DENDRIMERS AS NANOCARRIERS

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CONTENTS

ABSTRACT

Dendrimers are mono disperse macromolecules with a large number of surface functionalities and can be synthesized with great structural control. Research is ongoing about functionalizing dendrimers as nanocarriers. In this paper, different approaches for functionalizing dendrimers as nanocarriers are evaluated and also compared with other potential nanocarriers. The pH responsiveness of poly(amidoamine)s is found to be promising. On the other hand, targeting via an external stimulus such as electromagnetic radiation is far from any application, as not many photochemical reactions of dendrimers have been found yet and wavelengths used are strongly absorbed by human tissue. An interesting feature, however, is the ability to tune a dendrimers photo reactive wavelength by tuning its external framework. Targeting via an internal stimulus such as an enzyme, however, does have a high potential for nanodrug applications. However, such systems are highly specific for particular tumors and specific enzymatic activity and can not be used for several different types of tumors.

1 INTRODUCTION

Specific cell targeting with high efficiency is one of the main interests in the medical industry. However, pure technical processes of tackling tumors, such as proton beam therapy or X-ray therapy, often cannot be as thorough as needed due to technological limitations on resolution. On the other hand, drug targeting can, if the drugs efficacy is high enough, be a superior method for tackling tumors or the destruction of diseased cells. Promising results have been obtained using nano medicine [1], medicine utilizing nanosized molecules and systems, for modulating the pharmacokinetic (PK) and pharmacodynamic (PD) profile of a therapeutic substances to improve its efficacy and/or reduce its toxicity. Therefore, a shift from traditional small pharmaceutical molecule drugs to nanosized drug investigations has taken place as these larger drugs benefit from the ability to engineer and enhance more favorable PK and PD properties. Nanosized systems can act as nanocarriers for traditionally sized drugs, DNA/RNA and proteins or as intrinsically active nanoscale drugs[2],[3]. Size, shape, surface chemistry, flexibility/rigidity, architecture and elemental composition are several powerful parameters for controlling a nano medicine's functionality.

An effective drug is soluble and stable, reaches high concentrations at the target site, is able to cross the cellular membrane, localize the correct cellular membrane, extravasate into and distribute across the interstitium. Nanosized systems can help to obtain such qualities. These qualities are used in two ways to increase target site accumulation and decrease off-target toxicity: by passive and active targeting both of which will be discussed in this article. In the field of nanomedicine, nanosystems are classed in several categories such as colloids, micelles, protein assemblies, DNA/RNA and dendrimers, each with specific characteristics and approaches to improve drug efficacy[5].[6]. The latter category will be the main topic of this paper as several ways to functionalize dendrimers for practical implementation are compared.

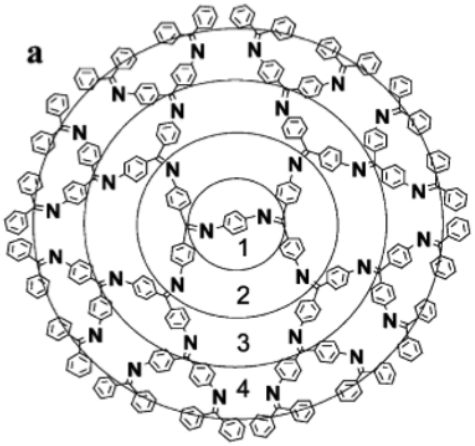

Figure 1: Dendritic Polyphenylazomethine, as an example of a dendrimer, the different generation layers are marked 1,2,3 and 4. [3]

2 DENDRIMER STRUCTURE

Dendrimers are nanoscaled macromolecules synthesized based on an iterative three-dimensional, as opposed to one-dimensional in polymers, covalent bonding process. While polymers form many hyperbranches resulting in species of different molecular weights, dendrimers, on the other hand, are mono disperse. Dendrimers built up around a central core in branched layers of monomers called dendrons[7]. In each iterative step a new layer is added to the macromolecules, allowing a precise control of its size and weight and ensuring discreteness. Growth of dendrimers is therefore often called generation by generation growth. Present at each branch-end are peripheral surface groups which can be modified to capture target molecules, attach molecules or improve hydrophobicity/hydrophilicity. Current investigations focus on synthesis and modification of dendrimers to function as a nanocarrier but also on stimuli-sensitive dendrimers [8],[9] a development which can possibly expand the scope of dendrimers by usage of a physical parameter to switch the dendrimer's characteristics.

3 APPLICATIONS

A broad range of applications resulting from research on dendrimers can be thought of. Applications as nano medicine, host-guest chemistry, catalysis, sensor technology or as new material, are investigated. For instance, Duan et al. have used the self assembly of fluorinated tapered dendrons to drive the formation of liquid crystals. By attaching donor or accepting groups to the apex of the dendrons, a supramolecular column of pi-stacks of donors, acceptors or donor-acceptor complexes was synthesized, resulting in a high mobility material suitable for electronics or optoelectronics applications. [11] Besides a function as building blocks for a supramolecular material, dendrimers as single molecules on itself are, due to their high number of functional surface groups, useful as sensors. Vögtle et al. have used poly (propylene amine) dendrimers with dansyl units at the periphery as a fluorescent chemosensor. Aliphatic amine groups present in the interior of the dendrimer coordinate metal ions leading to quenching of the highly fluorescent dansyl groups. Due to the large number of surface dansyl groups, a dendrimer sensor displays much stronger quenching than traditional fluorescent chemosensors. [12] Other research groups made use of the large number of surface functionalities of dendrimers to act as catalysts. Groups have used peripheral functional groups to catalyze several chemical reactions such as the Kharasch addition or the Heck reaction. [13] However, not only for catalysis but also for host-guest chemistry dendrimers proved to be good candidates. Meijer et al. have shown the topological trapping by core-shell molecules of dendrimers, by creating a flexible core which allows guests molecules to be trapped and around this core a rigid shell preventing them to diffuse out. These so-called dendritic boxes can only release their guests when the rigid shell is destroyed. [14] The diverse possibilities with dendritic boxes is big, but as one can imagine, the overall opportunities with dendrimers is enormous. In this paper, therefore, the focus will be on dendrimers as nanocarriers.

