Dendritic Polymers in Biomedical Applications: From Potential to Clinical Use in Diagnostics and Therapy

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Dendrimers are characterized by a combination of high end-group functionality and a compact, precisely defined molecular structure. These characteristics can be used in biomedical applications, for example, for the amplification or multiplication of effects on a molecular level, or to create extremely high local concentrations of drugs, molecular labels, or probe moieties. A brief summary of the current state of the art in the field is given, and focuses on the application of dendrimers both in diagnostics as well as in therapy. In diagnostics, dendrimers that bear Gd^{III} complexes are used as contrast agents in magnetic resonance imaging. DNA dendrimers have potential for routine use in high-throughput functional genomic analysis, as well as for DNA biosensors. Dendrimers are also being investigated for therapeutics, for example, as carriers for controlled drug delivery, in gene transfection, as well as in boron neutron-capture therapy. Furthermore, the antimicrobial activity of dendrimers has been studied.

1. Introduction

Dendrimers are perfectly cascade-branched, highly defined macromolecules, characterized by a combination of high end-group functionality and compact molecular structures. Since the seminal works in this field,[1] a large number of dendrimer structures have been developed and has become a subject of intense interdisciplinary research efforts, bringing together scientists from entirely different areas. The perfectly branched, monodisperse structure of dendrimers have established them as valuable model compounds to study fundamental electrochemical, photophysical, and supramolecular properties.[2]

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Based on the rapid advances in this area over the past 15 years, the quest for practical applications for dendrimers is becoming increasingly intense. Owing to the tedious stepwise synthetic construction of dendrimers, future applications will have to be highly specific and a precise nanoengineered structure will be a crucial prerequisite for the targeted application. Bulk applications, for example, as processing additives, specialty coatings, or as rheology modifiers will be realized with random cascade-branched hyperbranched materials, which are structurally less defined but can be prepared in a single polymerization step.[3]

The structural precision of dendrimers has motivated numerous studies aimed at biomedical applications, for example, the amplification of molecular effects or the creation of extremely high local concentrations of drugs, molecular labels, or probe moieties. In this brief account, we summarize the current state of the art in the field of medical application of dendrimers and highlight the most promising recent developments.

2. Medical Diagnostics

Magnetic resonance imaging (MRI) is a powerful technique in modern medical diagnostics[4] and is used to visualize soft tissue, for example, organs and blood vessels. MRI is based on subtle differences of environment-sensitive ^{1}H NMR resonances (mainly of H_2O) in living systems, enhanced by the administration of a paramagnetic contrast agent. The contrast agents (most widely approved for clinical use are Gd^{III} complexes such as $[Gd(DTPA)]^{2-}$, $[Gd(DOTA)]^-$ (Dotarem), [Gd(HP-do3a)] (Prohance)[5]) serve to modify the T_1-relaxation rate of protons in the H_2O molecules and thus the quality of visualization.[6a] Crucial properties of Gd^{III} complex based MRI contrast agents include good biocompatibility of the chelating ligands, lower toxicity than the corresponding metal salts, and high relaxivity.[7] Furthermore, excellent solubility and a low dose requirement for diagnosis ($0.1-0.001\ mg\,kg^{-1}$) as well as good excretion of the complexed metal ions from the biological system in combination with high thermodynamic and kinetic stability are important requirements for MRI contrast agents. A major drawback of currently used low molecular weight contrast agents is their rapid diffusion into the extracellular matrix and consequently fast elimination from the blood circuit. This necessitates

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relatively high doses and injection rates. Attachment of low molecular weight Gd^{III} chelates to linear macromolecules such as polylysine and poly(ethylene glycol) (PEG) and also to polysaccharides has been explored. However, the relaxivities of these polymers were low and independent of temperature at all field strengths, which was ascribed to the flexibility and segmental mobility within the polymer chain or the spacer arms.[6b]

In pioneering work, Wiener et al. reported dendrimer-based Gd^{III} chelates that consist of polyamidoamine (PAMAM) dendrimers of generations 2 and 6 ([G2] and [G6]), which possess 12 and 192 reactive terminal amines, respectively, conjugated to the chelating ligand 2-(4-isothiocyanatobenzyl)-6-methyldiethylenetriaminepentaacetic acid (dtpa) through a thiourea linkage (Figure 1).[8] In vivo experiments

