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Dendrimer Structure Diversity and Tailorability as a Way to Fight Infectious Diseases

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Additional information is available at the end of the chapter

Abstract

Dendrimers represent a distinct class of polymers—highly branched and uniform, with a relatively small size when compared to their mass. They are composed of the core, from which branched polymeric dendrons diverge and they are end-capped with selected terminal groups. Recently, dendrimers have attracted considerable attention from medicinal chemists, mostly due to their well-defined and easy-to-modify structure. This chapter aims to compile dendrimer applications and activities especially for prevention and fighting off infections caused by bacteria and fungi, viruses, and parasites/protozoa. Our goal in this review is to discuss selected modifications of dendrimers of potential value for pharmaceutical chemistry.

Keywords: antibacterial, antimycotic, dendrimer, nanomaterials, polymers

1. Introduction

Dendrimers are spherical nanosized polymers that branch in a well-defined manner. They were first synthesized and described by Franz Vögtle in 1978 [1]. For the last 30 years, they have gained a lot of attention, mainly due to the discovery of their stunning complexation abilities. In medicinal chemistry, they reveal interesting pharmacological properties of potential value for various medical fields. In pharmaceutical sciences, these nanostructures are particularly interesting as they can be potentially useful in pharmaceutical technology for preparation of water-soluble complexes with poorly soluble active pharmaceutical ingredients (API). It is worth noting that at the same time a decrease in API's overall toxicity is observed.

In this chapter, the aim is to describe the potential use of dendrimers in fighting off infectious diseases. Infectious diseases continue to constitute a problem around the globe, and a proper surveillance is required, as the amount of reports regarding occurrence of bacteria and fungi resistant to all clinically used antibiotics and antimycotics is growing. From one year to the next, the number of potentially useful antimicrobials is slowly decreasing, while only a few APIs have been introduced to clinical practice in recent years. Therefore, even treatment of common diseases has the potential to become a serious problem in the near future. The link between pollution and health although complex is obvious. An increasing pollution of the environment with pharmaceuticals intended to fight infectious diseases as well as their enhanced consumption over the last 70 years has led to the development of resistance mechanisms. Additionally, the treatment of infectious diseases in developing countries is quite problematic due to the lack of regulations in drug marketing. Another factor is the price of what is often referred to as last resort medicines; the contribution of public funding is often essential for the implementation of therapy with such medicinal products. Moreover, decreased hygiene level and underdeveloped sanitation favour the occurrence of bacterial, fungal and parasitic infections [2, 3].

Currently, the amount of novel antibiotic classes seems to be constant. In parallel, the propagation of multidrug resistant microbes indicates the necessity of searching for novel agents and methods, which can be used as a revolutionary approach. Therefore, present research wrestles with the problem of whether novel dendrimer nanoparticles alone or in complexes with APIs can be of potential usage in medicinal and pharmaceutical chemistry. The goal is to develop novel antibiotics, antimicrobial and antiparasitic/protozoa therapies. Currently, there are many ongoing attempts that aim at increasing the efficiency of strategies against bacteria and fungi in planktonic and biofilm modes of growth. There are researched methods that lead to biofilm growth inhibition, disruption or eradication. These approaches include APIs with new mechanisms of action, like enzymes, salts, metal nanoparticles, antibiotics, acids, plant extracts or antimicrobial photodynamic therapy. Regarding all this, dendrimers could be a material that might help to reach this goal [4].