4 CURRENT CLINICAL STATUS OF DENDRIMERS

Type of nanostructure for confirmed and likely nanomedicine applications and products, by developmental status

Nanocomponent	Investigational			Commercial		
	Therapeutic	Device	Total	Therapeutic	Device	Total
Hard NP	3	12	15	0	28	28
Nanodispersion	5	0	5	1	1	2
Polymeric NP	23	0	23	9	0	9
Protein NP	4	0	4	2	0	2
Liposome	53	0	53	7	1	8
Emulsion	18	1	19	9	0	9
Micelle	8	0	8	3	1	4
Dendrimer / Fleximer	2	2	4	0	3	3
Virosome	6	0	6	2	0	2
Nanocomposite	0	0	0	0	18	18
NP Coating	0	2	2	0	6	6
Nanoporous Material	0	3	3	0	2	2
Nanopatterned	0	2	2	0	2	2
Quantum Dot	0	1	1	0	4	4
Fullerene	0	1	1	0	0	0
Hydrogel	0	0	0	0	1	1
Carbon Nanotube	0	1	1	0	0	0
Totals	122	25	147	33	67	100

Figure 2: The types of nanostructure materials used in nanomedicine by Arthur et al.[18]

Many review articles on nanodrugs speak of an emerging field in the pharmaceutical industry. As Sanjeeh et al. states:"The emergence of nanotechnology is likely to have a significant impact on the drug-delivery sector and nanoparticles (NPs) are at the leading edge, with many potential applications in clinical medicine and research." [17] Also, Arthur et al. predict: "Developments in nanomedicine are expected to provide solutions to many of modern medicine's unsolved problems"[18] . However, when looking at the current investigations in nanomedicine, the focus seems to be primarily on other nanosystems than dendrimers.

As shown in Figure 2, the number of dendrimer clinical studies is low compared to liposomes and polymerics, also the amount of commercially available products comprised of dendrimers is lower than liposomes, polymerics and inorganic nanoparticles. An important difference of dendrimers with the other nanoparticles, however, is that they are harder to obtain for clinical studies. An inorganic nanoparticle, for instance, is easy to obtain commercially whereas the synthesis of a dendrimer requires the expertise of a synthetic chemist. Dendrimers are therefore currently mainly studied within academic labs and not yet much used for clinical studies.

In this paper the aim is to assess different approaches for functionalizing dendrimers as nanocarriers, making use of their monodispersion, large number of surface functionalities and great structural control. The synthesis, current and future functionalities and applications of dendrimers in nanomedicine will be discussed. The aim is to explain the chemistry/physics behind the dendrimer's functionalities and their potential role as nanodrugs, so that one can understand the current research performed in this field and critically assess different approaches towards dendrimers as nanocarriers. Also, briefly, other types of nanosystems will be evaluated in order to make a comparative study.

5 SYNTHESIS

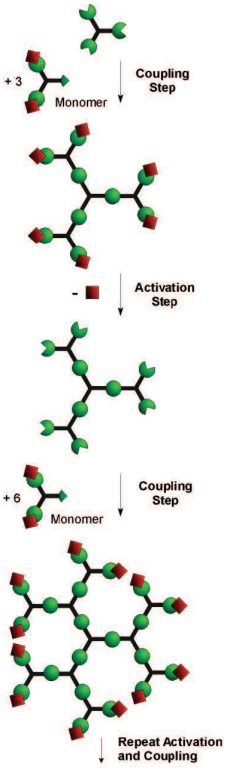

Figure 3: Schematic illustration of divergent synthesis of dendrimers[7]

The very first synthesis and characterization of dendrimers was done in 1985 by Tomalia et al. [10] , the dendrimers were coupled covalently to form so-called "Starburst Polymers". These dendrimers were divergently synthesized, another type of synthesis of a dendrimer is convergent synthesis. The divergent approach starts at what will become the core of the dendrimer, from here it will iteratively grow outwards in coupling and activation steps to become a globular macromolecule as shown in Figure 3. An exponential increase of reactions at the periphery occurs as the dendrimers grow.

In a convergent synthesis of a dendrimer, first several building blocks or dendritic fragments are generated via a coupling and activation step. After the coupling step, the single functional group at the tip of the dendritic fragment is used to attach it to the dendrimer's core. Convergent synthesis offers a more versatile way of producing functional macromolecules because of the ability to modify dendrons at both the focal point and the chain ends.[7]

5.1 Divergent synthesis

When performing a divergent synthesis, to prevent uncontrollable hyperbranching, each monomer's (also called dendron) functionalities at the periphery are unable to link to the core functionalities at first as illustrated in Figure 3. After completing the first coupling step, however, these peripheral functionalities can be activated by coupling to another molecule, removement of a protective group or converting into a reactive functionality. An activated peripheral group than initiates in a new coupling reaction resulting into a new generation layer. After a few of such steps, the dendrimer starts to posses a fair amount of mass, making them easy to separate from its reagents. Although the synthesis might seem easy, special care must be taken to ensure each coupling and activation is driven to completion to prevent a polydisperse final product. If done improperly, the side products obtained might be hard to separate from the intended product as they are probably fairly similar in molecular weight. As an exponential growth of peripheral groups also increases the probability of incomplete coupling or activation reactions, large divergently synthesized dendrimers show more polydispersity than their convergent analogues. For these first mentioned dendrimers, it is therefore always a necessity to preform a good characterization of the final product.