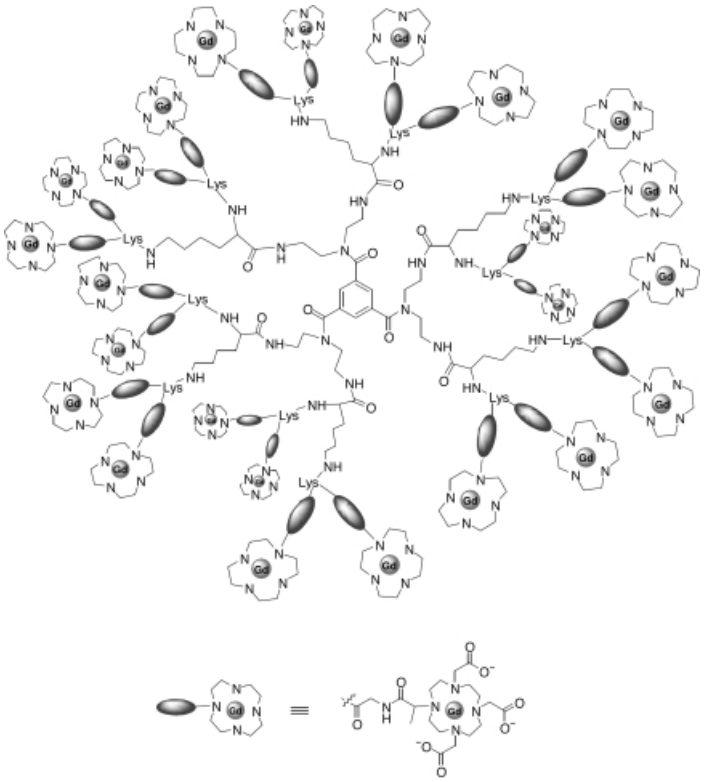

Figure 1. Structure of the dendritic Gd complex gadomer 17,[11] which is employed in MRI.

on rabbits showed excellent MRI images of blood vessels and long blood circulation times (>100 min) upon intravenous injection of larger dendrimer conjugates, for example, [G6]-PAMAM-TU-Gd^{III}-dtpa. These results are further confirmed by recent studies in which even larger PAMAM dendrimers ([G9] and [G10]) were used.[9a] In this case, although a saturation of relaxivity was observed per Gd ion with dendrimer generation, the total molecular relaxivity increased strongly. A linear increase in the relaxivity as a function of molecular weight was also found in another study, which demonstrated prolonged half-lives of dendrimer polychelates from [G3] to [G5] in blood. These dendrimer polychelates were exploited for high-quality MR angiography (MRA) images up to 60 minutes post injection. Another feature of these MRI dendrimers is the retention of up to 40% of larger derivatives in the liver seven days after administration. Upon incorporation of PEG subunits into Gd^{III}-chelated PAMAM dendrimers, liver retention after seven days decreased to 1–7%.[9b] Further studies that confirm these trends have been reported, and emphasize the importance of the lowered water exchange rates for PAMAM-based Gd contrast agents.[10]

The potential of this application area is mirrored in the intense efforts by pharmaceutical companies to introduce such contrast agents. For example, the 24-Gd-dtpa cascade polymer and gadomer 17 (Figure 1) have been introduced.[11] These compounds consist of a trimesic acid central moiety with [G2]-polylysine dendron substituents that bear 24 dtpa and 24 do3a chelating groups, respectively.