2. Dendrimer structural versatility

Dendrimers do not form a uniform group based on their chemical structure. They are different from other dendritic structures such as dendronic and dendritic surfaces, dendronized polymers, dendriplexes and dendrigrafts. Schematic representation of dendrimeric nanoparticle, which constitutes the main subject of this short review, is presented in **Figure 1** [5]. Generally, dendrimers are composed of three elements: (*i*) a core, (*ii*) branched dendrons and (*iii*) terminal groups. They are most commonly synthesized using two different synthetic pathways: (*i*) divergent or (*ii*) convergent (**Figure 2**). In the divergent approach, firstly the synthesis of the functionalized core (or inner part of the dendrimer) takes part. Then this structure serves as a scaffold for the synthesis of higher generation dendrimers. Convergent approach relies on the synthesis of dendrons, which can be further attached to the earlier synthesized core. Some synthetic chemistry techniques, including *click chemistry* are especially useful for the synthesis of dendrimers [6].
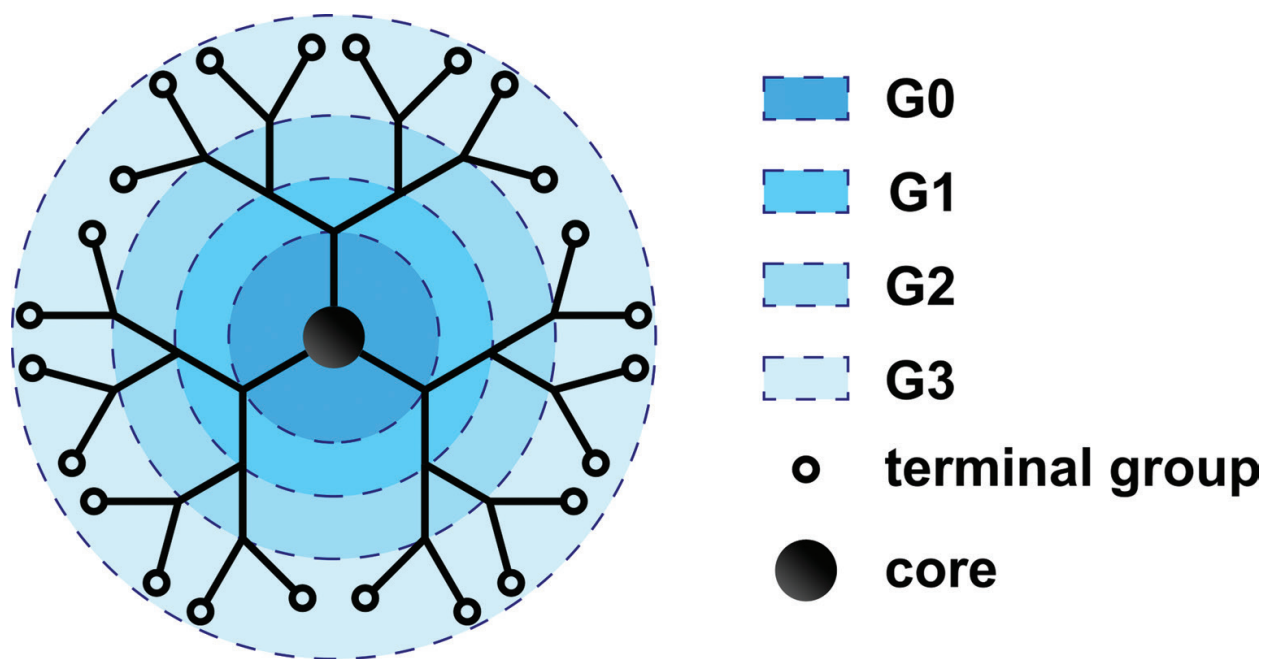
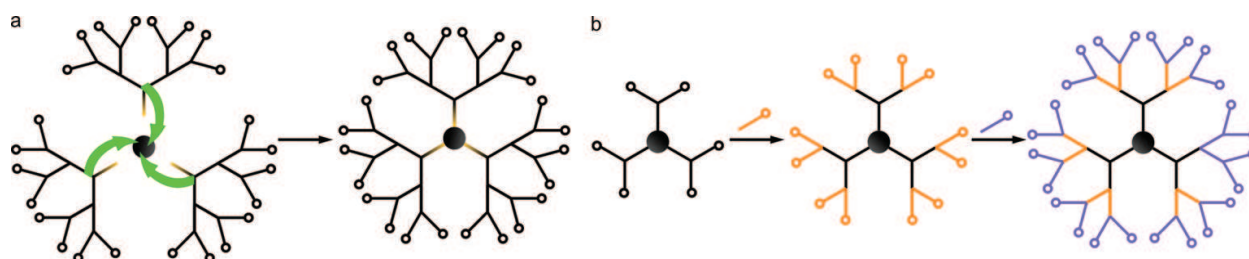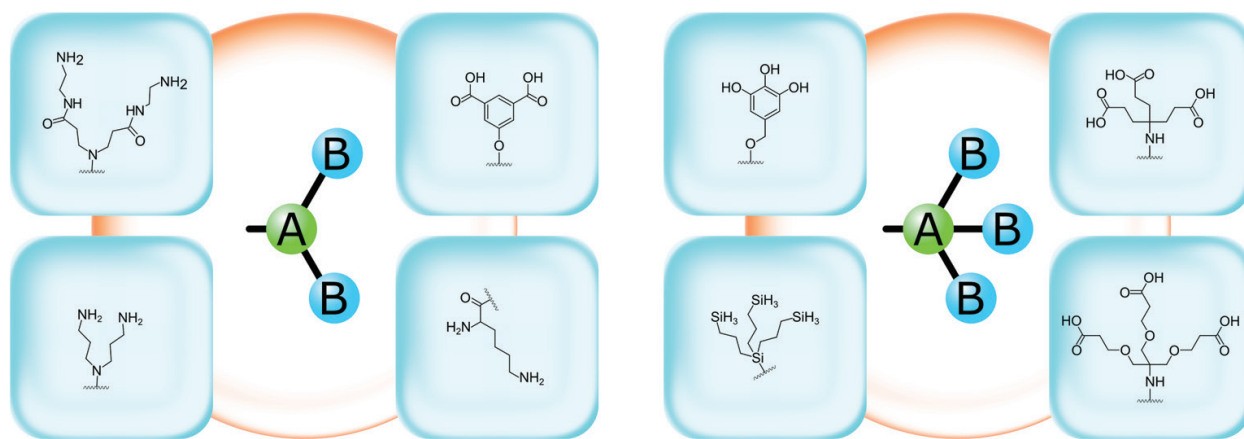**Figure 1.** Schematic representation of dendrimer structure.

