environment, such as pH or ionic strength. An extra advantage of dendritic nanocarriers is their strong binding to cells resulting from multivalent interactions of dendritic functional groups with cell complementary receptors.

5. Expert opinion

Multifunctional dendrimers and hyperbranched polymers have been designed and synthesized to afford drug delivery systems that can be loaded with bioactive compounds either covalently or non-covalently. Following initial studies, where simple functionalization was performed, investigations were undertaken, which, under proper selection of basic dendritic scaffolds and application of multifunctionalization strategy, led to drug delivery systems that are simultaneously biodegradable and non-toxic, show relative stability in the biological environment, show specificity to certain cells and, in certain cases, can penetrate cell membrane. In addition, owing to the molecular features of the interior of dendritic polymers, it is possible to encapsulate a diversity of compounds, the release of which can be controlled and/or tuned in the biological environment, for example by pH or ionic strength.

Analyzing these properties further, it was possible through partial functionalization of the surface groups with PEG chains to enhance significantly water solubility, decrease toxicity and increase stability of dendritic nanocarriers in the biological milieu. On the other hand, targeting ligands attached on the dendritic scaffold, for example folate moiety, or RGD peptides, the application of which has been discussed in detail in this review, enhance carriers' specificity. Furthermore, their binding to cell receptors is amplified by taking advantage of the multivalent effect, which enhances the effectiveness of binding of nanocarriers to cells, as more than one of the cell receptors can bind to multiple

complementary groups on the surface of a dendritic polymer. Transport through cell membranes has also been facilitated by attaching molecular transporting moieties on nanocarriers. In fact, the introduction of cell-penetrating peptides and specifically arginine-rich derivatives enhances translocation ability of dendritic nanoparticles. In addition, other structural features fine-tune the transport of dendritic carriers and even determine their subcellular destination. It should be noted that although the emphasis with dendritic nanocarriers is rather placed on their targeting property, transport through cell membranes should not be disregarded if an effective drug delivery system would be obtained. Summarizing, an optimal guanidinylated molecular transporter [110] acting as a drug delivery system should have appropriate size and degree of functionalization, flexibility of the functional moiety and an appropriate balance between hydrophilic and hydrophobic moieties on the dendritic surface. These parameters should be taken into consideration in designing multifunctional dendritic systems in order to be internalized, together with their drug load, to specific subcellular sites [111]. This is the main challenge and efforts should be directed towards this end.

In view of these promising prospects, further structural elaboration of dendritic polymers will be pursued towards the development of effective, biocompatible and less toxic systems, which is certainly a prerequisite for *in vivo* applications. The strategy for their design and synthesis has been established, but further work is needed to elucidate internalization mechanisms, aiming at discovering the structural features leading to the preparation of effective dendritic drug delivery systems. Finally, it is crucial that all the beneficial properties of dendritic carriers should be fulfilled simultaneously.

Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Vogtle F, Gestermann S, Hesse R, et al. Functional dendrimers. Prog Polym Sci 2000;25:987-1041
   • **A classical review article on functional dendrimers.**
2. Frechet JMJ, Tomalia DA. Dendrimers and other dendritic polymers. Chichester: J Wiley & Sons; 2001
3. Newkome GR, Moorefield CN, Vogtle F. Dendrimers and dendrons. Concepts, syntheses, perspectives. Weinheim: Wiley-VCH; 2001
4. Svenson S, Tomalia DA. Dendrimers in biomedical applications – reflections on the field. Adv Drug Deliv Rev 2005;57:2106-29
5. Lee CC, MacKay JA, Frechet JMJ, Szoka FC. Designing dendrimers for biological applications. Nat Biotechnol 2005;23:1517-26
   •• **An excellent review article highlighting biological aspects on dendritic polymers.**
6. Newkome GR, Shreiner CD. Poly (amidoamine), polypropylenimine, and related dendrimers and dendrons possessing different 1 → 2 branching motifs: an overview of the divergent procedures. Polymer 2008;49:1-173
7. Tomalia DA, Frechet JMJ. Discovery of dendrimers and dendritic polymers: a brief historical perspective. J Polym Sci Part A: Polym Chem 2002;40:2719-28
8. Frechet JMJ, Hawker CJ, Gitsov I, Leon JW. Dendrimers and hyperbranched polymers: two families of three-dimensional macromolecules with similar but clearly distinct properties. J Macromol Sci Part A: Pure Appl Chem 1996;33:1399-425
9. Mammen M, Choi SK, Whitesides GM. Polyvalent interactions in biological systems: Implications for design and use of multivalent ligands and inhibitors. Angew Chem Int Ed 1998;37:2755-94
   •• **A seminal review article on multivalency.**
10. Kitov PI, Bundle DR. On the nature of the multivalency effect: a thermodynamic model. J Am Chem Soc 2003;125:16271-84
11. Badjic JD, Nelson A, Cantrill SJ, et al. Multivalency and cooperativity in supramolecular chemistry. Acc Chem Res 2005;38:723-32
12. Galeazzi S, Hermans TM, Paolino M, et al. Multivalent supramolecular dendrimer-based drugs. Biomacromolecules 2010;11:182-6
13. Rolland O, Turrin CO, Caminade AM, Majoral JP. Dendrimers and nanomedicine: Multivalency in action. New J Chem 2009;33:1809-24
14. Dhanikula RS, Hildgen P. Synthesis and evaluation of novel dendrimers with a hydrophilic interior as nanocarriers for drug delivery. Bioconjugate Chem 2006;17:29-41
15. D' Emanuele A, Attwood D. Dendrimer-drug interactions. Adv Drug Delivery Rev 2005;57:2147-62
   • **A very good review article on covalent and non-covalent interactions between dendritic polymers and drugs.**
16. Gajbhiye V, Kumar PV, Tekade RK, Jain NK. Pharmaceutical and biomedical potential of PEGylated dendrimers. Curr Pharm Des 2007;13:415-29
17. Paleos CM, Tsiourvas D, Sideratou Z. Molecular engineering of dendritic polymers and their application as drug and gene delivery systems. Mol Pharm 2007;4:169-88
   • **A well-organized review article on dendritic drug and gene delivery systems.**
18. Paleos CM, Tsiourvas D, Sideratou Z, Tziveleka L-A. Multifunctional dendritic drug delivery systems: Design, synthesis, controlled and triggered release. Curr Top Med Chem 2008;8:1204-24
19. Stiriba S-E, Frey H, Haag R. Dendritic polymers in biomedical applications: From potential to clinical use in diagnostics and therapy. Angew Chem Int Ed 2002;41:1329-34
20. Beezer AE, King ASH, Martin IK, et al. Dendrimers as potential drug carriers: Encapsulation of acidic hydrophobes

within water soluble PAMAM derivatives. Tetrahedron 2003;59:3873-80

21. Kolhe P, Misra E, Kannan RM, et al. Drug complexation, in vitro release and cellular entry of dendrimers and hyperbranched polymers. Int J Pharm 2003;259:143-60
22. Ambade AV, Savariar EN, Thayumanavan S. Dendrimeric micelles for controlled drug release and targeted delivery. Mol Pharm 2005;2:264-72
23. Gillies ER, Frechet JMJ. Dendrimers and dendritic polymers in drug delivery. Drug Discov Today 2005;10:35-43
24. Lim J, Simanek EE. Toward the next-generation drug delivery vehicle: Synthesis of a dendrimer with four orthogonally reactive groups. Mol Pharm 2005;2:273-7
25. Yang H, Kao WYJ. Dendrimers for pharmaceutical and biomedical applications. J Biomaterials Sci – Polymer edition 2006;17:3-19
26. Baker JR Jr. Dendrimer-based nanoparticles for cancer therapy. In Hematology, American Society of Hematology; 2009. p. 708-19
27. Choi SK, Thomas T, Li MH, et al. Light-controlled release of caged doxorubicin from folate receptor-targeting PAMAM dendrimer nanoconjugate. Chem Commun (Camb) 2010;46:2632-4
28. Samad A, Alam Mi, Saxena K. Dendrimers: a class of polymers in the nanotechnology for the delivery of active pharmaceuticals. Curr Pharm Des 2009;15:2958-69
29. Nanjwadea BK, Bechraa HM, Derkar GK, et al. Dendrimers: Emerging polymers for drug-delivery systems. Eur J Pharm Sci 2009;38:185-96