Gadomer 17 is suitable for blood-pool imaging (Figure 2), similar to the known linear Gd-dtpa-polylysine, but shows a superior elimination rate, presumably as a result of the globular nature of the dendrimer derivative.[12a] This

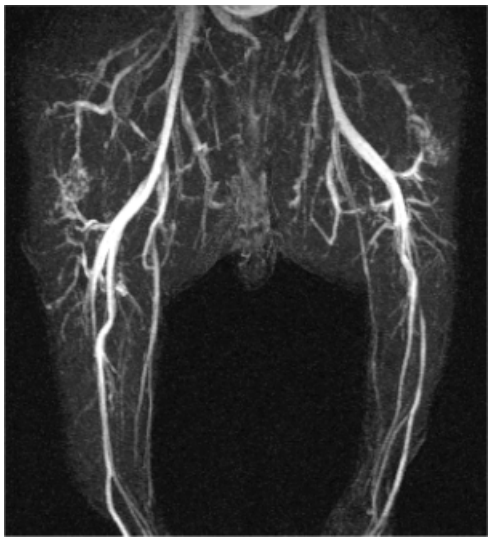

Figure 2. Contrast-enhanced magnetic resonance image of the peripheral blood vessels of a dog after injection with gadomer 17 (dose: 50 μmol per kg body weight; ≈3 min post injection).

dendrimer-based contrast agent also extends the temporal window of dynamic contrast-enhanced MRI,[12] as confirmed in several recent pharmacokinetic studies of gadomer 17 and related compounds.[13] Clinical trials (phase I) are currently underway. A recent alternative approach is based on the utilization of a glycodendrimer framework that is anchored to dtpa as functionalized ligands and in which the Gd^{III} ions are coordinated at the center of the dendrimers.[14]

Another interesting field is the investigation of DNA dendrimers,[15] which are constructed by sequential hybridizations of partially complementary heteroduplexes ("DNA monomers"). After each generation, the structure is fixed by a "crosslinking" step. DNA dendrimers with up to two million terminal oligonucleotide strands have been reported. These DNA dendrimers (commercialized as 3DNA-technology[16]) offer numerous possibilities for oligonucleotide detection,

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since the terminal polynucleotide strands can be varied and also be labeled with hundreds of radioactive or fluorescent labels (Figure 3), thus leading to enhanced detection sensitivity. Such DNA dendrimers have been used to identify oligonucleotides directly in chick embryo tissue and also as a specific probe to detect the Epstein–Barr virus in post-transplant patients by recognition of specific RNA strands.[17] Polynucleotide dendrimers with fluorescent labels have also been used for signal amplification in DNA microarray technology, and are promising candidates for routine use in high-throughput functional genomic analysis.[18]

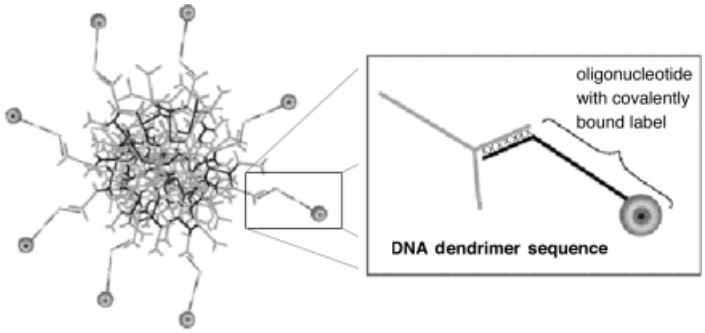

Figure 3. Multifunctional DNA dendrimers with multiple fluorescent labels are used for DNA hybridization and sensor applications.[16]

Biosensors for DNA hybridization hold great promise for the rapid diagnosis of genetic and pathogenetic diseases. Such sensors rely on the immobilization of single-stranded oligonucleotide probes that selectively recognize their complementary target sequence through hybridization. Biosensors based on a quartz-crystal microbalance and on DNA dendrimers make use of the increased hybridization capacity and detection capability of polyfunctional DNA molecules and have been shown to exhibit significantly enhanced sensitivity in combination with very low detection limits.[19]

In a similar manner, radiolabeled monoclonal antibodies with high specific activity have been prepared by attachment of PAMAM dendrimers loaded with ^{111}In or ^{153}Gd complexes.[20] A further concept for DNA detection was introduced by Fréchet and co-workers and is based on the antenna effect of a dendronized polymer with a single DNA strand at the core. The dendritic architecture is equipped with dye molecules on each dendritic arm, which transfer their energy to a complementary fluorescently labeled DNA strand upon hybridization.[21]