5.2 Convergent synthesis

The initial monomers used in convergent synthesis will not later become the core of the dendrimer but instead will be the most outer generation layer of the dendrimer. As illustrated in Figure 4, the outer molecules of the dendrimer couple to two branches of the monomer in a coupling reaction. Thereafter, the new dendritic fragment (dendron) hereby formed activates

its functional group in an activation reaction. Coupling of the activated dendrons with, again, a monomer leads to a new dendron with one inactivated functionality. Repetition of these steps eventually leads to dendrons of higher generation, these can be attached to a core molecule to form a globular dendron.

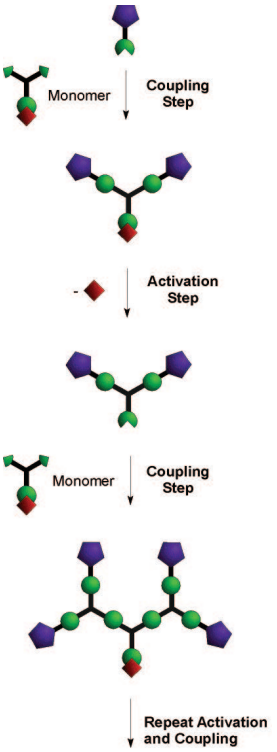

Figure 4: Schematic illustration of convergent synthesis of dendrimers[7]]

A general difference with the divergent synthesis is the number of transformations in each step (coupling or activation), in a convergent synthesis less transitions per step occur. Therefore, the reactions can easily be brought to completion using a small excess of reagent resulting in much greater structural control. Also, the small number of components at the final stage allows for a chromatographic purification.[3] Therefore, a convergent synthesis ensures dendrimers much less polydisperse than those synthesized following a divergent path. Using dendrons as building blocks has another important advantage over a divergent synthesis: till the final coupling to the core molecule, both the chain ends as well as the focal points can still be modified. It allows, for instance, to vary the number of functional peripheral groups on the final dendrimer or to attach different dendrons to the same core molecule.

A disadvantage, however, of convergent synthesis is the long time needed to prepare dendrimers. To speed up dendrimer formation several techniques have been developed. An example is, for instance, orthogonal synthesis, a convergent growth of two different monomers. Two monomers with selected functionalities that only react with the peripheral groups of the other monomer but not with its own functional groups. The activation step can in this case be skipped in the formation of third generation dendrons. Other acceleration approaches for dendrimer formation will not be discussed in this paper.

6 PHARMACOKINETIC AND PHARMACODYNAMIC PROFILE

In order to understand what functionalities for dendrimers are of importance, knowledge about what parameters can be tuned to obtain a good nanodrug, is necessary. When introducing a drug to the body, an effect of the drug on the body and vice versa is expected. Therefore, it is of importance to protect the body from the drug, but at the same time protect the drug from the body. The pharmacokinetic (PK) and pharmacodynamic (PD) profile of a drug must be altered in such a way to increase target site accumulation, however, at the same time decrease off target toxicity.

Before introducing a drug on the market, a proper understanding of its PKs and PDs is necessary. Both determine the efficacy of the drug but drug targeting research mostly focuses on modulating the PK profile. A collection

of properties of the drug determine such a profile: the absorption, distribution, metabolism and excretion (ADME) rates. In the pharmaceutical industry, safety evaluations include PK studies designed to determine ADME rates. [15] Several ways exist to modify dendrimers in order to change these rates.

Favorable ADME rates are obtained if the accumulation and residence time at the target site, solubility, stability and circulation time of the drug are increased within the body while excretion, metabolic breakdown and accumulation at non-target sites are decreased. Objectives which can be either obtained by passive targeting or active targeting approaches.

6.1 Passive targeting

Some diseased cell aggregations (such as tumors or arthritis aggergations), in their tendency to grow rapidly, contain many disorganized vessels and a large number of pores causing extravasation of nanosized macromolecules or nanoparticles into the tumor tissue while traditionally sized drugs tend not to. Also, cell aggregations typically lack lymphatic drainage necessary to transport waste materials away from tumor tissue back to the heart.

Nanosized systems can therefore be useful to carry known functional drugs to such cell aggregations. More than that, some types of tumors display slightly acidic environments and a low oxygen concentration in their tissue matrix, [19] abnormalities which could be of use when designing particular nanocarriers. Besides passive targeting, active targeting is a different type of targeting a tumor aggregation.

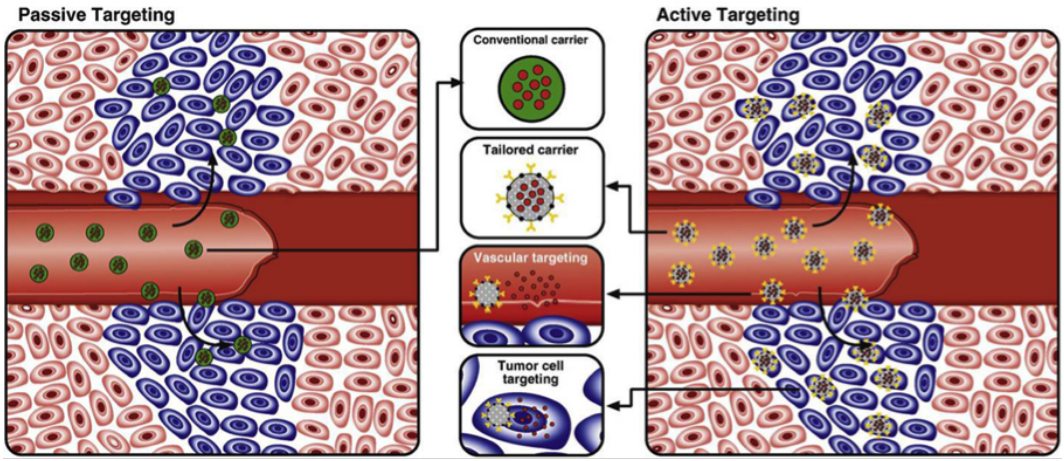

Figure 5: Illustration of the difference in active and passive targeting[16]