• **A very good condensed general review article on dendritic polymers.**

30. Svenson S. Dendrimers as versatile platform in drug delivery applications. Eur J Pharm Biopharm 2009;71:445-62

•• **An excellent review article highlighting the application of dendritic polymers encapsulating a diversity of drugs.**

31. McCarthy TD, Karellas P, Henderson SA, et al. Dendrimers as drugs: discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention. Mol Pharm 2005;2:312-18
32. Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. Adv Drug Delivery Rev 2004;56:1649-59
33. Fahmy TM, Fong PM, Goyal A, Saltzman WM. Targeted for drug delivery. Nanotoday 2005;8:18-26
34. Couvreur P, Gref R, Andrieux K, Maivy C. Nanotechnologies for drug delivery: Application to cancer and autoimmune diseases. Prog Solid State Chem 2006;34:231-5
35. Gu FX, Karnik R, Wang AZ, et al. Targeted nanoparticles for cancer therapy. Nano Today 2007;2:14-21
36. Beduneau A, Saulnier P, Benoit JP. Active targeting of brain tumors using nanocarriers. Biomaterials 2007;28:4947-67
37. Mishra B, Patel BB, Tiwari S. Colloidal nanocarriers: a review on formulation technology, types and applications toward targeted drug delivery. Nanomedicine-Nanotechnol Biol Med 2010;6:9-24
38. Tung C-H, Weissleder R. Arginine containing peptides as delivery vectors. Adv Drug Deliv Rev 2003;55:281-94
39. Futaki S. Membrane-permeable arginine-rich peptides and the translocation mechanisms. Adv Drug Delivery Rev 2005;57:547-58
40. Rothbard JB, Jessop TC, Wender PA. Adaptive translocation: the role of hydrogen bonding and membrane potential in the uptake of guanidinium-rich transporters into cells. Adv Drug Deliv Rev 2005;57:495-504
41. Wender PA, Kreider E, Pelkey ET, et al. Dendrimeric molecular transporters: Synthesis and evaluation of tunable polyguanidino dendrimers that facilitate cellular uptake. Org Lett 2005;7:4815-18
42. Foged C, Nielsen HM. Cell-penetrating peptides for drug delivery across membrane barriers. Expert Opin Drug Deliv 2008;5:105-17
43. Bonduelle CV, Gillies ER. Dendritic guanidines as efficient analogues of cell penetrating peptides. Pharmaceuticals 2010;3:636-66

•• **An excellent review article on guanidinium dendritic polymers as molecular transporters.**

44. Tsogas I, Tsiourvas D, Nounesis G, Paleos CM. Modeling cell membrane transport: Interaction of guanidinylated Poly(propylene imine) dendrimers with a liposomal membrane consisting of phosphate-based lipids. Langmuir 2006;22:11322-8
45. Tsogas I, Sideratou Z, Tsiourvas D, et al. Interactive transport of guanidinylated poly(propylene imine)-based dendrimers through liposomal and cellular membranes. ChemBioChem 2007;8:1865-76
46. Tsogas I, Theodossiou T, Sideratou Z, et al. Interaction and transport of poly(l-lysine) dendrigrafts through liposomal and cellular membranes: the role of generation and surface functionalization. Biomacromolecules 2007;8:3263-70
47. Liu MJ, Kono K, Frechet JMJ. Water-soluble dendrimer-poly(ethylene glycol) starlike conjugates as potential drug carriers. J Polym Sci Part A: Polym Chem 1999;37:3492-503
48. Liu MJ, Kono K, Frechet JMJ. Water-soluble dendritic unimolecular micelles: Their potential as drug delivery agents. J. Control Release 2000;65:121-31
49. Veronese FM. Peptide and protein PEGylation: a review of problems and solutions. Biomaterials 2001;22:405-17
50. Roberts MJ, Bentley MD, Harris JM. Chemistry for peptide and protein PEGylation. Adv Drug Deliv Rev 2002;54:459-76
51. Vandermeulen GWM, Klok H-A. Peptide/protein hybrid materials: enhanced control of structure and improved performance through conjugation of biological and synthetic polymers. Macromol Biosci 2004;4:383-98
52. Yang H, Morris JJ, Lopina ST. Polyethylene glycol-polyamidoamine dendritic micelle as solubility enhancer and the effect of the length of polyethylene glycol arms on the solubility of pyrene in water. J Colloid Interface Sci 2004;273:148-54
53. Vonarbourg A, Passirani C, Saulnier P, Benoit J-P. Parameters influencing the stealthiness of colloidal drug delivery systems. Biomaterials 2006;27:4356-73
54. Kumar PV, Agashe H, Dutta T, et al. PEGylated dendritic architecture for development of a prolonged drug delivery system for an antitubercular drug. Curr Drug Deliv 2007;4:11-9