Figure 2. Schematic representation of convergent (a) and divergent (b) method of dendrimer synthesis.

A system was proposed describing the specific branching architecture of dendrimers, with general abbreviation AB_n—where n stands for new branches that arise from a node. For example, AB_2 and AB_3 states for two and three branches outgoing from each node, respectively. For graphical description, see **Figure 3** [6]. The most common dendrimers that can be found in the literature are built of AB_2 building blocks. Among those to the most popular belong **p**oly(**am**ido**am**ine)—PAMAM, **p**oly(**p**ropylene**i**mine)—PPI (also called **po**ly(**p**ropylene**am**ine)—POPAM), carbosilane and **p**oly(**L**-**l**ysine)—PLL dendrimers [7].

The core part of dendrimers may be a variety of molecules—starting with single atoms (like nitrogen in some PAMAMs), aliphatic chains, alicyclic or aromatic rings through polyaromatic moieties, inorganic frameworks, and ending with other polymers and peptides. The core of a dendrimer can be simply a scaffold to which dendrons are attached. However, in some cases, the core is a molecule that expresses its own activity and added dendrons modify the periphery of the central molecule, thus affecting its physico-chemical properties (solubility, photochemical and electrochemical properties, protection from enzymes, etc.) [8–12].

Figure 3. Schematic depiction of dendrimer branching modes with examples.

Dendrons serve mainly as carriers for other compounds. The controlled release of drugs from dendron-drug complexes can be modulated at certain pH values present in the environment of living organisms. An acidic environment is often associated with cancerous tissues, which was the subject of research by Wang *et al.* [13]. In this study, a potent and specific proteasome inhibitor bortezomib was released from biocompatible modified PAMAM dendrimer complex when triggered by the acidic environment of the tumour. End-group or terminal group of a dendrimer is a peripheral functionalization of dendrimer. The outstanding feature that makes dendrimers so special is the enormous amount of terminal groups in such a small molecule. The number of end-groups increases in exponential manner while the round molecule size increases linearly. This is depicted in **Table 1** for PAMAM dendrimer. Quite often the structure of end-groups depends on the synthetic method applied (harsh reaction conditions, protective groups) and the nature of the dendrimer building blocks [6].

Dendrimer end-groups can be easily modified. Modification changes their polarity and solubility in different solvents. In this way, high toxicity associated with many free amino groups in PAMAM and PPI dendrimers may be overcome by substituting them partially with non-toxic moieties [15, 16]. Alternatively, appending the end-groups with hydrophobic substituents may be considered, when they are intended to be utilized as carriers in hydrophobic formulations. In this way, the toxicity of prepared dendrimer is kept at bay and its complexation capabilities in hydrophobic mediums are increased. Utilizing this method, Hamilton *et al.* [17], while studying potential proteomimetics for ophthalmic use, decorated hydroxyl-terminated G5 PAMAM dendrimers with either dodecyl or cholesteryl moieties. Interestingly, use of dodecyl chains highly increased the overall molecule toxicity, as assessed on Chinese Hamster ovarian cells and pig lens epithelial cells. Cholesteryl-modified G5 PAMAM expressed toxicity to a lesser extent and was less toxic against lens epithelial cells than unmodified dendrimer. In another study, appending the hydrophobic Fréchet-type dendrimers with highly water-soluble carboxylic salt groups enabled the use of them in hydrophilic solvents [8].