6.2 Active targeting

This type of targeting , as shown illustratively in Figure 5, utilize receptor specific ligands to attach to diseased cells. Molecular recognition of diseased cells by various signature molecules overexpressed at the diseased site either by ligand-receptor or anitbody-antigen interactions is necessary. [17] The drug molecule can be actively targeted by conjugating receptor specific ligands to a nanocarrier. Mateja et al. for instance have shown the specific targeting with polylactic-co-glycolic acid (PLGA) immuno-nanoparticles onto which monoclonal antibody was covalently or non-covalently attached to

the nanoparticles as an antigen specific antibody. Results indicate that nanoparticles coated with specific antibodies can indeed target specific cells. [20] However, despite of their ability to target, these nanoparticles show a lower tumor uptake than non-targeted nanoparticles because non-targeted nanoparticles have a prolonged circulation time while targeted nanoparticles are rapidly cleared by liver and spleen. [21] It therefore seems active targeting might not be so effective, but Talleli et al. have shown that active targeting can be advantageous if it confers intrinsic pharmacological activity. [22] Also, research of Davis et al. suggests that for drugs which cannot enter the cells, a receptor specific ligand or antibody is even necessary. [23]

7 NANOCARRIER FUNCTIONALITIES

As active targeting seems to be only effective in specific cases, the main focus of this article is passive targeting and targeting via an external stimulus. The main goal of such a targeting process is to encapsulate small molecule drugs and thereby enhancing its pharmacokinetic profile.

Several problems are repeatedly encountered with small molecule drugs. For instance, a small molecule drug might be perfect for the job of destroying cancer cells, however, its function does not only affect the cancer cells but also healthy tissue. Also, the solubility of the drug is often too low for practical use or not stable inside the body. Many more of such problems can be thought of, but the use of carrier molecules can provide a way to overcome them. In several ways a dendrimer can be useful as drug delivery vehicle: a drug molecule can be attached to one of the peripheral groups, a drug molecule can be encapsulated within the dendrimers cavities or a drug can be coordinated to the peripheral groups via ionic interactions. Within this paragraph, I will first talk about dendrimers ability to make an insoluble drug still usable within the body. Secondly, about dendrimers which make use of pH or redox to change structurally or chemically. Finally, a number of ways using responsiveness to external and internal stimuli will be discussed.

7.1 Solubility

The supramolecular noncovalent assembly of molecule-dendrimer as a guest-host pair can be used to transport drugs while maintaining full pharmacologically functionality of the drug molecule. Current drugs usually have to be synthesized as an ammonium salt to make them soluble, however, such a synthesis is not possible for natural products, chemical substance found in nature (produced by a living organism for instance). Natural products are the most consistently successful source of drug leads, both historically and currently.[36] Therefore, dendrimers could open up a large number of possibilities for reintroducing poorly soluble natural products as some dendrimers are able to make such drugs soluble. Regardless of passive or active targeting, using dendrimers in such a way increases the circulation time of a drug in the body, thereby increasing its efficacy.

An example for increased solubility of a natural product by dendrimers was seen in Combretastatin A4 (CA4) a potent anticancer and antiangiogenesis substance isolated from the South African tree Combretum caffrum. [37] CA4 can disrupt the vascular system within tumors, whereas healthy vasculature stays intact as the endothelial cells in tumors are much more sensitive

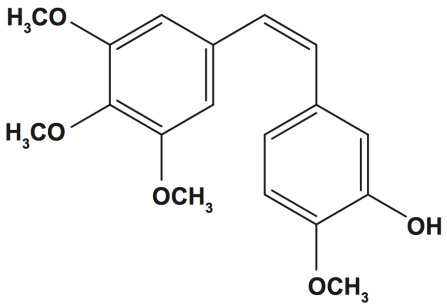

Figure 6: Combrestastatin A4 a potent ancticancer drug

to antimicrotubular effect (blocking of mitose) of CA4. Although this drugs has a high potential for the destruction of tumors, it is a poorly soluble drug which reduces bioavailability. While synthesis of soluble analogues was possible, this approach was not ideal as such substances displayed a decreased lifetime which reduced its efficacy. Besides, these soluble analogues caused several side effects. [38]

To improve the drugs solubility it was encapsulated in a generation 5 dendrimer, this showed a 20 fold improvement of solubility of the drug. The CA4 could reside in the dendrimer's hydrophobic cavities and was gradually released while maintaining the same vascular effect on tumors as was observed with free CA4.

Also, several studies are done by Xu et al. on the topic of host-guest chemistry of dendrimers. [40], [41], [42], [43] The group has for instance shown that the encapsulation of charge bearing molecules show a much stronger ability to capsulate when the poly(amidoamine)s were acetylated. [43]

Besides only improving the solubility, a dendrimer can have a dual effect: increasing the solubility of a drug and passively targeting a tumor site. An example of such an attempt is in the case of antitumor campthothecins, one of the natural products used for the destruction of tumor cells. Its unique mode of operating, which is poisoning of the DNA topoisomerase 1 during DNA relaxations, makes these molecules suitable for quick destruction of cells.[24] However, this natural product, which appears in nature in the forms of 10-hydroxycamptothecin and 7-butyl-10-aminocamptothecin, is poorly soluble in physiological conditions.

Polyester dendrimers, divergently synthesized, are used for the encapsulation of campthothecins. Forming a non-covalent macromolecular assembly of host and guest, these complexes were tested for intracellular accumulation and retention and compared with the single molecule drugs. Results show that the delivery of hydrophobic molecules such as camptothecins, can be accomplished using biodegradable, biocompatible dendrimers that increase aqueous solubility by approximately an order of magnitude without attaching functional groups to the camptothecins. [24] The dendrimer enhance both uptake and retention, making such dendrimers promising as a drug targeting vehicle. Besides polyesters, more of such dendrimers can be found which by increasing solubility of a traditional drug and an increasing retention, increases its efficacy.