55. Ryan SM, Mantovani G, Wang X, et al. Advances in PEGylation of important biotech molecules: Delivery aspects. Expert Opin Drug Deliv 2008;5:371-83

56. Kojima C, Regino C, Umeda Y, et al. Influence of dendrimer generation and polyethylene glycol length on the biodistribution of PEGylated dendrimers. Int J Pharm 2010;383:293-6

57. Lasic DD, Needham D. The 'Stealth' liposome: a prototypical biomaterial. Chem Rev 1995;95:2601-28

58. Needham D, Kim DHH. PEG-covered lipid surfaces: Bilayers and monolayers. Colloids Surf B 2000;18:183-95

59. Silvander M, Hansson P, Edwards K. Liposomal surface potential and bilayer packing as affected by PEG-lipid inclusion. Langmuir 2000;16:3696-702

60. Kaasgaard T, Mouritsen OG, Jorgensen K. Screening effect of PEG on avidin binding to liposome surface receptors. Int J Pharm 2001;214:63-5

61. Pantos A, Tsiourvas D, Sideratou Z, Paleos CM. Interactions of complementary PEGylated liposomes and characterization of the resulting aggregates. Langmuir 2004;20:6165-72

62. Paleos CM, Tsiourvas D, Sideratou Z, Tziveleka L-A. Acid- and salt-triggered multifunctional poly(propylene imine) dendrimer as a prospective drug delivery system. Biomacromolecules 2004;5:524-9

- **The first publication in which the issue of design and synthesis of a multifunctional dendrimeric polymer was set forward.**

63. Calderon M, Quadir MA, Strumia M, Haag R. Functional dendritic polymer architectures as stimuli-responsive nanocarriers. Biochimie 2010;92:1242-51

64. Paleos CM, Tziveleka L-A, Sideratou Z, Tsiourvas D. Gene delivery using functional dendritic polymers. Expert Opin Drug Deliv 2009;6:27-38

65. Malmstrom E, Johansson M, Hult A. Hyperbranched aliphatic polyesters. Macromolecules 1995;28:1698-703

66. Zagar E, Zigon M. Characterization of a commercial hyperbranched aliphatic polyester based on 2,2-bis(methylol) propionic acid. Macromolecules 2002;35:9913-25

67. Zagar E, Zigon M, Podzimek S. Characterization of commercial aliphatic hyperbranched polyesters. Polymer 2006;47:166-75

68. Gupta U, Agashe HB, Asthana A, Jain NK. A review of in vitro-in vivo investigations on dendrimers: The novel nanoscopic drug carriers. Nanomed Nanotechnol Biol Med 2006;2:66-73

69. Malik N, Wiwattanapatapee R, Klopsch R, et al. Dendrimers: relationship between structure and biocompatibility in vitro, and preliminary studies on the biodistribution of 125I-labelled polyamidoamine dendrimers in vivo. J Control Release 2000;65:133-48

70. Jain K, Kesharwani P, Gupta U, Jain NK. Dendrimer toxicity: Let's meet the challenge. Int J Pharm 2010;394:122-42

71. Sideratou Z, Tsiourvas D, Paleos CM. Solubilization and release properties of PEGylated diaminobutane poly (propylene imine) dendrimers. J Colloid Interface Sci 2001;242:272-6

72. Guyton AC, Hall JE. The body fluid compartments: extracellular and intracellular fluids; interstitial fluid and edema. In textbook of medical physiology, Saunders WB Co, Philadelphia; 2000. p. 264-7

73. Yellepeddi VK, Kumar A, Palakurthi S. Surface modified poly(amido)amine dendrimers as diverse nanomolecules for biomedical applications. Expert Opin Drug Deliv 2009;6:835-50