3. *Therapeutic Applications*

The most active area in dendrimer-based therapeutics is gene transfection by dendrimers as nonviral vectors. Polyamine dendrimers such as PAMAM and poly(propylene imine) (PPI) are commercially available and their complexation to DNA molecules has been studied.[22] In particular, their tendency to form compact polycations under physiological conditions (pH 7.4) has been used in gene therapy.[23] For instance, PolyFect is a commercially available in vitro transfection agent and is based on a dendritic PAMAM structure obtained by partial chemical hydrolysis of PAMAM dendrimers.[23b,24] Under physiological conditions, the branches bear positively charged ammonium end groups, which can interact with negatively charged phosphate groups of nucleic acids. The PolyFect reagent assembles DNA into compact toroidal structures, thus optimizing the entry of the DNA into cells. Once inside the cell, the dendrimers buffer the lysosomes after fusion with the endosomes (Figure 4). This leads to pH-controlled inhibition of lysosomal nucleases, thus ensuring the high stability of PolyFect–DNA complexes. However, the final penetration into the nucleus of the cell and the elimination of these cationic macromolecules are not fully understood yet.

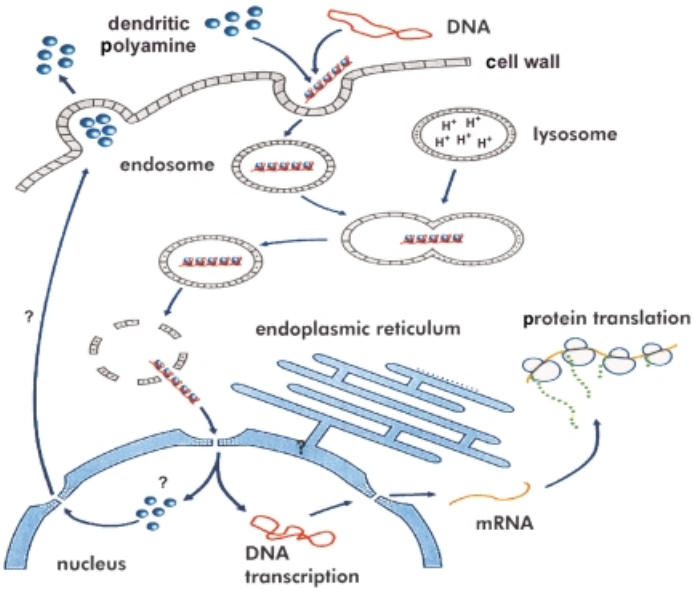

Figure 4. Hypothetical mechanism of DNA transfection with cationic dendrimers.[24,25]

Interestingly, the perfect dendritic structure (DB = 100 %) is a less effective transfection agent than the partially degraded (hyperbranched) structure that results from the partial cleavage (hydrolysis) of the branches.[23] This observation is also supported by the fact that the commercially available poly(ethylene imine), which is a hyperbranched polymer (DB ≈ 65 %), is also an efficient transfection agent.[25] However, it is still not clear whether these dendritic polyamine transfection agents can be employed in vivo, and further toxicity studies are required.[26] Only in cancer therapy with an Epstein–Barr virus-based plasmid vector complexed to a PAMAM dendrimer was a significant prolongation of survival observed in an animal model.[27]

Another area of current dendrimer research efforts is controlled drug delivery.[28] Topological encapsulation within dendrimers has been suggested as a means to transport drugs (Figure 5). Meijer and coworkers first reported the encapsulation of polar guest molecules into a dendritic core-shell architecture with a dense apolar shell, the so-called "dendritic box".[29] Depending on their molecular weight, guest molecules can be encapsulated and selectively released from this dendritic host.[30] The problem of this and similar systems[31] for drug delivery, however, is their high hydrophobicity. More recently, water-soluble drug-delivery systems with reversed