7.2 pH and redox as a stimulus for dendrimers

The behavior of dendrimers in different pH or redox environments might be of interest to investigate its nanocarrier functionalities. Several different dendrimers are of interest due to their ability of conformational changes in different pH environments. The poly(ethylene oxide)s (PEOs), poly (amidoamine)s (PAMAMS) and poly(β-aminoester) dendrimers display this kind of behavior. The latter even displays a dual thermal and pH sensitivity. In the upcoming paragraph current investigations on dendrimers at different pH environments are evaluated.

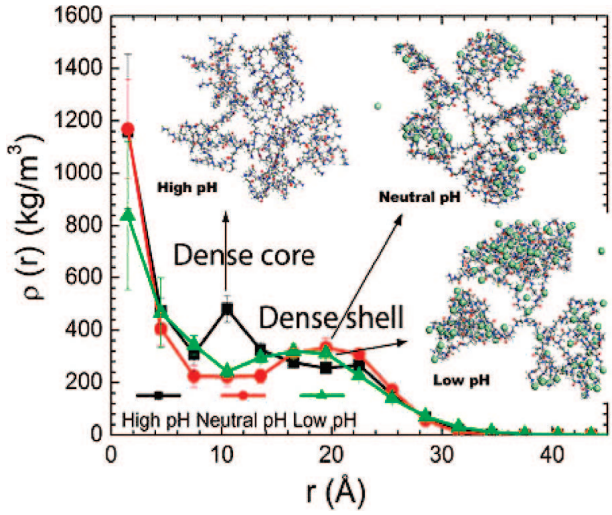

Figure 7: Radial mass distribution of a G4 PAMAM dendrimer[25]

PAMAM dendrimers, are useful due to their large number of surface amine groups which are easily functionalized by simple chemical reactions rendering dendrimers with many functionalities, more than that, they contain hydrophobic cores into which drug molecules can be stored. Also, they are thought to show conformational changes in different pH conditions, which has been simulated by Goddard et al. using atomistic molecular dynamics simulations. A fourth generation (G4) PAMAM dendrimer shows a shift of maximum density from 10 Å at high pH to 18 Å at neutral or low pH.[25] These simulations indicate there is a mass migration to the periphery of the dendrimers in acidic conditions. A probable cause for these mass migrations can be strong intramolecular hydrogen bonding within the water-dendrimer-counterion system, resulting in a dense outer shell. As can be seen from Figure 7, a denser core contains more container-like cavities useful for carrying a drug while in a denser shell structure the different branches originating from the dendrimers core pack together and create an easier pathway to diffuse out of the dendrimer's core.

A study with PAMAMs which are a bit more closer to go into clinical studies are the PAMAM-poly(ethylene glycol) conjugates. Poly(ethylene glycol) (PEG) is used to reduce the PAMAMs in vivo toxicity, however, can hinder the drug release due to steric hindrance. Chen et al. use a disulfide linker to attach the poly(ethylene glycol)s to the PAMAMs to yield pH and redox sensitive nanocarriers. Doxorubicin (DOX), a common cancer drug, was used to load the hydrophobic core of such dendrimers. Comparison studies with both disulfide linked PEGs and directly linked PEGs show that disulfide linked PEGs show an increased rate of drug release. [28] As these

studies were performed under glutathione concentration similar as that in living tissue, it is highly likely that reductive cleavage of the disulfide bond by glutathione eliminated the steric hindrance caused by PEG chains and allowed a better diffusion of DOX molecules out of the dendrimer.

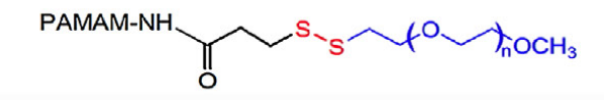

Figure 8: Structure of the PAMAM-PEG conjugate with disulfide bond

PAMAMs have many advantages, however, also some disadvantages such as in vivo toxicity and non-biodegradability. Therefore dendrimer research also focuses on other types, poly(ethyloxide)s (PEOs) for instance, are of interest in the field of nanocarriers due to several characteristics: they exhibit good solubility, nontoxicity and are not detected by the human immune system. Also, in combination with poly(acryl acids) (PAAs) they can be made pH sensitive. A useful property of dendrimers, relevant to drug delivery, is size adjustment in different pH environments. PEO(G5)(PAA)21(OBn)48 has shown to resize when in acidic conditions, these generation 5 PEO dendrimers form copolymers with the poly(acryl acids) by mutual hydrogen bonding. [27] At low pH the PAA chains contract, thereby they force the PEO branches (which are mutually hydrogen bonded) to shrink in size with them. The PAA chains are attached to functionalities at the core of the denrimer.

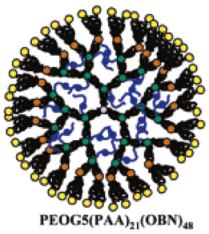

Figure 9: PEO dendrimer functionalized with PAA

For PAMAM's, surface groups can be of great importance, comparison of the surface groups on PAMAM dendrimers was preformed by Shi et al. to determine doxorubicin release kinetics. Acetyl-, glycidol hydroxyl- and carboxyl-terminated G5 PAMAM dendrimers were synthesized which, in NMR studies, showed an increase of dendrimer-drug interaction in that order. In more acidic environments, al the differently functionalized dendrimers showed an increased release pattern. [35] Also the release kinetics were in accordance with the dendrimer-drug interaction, the strongest interaction showed the slowest kinetics as expected and vice versa.