74. Majoros IJ, Thomas TP, Mehta CB, Baker JR Jr. Poly(amidoamine) dendrimer-based multifunctional engineered nanodevice for cancer therapy. J Med Chem 2005;48:5892-9

- **A systematic work illustrating multifunctionalization methodology with covalently attached drug.**

75. Thomas TP, Majoros IJ, Kotlyar A, et al. Targeting and inhibition of cell growth by an engineered dendritic nanodevice. J Med Chem 2005;48:3729-35

76. Kukowska-Latallo JF, Candido KA, Cao ZY, et al. Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. Cancer Res 2005;65:5317-24

77. Low PS, Henne WA, Doorneweerd DD. Discovery and development of folic-acid-based receptor targeting for imaging and therapy of cancer and inflammatory diseases. Acc Chem Res 2008;41:120-9

78. Zhao X, Li H, Lee RJ. Targeted drug delivery via folate receptors. Expert Opin Drug Deliv 2008;5:309-19

79. Low PS, Henne WA, Doorneweerd DD. Discovery and development of folic-acid-based receptor targeting for imaging and therapy of cancer and inflammatory diseases. Acc Chem Res 2008;41:120-9

80. Gabizon A, Shmeeda H, Horowitz AT, Zalipsky S. Tumor cell targeting of liposome-entrapped drugs with phospholipid-anchored folic acid-PEG conjugates. Drug Deliv Rev 2004;56:1177-92

81. Zhai G, Wu J, Xiang G, et al. Preparation, characterization and pharmacokinetics of folate receptor-targeted liposomes for docetaxel delivery. J Nanosci Nanotechnol 2009;9:2155-61

82. Kono K, Liu MJ, Frechet JMJ. Design of dendritic macromolecules containing folate or methotrexate residues. Bioconjugate Chem 1999;10:1115-21

83. Konda SD, Aref M, Wang S, et al. Specific targeting of folate-dendrimer MRI contrast agents to the high affinity folate receptor expressed in ovarian tumor xenografts. Magn Reson Mater Phys Biol Med 2001;12:104-13

84. Sideratou Z, Kontoyianni C, Drossopoulou GI, Paleos CM. Synthesis of a folate functionalized PEGylated poly (propylene imine) dendrimer as prospective targeted drug delivery system. Bioorg Med Chem Lett 2010;20:6513-17

85. Prabaharan M, Grailer JJ, Pilla S, et al. Folate-conjugated amphiphilic hyperbranched block copolymers based on Boltorn® H40, poly(l-lactide) and poly(ethylene glycol) for tumor-targeted drug delivery. Biomaterials 2009;30:3009-19

- **Detailed and systematic work based on multifunctional hyperbranched aliphatic polyesters.**

86. Sideratou Z, Tsiourvas D, Theodossiou T, et al. Synthesis and characterization of multifunctional hyperbranched polyesters as prospective contrast agents for targeted MRI. Bioorg Med Chem Lett 2010;20:4177-81

87. Dauty E, Remy JS, Zuber G, Behr JP. Intracellular delivery of nanometric

DNA particles via the folate receptor. Bioconjugate Chem 2002;13:831-9

88. Aronov O, Horowitz AT, Gabizon A, Gibson D. Folate-targeted PEG as a potential carrier for carboplatin analogs. Synthesis and in vitro studies. Bioconjugate Chem 2003;14:563-74

89. Zuber G, Zammut-Italiano L, Dauty E, Behr JP. Targeted gene delivery to cancer cells: directed assembly of nanometric dna particles coated with folic acid. Angew Chem Int Ed 2003;42:2666-9

90. Yoo HS, Park TG. Folate receptor targeted biodegradable polymeric doxorubicin micelles. J Control Release 2004;96:273-83

91. Kim SH, Jeong JH, Joe CO, Park TG. Folate receptor mediated intracellular protein delivery using PLL-PEG-FOL conjugate. J. Control Release 2005;103:625-34

92. Licciardi M, Tang Y, Billingham NC, Armes SP. Synthesis of novel folic acid-functionalized biocompatible block copolymers by atom transfer radical polymerization for gene delivery and encapsulation of hydrophobic drugs. Biomacromolecules 2005;6:1085-96