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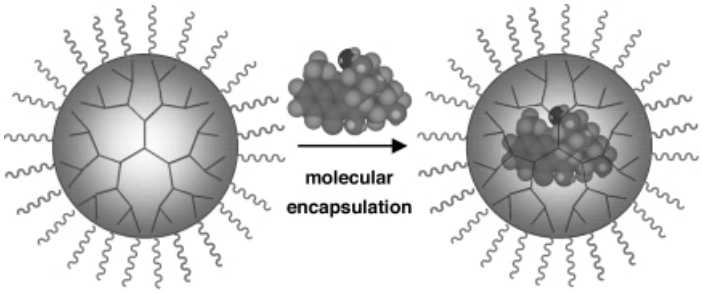

Figure 5. Schematic representation of the encapsulation of the cytostatic adriamycin in a PAMAM dendrimer with PEG end groups.

core-shell polarity based on dendritic polymers have been developed.[32] Thus antitumor cytostatics such as adriamycin and methotrexate have been encapsulated in such dendrimers (Figure 5).[32a]

Owing to their high surface functionality, dendritic polymers, particularly dendritic glycopolymers, have also been considered as scaffolds for polyvalent drugs.[33] Various dendrimer structures with multiple carbohydrate moieties attached at the periphery have been investigated. The so-called "sugar-coating" of dendrimers serve as multivalent recognition structures for sugar-binding proteins, for example, lectins.[34] For example, poly(L-lysine) dendrimers with two to 16 sialic acid units show enhanced binding affinities in the *Limax flavus* lectin precipitation assay and in the hemaglutination assay of erythrocytes, which tests for the influenza A virus.[35] In these systems, four to six sialic acid residues appeared to be the optimal number of functional groups for the most potent antiviral properties (ca. 200-fold increase in binding affinity relative to the monovalent ligand), probably as a result of the restricted accessibility of the end groups of higher functional dendrimers. Recently, a pentavalent starlike carbohydrate ligand has been reported to fit exactly into the binding pocket of the five subunits of the Shiga-like bacteria toxin (a close analogue of the cholera toxin).[36] The binding affinity of this pentavalent ligand is 10^7 times higher than that of the monovalent ligand.

Boron neutron-capture therapy (BNCT) is yet another therapeutic application of multifunctional dendritic macromolecules and has been used in the treatment of cancer. This method is based on the $^{10}B(n,\alpha)^7Li^{3+}$ reaction. If ^{10}B can be delivered in sufficient quantities ($>10^9$ ^{10}B atoms) to the tumor tissue, subsequent irradiation with thermal or epithermal neutrons produces highly energetic α particles and $^7Li^{3+}$ ions that damage the mitotic potential of the tumor cells.[37] A central issue for BNCT is the precise localization of the boron-containing therapeutics in the tumor. BNCT has been improved by using caged sulfhydryl borohydrides such as $Na_2B_{12}H_{11}SH$. For applications in BNCT, the reagents must have a tumor/blood partition ratio at least greater than 1 (and preferably higher during the radiation process) and the compounds used should have a low toxicity.[37]

The incorporation of boron into polymer scaffolds represents a promising concept to enhance the activity of boron compounds in order to target a large quantity of ^{10}B in the tumor tissue. PAMAM dendrimers have been used to prepare boron-containing immunoconjugates to give high concentrations of boron. [G2] and [G4] PAMAM dendrimers were linked to the isocyanatoborane $Na(CH_3)_3NB_{10}H_8NCO$. This compound was attached to monoclonal antibodies to give an immunoconjugate for selective tumor targeting (Figure 6). The immunoconjugate retained a high level of immunoreactivity, and had a strong propensity to localize in the liver and spleen.[38]