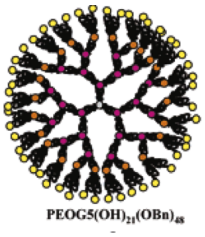

Figure 10: PEO dendrimer

Poly(β-aminoester)s are another type of dendrimers showing an enhanced drug release in acidic environments accompanied with a size dependence.

The main advantage of this kind of dendrimer is that it is biodegradable. Fan et al. have shown an increased release of the DOX drug from these dendrimers in acidic conditions. The dendrimer contains cavities easily loaded with a hydrophobic drug. In acidic conditions, the protonation of the inner amines causes a quick release of guest molecules. Besides their acidic sensitivity, also an increased drug loading effect at low temperatures has been reported.[29]

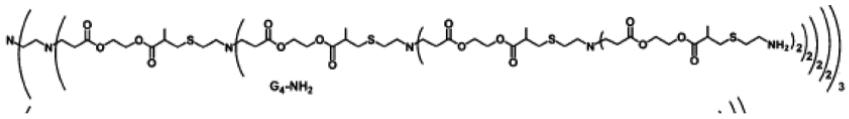

Figure 11: A fourth generation Poly(β -aminoester)

7.3 Nanoparticles responsive to external stimuli

As already mentioned, pure technological processes for the destruction of cancer or diseased cell aggregations such as proton beam therapy or X-ray therapy come often with limited resolution and therefore can not be as effective as a drug can be. Besides, the use of such sources is often harmful for tissue lying in the beam path. It could be an idea to merge both worlds, by using a nanoparticle as a carrier which is made responsive to an external non-damaging stimulus. An example of such an external stimulus is near-infrared (NIR) light, due to its low absorbance in skin and tissue it has a deep penetration depth. Gold nano rods can act as NIR to heat transducers and can therefore be used for thermotherapy in tumors. A more interesting and effective approach for the destruction of cells, however, was introduced by Farokhzad et al. recently. They synthesized nano rods attached with PEG layers for increased circulation time and DNA strands for drug loading. [30]

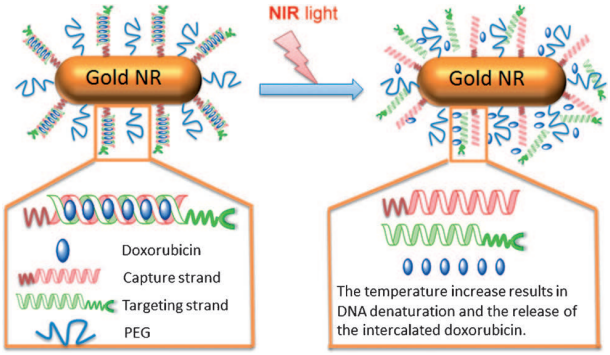

Figure 12: Mechanism of stimulated drug release by NIR light of gold nano rods attached with DNA, PEG and DOX[30]

As shown in Figure 12, upon irradiation with NIR light the gold's temperature increases resulting in denaturation of the DNA and thereby release of the intercalated DOX. This combination of thermotherapy, targeted delivery and stimulated release, was demonstrated in in-vivo anti-tumor efficacy tests to be superior to gold nano rods using only thermotherapy.

Also dendrimers can be triggered by an external physical stimulus such as infrared light. However, when inducing low-frequency molecular vibration by infrared light the excitation energy mostly rapidly dissipates rather

than inducing photochemical changes. Aida et al. have prepared aryl ether dendrimers with an azobenzene core capable of photoisomerization. [31] Such an isomerization of the core is illustrated in Figure 13, upon radiation it was found that a cis-L5AZO dendrimer showed isomerization to the trans state at wavelengths of stretching vibrational bands for aromatic rings. The lower generation dendrimers, did not display such an isomerization indicating that the dendrimer outer framework is strongly depending the isomerization of its azobenzene core. [31] The cause for this dependence is suggested to be intramolecular energy transfer from the dendrimer matrix to the core.

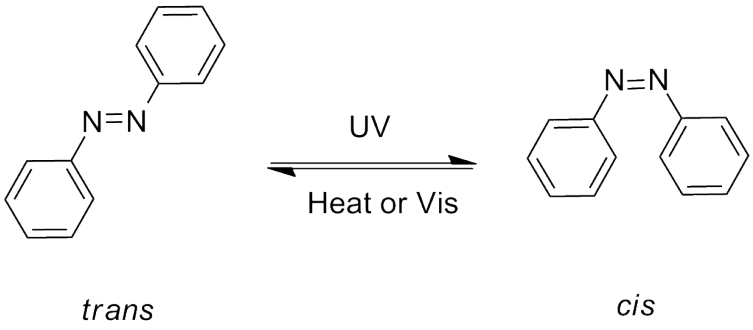

Figure 13: Isomerization of azobenzene core upon UV radiation[31]

An example of a light sensitive dendrimer in a more progressed stage as a drug nanocontainers are amphiphilic biaryl dendrimers which contain both lipophilic and hydrophilic functionalities in its dendrimer backbone. These backbones form micelle type aggregates in water at a critical concentration and can bind lipophilic guest molecules. Upon excitation by light, the photolabile 2-nitrobenzyl ester group is cleaved off as illustrated in Figure 14 resulting in the disassembly of the micelle type aggregate and the release of its guest molecules. [32]

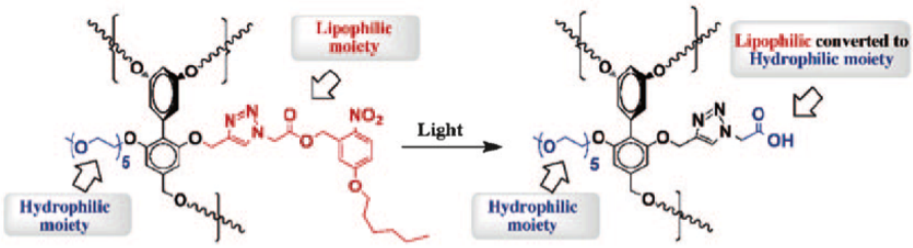

Figure 14: Cleaving of the lipophilic moiety due to photon excitation Enzymatic stimuli[32]