93. Wang CH, Hsiue GH. Polymer-DNA hybrid nanoparticles based on folate-polyethylenimine-block-poly(L-lactide). Bioconjugate Chem 2005;16:391-6

94. Liu Y, Li K, Pan J, et al. Folic acid conjugated nanoparticles of mixed lipid monolayer shell and biodegradable polymer core for targeted delivery of Docetaxel. Biomaterials 2010;31:330-8

95. Majoros IJ, Myc A, Thomas T, et al. PAMAM dendrimer-based multifunctional conjugate for cancer therapy: synthesis, characterization, and functionality. Biomacromolecules 2006;7:572-9

96. Shukla R, Thomas TP, Peters J, et al. Tumor angiogenic vasculature targeting with PAMAM dendrimer-RGD conjugates. Chem Commun 2005;14(46):5739-41

97. Hill E, Shukla R, Park SS, Baker JR Jr. Synthetic PAMAM-RGD conjugates target and bind to odontoblast-like MDPC 23 cells and the predentin in tooth organ cultures. Bioconjugate Chem 2007;18:1756-62

98. Lesniak WG, Kariapper MST, Nair BM, et al. Synthesis and characterization of PAMAM dendrimer-based multifunctional nanodevices for targeting alphaVbeta3 integrins. Bioconjugate Chem 2007;18:1148-54

- **A systematic work describing the synthesis and characterization of multifunctional dendrimeric nanodevices.**

99. Shukla S, Wu G, Chatterjee M, et al. Synthesis and biological evaluation of folate receptor-targeted boronated PAMAM dendrimers as potential agents for neutron capture therapy. Bioconjugate Chem 2003;14:158-67

- **A systematic work illustrating that the optimal modification of a drug delivery system is dependent on a variety of factors.**

100. Singh P, Gupta U, Asthana A, et al. Folate and Folate-PEG-PAMAM dendrimers: synthesis, characterization, and targeted anticancer drug delivery potential in tumor bearing mice. Bioconjugate Chem 2008;19:2239-52

101. Shukla R, Thomas TP, Desai AM, et al. HER2 specific delivery of methotrexate by dendrimer conjugated anti-HER2 mAb. Nanotechnol 2008;19/295102:1-7;doi:10.1088/0957-4484/19/29/295102

102. Calderon M, Quadir MA, Sharma SK, Haag R. Dendritic polyglycerols for biomedical applications. Adv Mater 2010;22:190-218

103. Wilms D, Stiriba SE, Frey H. Hyperbranched polyglycerols: from the controlled synthesis of biocompatible polyether polyols to multipurpose applications. Acc Chem Res 2010;43:129-41

104. Tziveleka L-A, Kontoyianni C, Sideratou Z, et al. C. M. Novel functional hyperbranched polyether polyols as prospected drug delivery systems. Macromol Biosci 2006;6:161-9

105. Wiseman H. Tamoxifen – new membrane-mediated mechanisms of action and therapeutic advances. Trends Pharmacol Sci 1994;15:83-9

106. Mocanu EV, Harrison RF. Tamoxifen in gynaecology. Rev Gynaecol Pract 2004;4:37-45

107. Arce E, Nieto PM, Diaz V, et al. Glycodendritic structures based on Boltorn hyperbranched polymers and their interactions with Lens culinaris lectin. Bioconjugate Chem 2003;14:817-23

108. Kontoyianni C, Sideratou Z, Theodossiou T, et al. A novel micellar PEGylated hyperbranched polyester as prospective drug delivery system for paclitaxel. Macromol Biosci 2008;8:871-81

109. Chen S, Zhang X-Z, Cheng S-X, et al. Functionalized amphiphilic hyperbranched polymers for targeted drug delivery. Biomacromolecules 2008;9:2578-85

- **A systematic work on folate functionalized hyperbranched aliphatic polyester.**

110. Theodossiou TA, Pantos A, Tsogas I, Paleos CM. Guanidinylated dendritic molecular transporters: prospective drug delivery systems and application in cell transfection. ChemMedChem 2008;3:1635-43

111. Albertazzi L, Serresi M, Albanese A, Beltram F. Dendrimer internalization and intracellular trafficking in living cells. Mol Pharmaceutics 2010;7:680-8

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