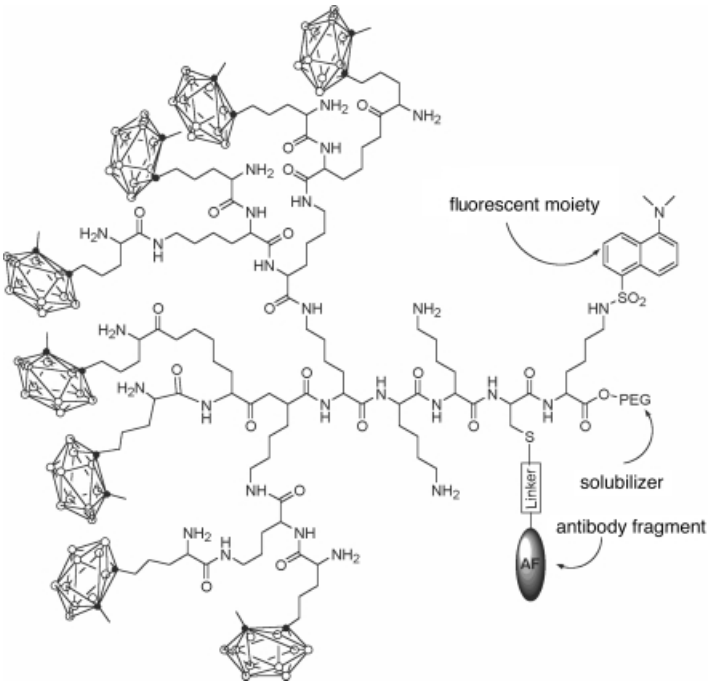

Figure 6. Multifunctional dendrimer conjugate linked to a specific antibody fragment, used in boron neutron-capture therapy (BNCT).

Tests of polylysine dendrons with 80 terminal boron atoms and linked to antibody fragments also revealed promising perspectives for applications in BNCT.[39] To achieve more effective tumor targeting with antibodies, the Epidermal Growth Factor (EGF), a single small polypeptide, was attached to a boronated [G4]-PAMAM dendrimer. In vitro studies with these bioconjugates indicated a specificity for the EGF receptor (EGFR) in brain tumors.[40]

Dendrimers with antimicrobial properties represent another facet of dendrimers in medicine. The enhanced interaction of dendritic polycations with cell walls can also be used to generate antimicrobial activity. Quaternary ammonium compounds (QACs) are widely used as disinfectants. Permanent polycationic structures have also been prepared by quaternization of polyamine dendrimers, for example, PPI dendrimers functionalized with dimethyldodecylammonium groups show strong antimicrobial activity.[41] Bioluminescence studies confirmed that these dendrimer biocides with 16 QAC groups on the surface are two orders of magnitude more potent against the Gram-negative bacteria, *Escherichia coli* than the monofunctional counterparts, and are also very effective against Gram-positive bacteria, such as *Staphylococus aureus*.[42] The polycationic structure facilitates the initial adsorption of the dendrimers at the cell wall and increases the permeability of cells towards foreign molecules. It was demonstrated that the

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antimicrobial activity depends on the dendrimer generation, the length of the hydrophobic group, and the counterion present.[42]

Dendrimer–silver complexes and nanocomposites prepared on the basis of dendrimer templates show greater in vitro activity than silver nitrate against various bacteria.[43] In contrast to silver nitrate solutions, the dendrimer–silver nanocomposites do not penetrate through membranes and might be useful for the antimicrobial treatment of topical wounds, such as burn wounds.

4. Conclusion

It is clear from the examples presented in this highlight that the unique structural features of dendrimer architectures have been used in medical applications in recent years. An increasing number of publications in medical journals have demonstrated the awareness and growing interest in these macromolecules in the medical community, both for advanced diagnostic tools as well as for therapeutic purposes. Progress has been made in MRI as well as in dendritic DNA for biosensor technology. In comparison, therapeutic application lags behind; however, efforts devoted to in vivo applications are long-term projects that require extensive testing and screening procedures with respect to toxicity, immunogenicity, and biodistribution.[44]

A central issue in this field will be a critical discussion concerning structural requirements: since most applications rely mainly on the high end group functionality of dendrimers and *not* on their unique structural perfection, randomly branched dendritic represent a promising alternative.[3] Although it has been demonstrated that the structural perfection of dendrimers is an important prerequisite for magnetic resonance imaging, a monodisperse structure may not be required for numerous other applications. Recently, novel biodegradable and biocompatible hyperbranched polymers that offer additional potential in this rapidly growing field have been developed, for example, polyaminoesters[45] and polyglycerol.[46] Since dendritic molecules have "infected" the medical community, further interdisciplinary research efforts and eventually new strategies for medical diagnostics and therapy are guaranteed. Since the submission of this Minireview further work on this theme has been reported.[47]

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