7.4 Nanosystems responsive to internal stimuli

Besides external physical stimuli, an internal stimulus such as for instance enzymatic activity can be a way to increase the efficacy of a drug. Dendrimers responsive to enzymes have been reported, the cleavage of a particular bond can lead to the disassembly of dendrimer molecules. [33] This characteristic of dendrimers can be useful for the controlled release of drug molecules when in the presence of particular enzymes. Thayumanavan et al.

have synthesized amphiphilic micelle structures of dendrimers which lose their micelle nature in response to an enzyme porcine liver esterase stimulus. [34] Amphiphilic micelles have a balanced hydrophilic-lipophilic ratio which can be destroyed by enzymatic cleavage, leading to the destruction of micelle disassembly as illustrated in Figure 14. The enzymatic activity was monitored by the release of a non-covalently attached molecule in the amphiphilic micelle.

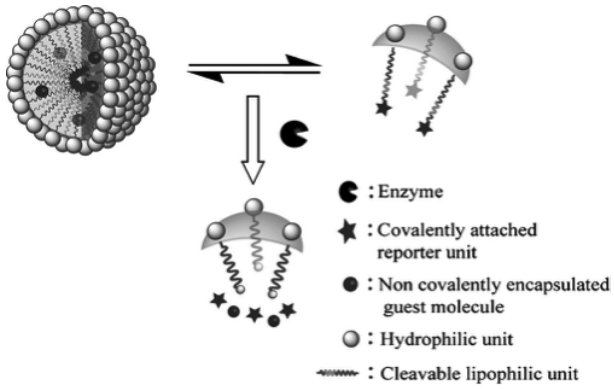

Figure 15: Schematic illustration of the enzymatic disassembly of amphiphilic micelle drendrimers[34]

In a similar way, enzymatic release of molecules is achieved using a molecular system consisting of primarily a skeleton made out of DNA origami. The structure consists of an origami "barrel" consisting of two parts connected by hinges as shown in Figure 13. The other end of the barrel can be connected by staples modified with DNA aptamer–based locks, which are oligonucleotide molecules that bind to a specific target molecule. A specific antigen key can dissociate the aptamer locks, afterwards the molecular system will undergo a transformation to the open state due to an increase in entropy. The DNA aptamer-based lock opens only due to a specific antigen key, only when both aptamer-based locks are opened the molecular system opens up. A payload of these nanosystems was selected to be fluorescently labeled antibody which would bind with specific surface cell antigens upon opening, thereby increasing the fluorescence. [34]

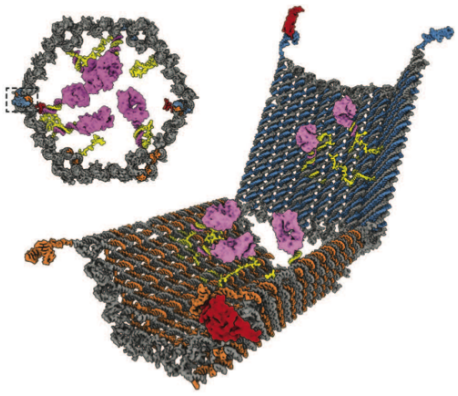

Figure 16: An illustration of the molecular system made with DNA origami for switchable opening and closing [34]

Nanosystems responsive to external stimuli are used in more specific cases of cancer destruction. When using such systems, enzymatic activity

in or near the tumor must be known of in order to succesfully deliver the drug to its target. A broader range of different tumors can be addressed by systems which make use of a more general characteristic of tumors such as disordered vasculature system or slightly acidic conditions.

8 DISCUSSION

Dendrimers were discovered in 1985 and praised for their high monodispersity, great structural control and large number of surface functionalities. Dendrimers are one of the nanostructures investigated in the field of nanomedicine. Nowadays, the field of nanomedicine is emerging as nanosystems enable scientists to enhance more favorable pharmacokinetic and pharmacodynamics properties and can even be used in combination with traditional drugs. Yet, not many nanomedicine have been commercialized, this can however, be mainly ascribed to the time consuming clinical studies in between academics and commercialization. On the other hand, the research in this area and more specific in the area of dendrimers as nanomedicine is very much alive. In this paper different approaches were described to functionalize dendrimers which might be of use in nanomedicine.

Dendrimers have the ability to improve the solubility tremendously of natural products. Several dendrimers are known to be able to capture such molecules in their hydrophobic cavities and make them soluble, examples are the PAMAM dendrimers which are also commercially available. A growing interest in encapsulating drug with the use of dendrimers is observered, as the area of new potential products spans all the natural products.

When looking at approaches to target specific diseased cell aggregations two main ways can be separated: active targeting and passive targeting. Several sources suggest that active targeting is very target specific: to be able to construct a proper nanosystem that actively targets a tumor, details about, for instance, the cellular intake pathways or composition of receptor molecules on tumor cells have to be known. Even than, nanosystems using specific ligand-receptor interactions often did not even display a superior uptake of drug in the tumor. Mainly due to metabolic breakdown upon recognition of the receptor by liver or spleen, reducing the circulation time.

Therefore the main focus in this paper was to address specific functionalities of dendrimers that could be useful passively targeting tumors. It is known that several types of tumors are slightly acidic within their tissue matrix, pH responsive dendrimers are therefore evaluated on their potential as a nanodrug. The PAMAM G4 denrimer, for instance, displays a conformational change in acidic environments leading to a more accessible pathway for diffusion out of the dendrimer's core. Although this is a useful interaction in acidic environments, "bare" PAMAMs are toxic in vivo due to their cationic shell. Besides, the conformational change in acidic environments has only been simulated using fourth generation PAMAM dendrimers. The toxicity of PAMAMs can be reduced to safe standards by functionalizing the dendrimers surface amines. Therefore, molecular dynamical simulations on higher generation PAMAMs and PAMAMs functionalized with non-toxic peripheral groups have to be conducted, for further proof that conformational changes are indeed the reason for a faster release rate in acidic conditions. Although, experimental studies did indicate faster release of PAMAMs functionalized with surface groups, the release of drugs could be a consequence of electrostatic interactions of deprotonated groups with drug

molecules (as for instance in poly(β aminoester)s) and not so much of the conformational changes of the dendrimer. The release due to conformational changes, though, is hoped to be the mechanism as such a mechanism can be applicable to release many kinds of non-polar molecules from dendrimers.

Another way in which dendrimers can be triggered to target pH sites, could be size adjustment. As discussed earlier, a nanosystems larger size than traditional drugs can enhance trapping on the right places, namely in tumors with disordered blood vessels and no lymphatic drainage system. It is not precisely known how trapping of such kind depends on the size of the nanoparticle but one can image that by adjusting a dendrimer's size in the target area, trapping can be enhanced in narrow and disorder vascular systems or size adjustment can cause an increased diffusion of guest molecules out of the dendrimer due to conformational changes. PEO dendrimers functionalized with PAA chains have demonstrated to shrink in acidic conditions. Unknown is, whether PEO is capable of encapsulating drug molecules and if so, whether the same mechanism that causes the dendrimer to shrink is still intact after encapsulation. Although this result indeed shows that size adjustments in acidic environments are accomplished, it is just the first step towards usage as a nanocarrier.

Besides pH as a stimulus, also external stimuli could be used for the purpose of drug targeting. Current technologies using radiation usually are destructive not only for tumor tissue but also for healthy tissue. However, when using a non-damaging stimulus to trigger nanocarriers to release a certain drug, damage to healthy tissue can be limited. Gold nano rods have for instance been successfully used in combination with DNA to encapsulate a drug which is released under irradiation with NIR light. Although light can be used as a stimulus for dendrimers as well, this energy usually dissipates rapidly rather than inducing actual photochemical changes. Still, several research groups showed how dendrimers might be used for light sensitive drug delivery. It has for instance been shown that upon excitation with UV an isomerization can occur in an aryl ether dendrimer. They found out, however, that the dependence for isomerization is strongly dependent on the outer framework of the dendrimer due to intramolecular energy transfer from the dendrimer matrix to the core. Also amiphiphilic biaryl dendrimers forming micelle aggregates can be made light sensitive, upon excitation they disassemble releasing a guest molecule. Although these particular examples indeed show that radiation can be used as the activation energy for a photochemical reaction in dendrimers, the wavelengths used are strongly absorbed by human tissue, making it difficult to reach deep lying tumors. Besides, it is not directly clear yet which photochemical reactions might be useful for nanocarriers. Gold nanorods are much more enhanced, as they act as a heat transducer for NIR, a type of radiation able to penetrate deeper inside the body. For dendrimers however, the interesting part is that the conversion of electromagnetic wave to photochemical reaction is dependent on dendrimer framework, meaning that by tuning its framework it could be made photoreactive to lower wavelengths such as IR with a deep penetration depth. Currently, however, a radiative stimulus as a means to target specific areas is still far away from any practical implication.

Besides external stimulus, likewise an enzyme or specific antigen key can be used as a trigger to release guest molecules. This has been done using a barrel made out of DNA origami with locks that respond only to specific antigen keys. Also, amphiphillic structures of micelles show to disassemble

when cleaved by an enzyme. Such techniques might be promising, however, they are highly specific for only certain tumors. As the exact enzymatic activity in tumors or near tumors should be known, or the specific receptor composition on a tumor cells.

9 CONCLUSION

An introduction to several ways in which dendrimers can be used potentially as a nanocarrier were evaluated. Not only by increasing the solubility of regular sized drugs dendrimers can increase a drugs efficacy, also active or passive targeting could lead to an improvement of efficacy. As tumors often show slightly acidic environments, dendrimers responsiveness to pH was assessed indicating that PAMAMs show an increased release in slightly acidic conditions. This might be due to conformational changes of the PAMAM dendrimer, however, it can also be a result of electrostatic interactions between host and guest molecule. Besides increased release of drugs in PAMAMS, other dendrimers such as PEO display a resizing effect when in acidic conditions. This might be of interest for drug delivery, however, relatively little is known of PEO as a host dendrimer for drug molecules and whether the same effect is seen when encapsulating a drug molecule. Besides, it is not known whether such an effect can improve the efficacy of drug molecules by an improved retention effect. For pH responsiveness therefore, PAMAM dendrimers are most promising and further research on their behavior in different acidic conditions and varying surface group is necessary for nanomedicine applications.

Another way of targeting is by using an external stimulus such as electromagnetic radiation. Several ways in which a dendrimer has been made responsive to such radiation are reported. However, wavelengths were used which are strongly absorbed by human tissue. Besides, several photochemical reactions have been shown but it is not clear how they would eventually lead to a responsive nanocarrier. Therefore a radiative responsive dendrimer to target tumors are still far from any practical implication. Other nanosystems such as gold nanorods are closer to such an application. First step for dendrimers would be to make them reactive to low energy wavelenghts which are not so strongly absorbed by the human body. This can possibly be done by tuning the dendrimer framework.

An internal stimulus such as an enzyme is more promising as there are already dendrimer structures which disassemble when near specific liver enzymes. Also in the field of DNA origami such systems are used and very promising. These systems, however, are very specific for particular types of tumors and enzymatic activity and cannot be of use on a broader variety of tumors.

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