Alternatively, the end-groups may be substituted with active substances, targeting molecules and others, that are relevant and needed for modern applications. Najlah and D'Emanuele reviewed the literature on the subject of dendrimer-drug conjugates [18]. The main benefit

| Dendron generation	Number of end-groups			PAMAM dendrimer size [nm]	
	AB_2 dendron	AB_3 dendron	PAMAM dendrimer [7]	Calculated [14]	Experimental hydrodynamic diameter [7]
0	1	1	4	N/A	1.5
1	2	3	8	1.0	2.2
2	4	9	16	1.4	2.9
3	8	27	32	1.6	3.6
4	16	81	64	2.1	4.5
5	32	243	128	2.8	5.4
6	64	729	256	3.6	6.7
7	128	2187	512	4.5	8.1
8	256	6561	1024	5.7	9.7
9	512	19683	2048	6.8	11.4
10	1024	59049	4096	8.6	13.5

Table 1. Number of end-groups of dendrons for AB_2, AB_3 and PAMAM dendrimers and the size of PAMAM dendrimers.

from combining APIs with dendrimers in such manner is that dendrimer-API conjugates are more stable in various conditions as compared to their complexes based on non-covalent bonds. A good example for covalent bonding of APIs to dendrimer surface groups is the use of dendrimers as carriers for immunoactive peptides in the formation of vaccines. Such approach has been successfully tested by Skwarczynski *et al.* [19]. They have synthesized G1 triazole-based dendrons bond to a tetravalent core and with B-cell epitopes as end-groups. As this structure of amphiphillic properties was introduced to water solution, it assembled to form micelles with hydrophobic inner shell and hydrophilic—peptide—outer shell. Another type of terminal group modification concerns the use of targeting molecules, such as folic acid for cancer cells. Majoros *et al.* [20] designed and synthesized PAMAM dendrimer that was appended with several moieties—covalently bound drug (paclitaxel), target molecule (folic acid) and an imaging agent (fluorescein isothiocyanate). It is worth noting that not all terminal groups have to be modified at the same time. There are examples in the literature when the synthesis of dendrimers was performed in a statistical manner, so that only part of end-groups in dendrimer was modified, while the rest remained unchanged. Alternatively, convergent synthetic approach enables to combine various dendrons or polymers into one dendrimeric molecule. Such approach was successfully implemented by Albertazzi *et al.* [21]. Researchers managed to obtain hybrid dendritic-linear block copolymers based on a 4-arm and 2-arm polyethylene glycol (PEG) core. The 4-arm-based dendrimer showed significantly improved DNA binding and gene transfection capabilities in comparison with the 2-arm derivative.

The plethora of different modifications that can be proposed makes dendrimers perfect molecules for any chosen application with endless possibilities. For more insight into the

potential applications of dendrimers and a broad spectrum of different properties of these nanosized polymers, the reader can refer to the comprehensive reviews such as Ref. [5] or Ref. [22]. Astruc *et al.* [5] broadly reviewed dendrimers designed for various functions and they discussed many examples of dendrimers and indicated how their physical, photophysical and supramolecular properties influence further applications in sensing, catalysis, molecular electronics, photonics and nanomedicine. The biomedical applications of dendrimers refer to the following areas: drug delivery, boron neutron capture therapy, photodynamic therapy, photothermal therapy based on gold and iron oxide nanoparticles, medical diagnostics and molecular probes (biosensors). It is worth noting that biomedical applications of dendrimers have gained a lot of attention during the last quarter of the century. In addition to dendrimers' own pharmacodynamic properties, especially promising is their possibility to be used as nanocarriers of improved solubility in water, biodistribution, ADME profile and pharmacokinetics with many other desired properties including extended circulation time and enhanced penetration and retention (EPR) property. Lately, dendrimers have been considered as agents of anti-amyloidogenic potential, as non-viral vectors of oligonucleotides and siRNA for gene therapy purposes and towards detection of IgE-mediated drug allergy reactions [5].

For pharmaceutical technology, dendrimers are mostly known for their carrier abilities. They exhibit great complexation potential for biologically active compounds, drugs, dyes and metal ions. Dendrimer carrier abilities for various chemical molecules (drugs, pigments, salts) comprise both drug encapsulation and chemical bonding to the periphery (**Figure 4**). Dendrimers have already found some commercial applications, for example, as a component of sexually transferred diseases preventing gel (*VivaGel*), a non-viral transfection agent (*Superfect*) and a contrast agent for MRI (*Gadomer 17*). Regardless of many potential uses and outstanding properties, there are also some drawbacks associated with dendrimers and their broader commercial use—these are usually time-consuming and expensive synthesis and purification of higher generations of dendrimers. Also high concentration of functional groups on the periphery debilitates its efficient modification [5, 18].
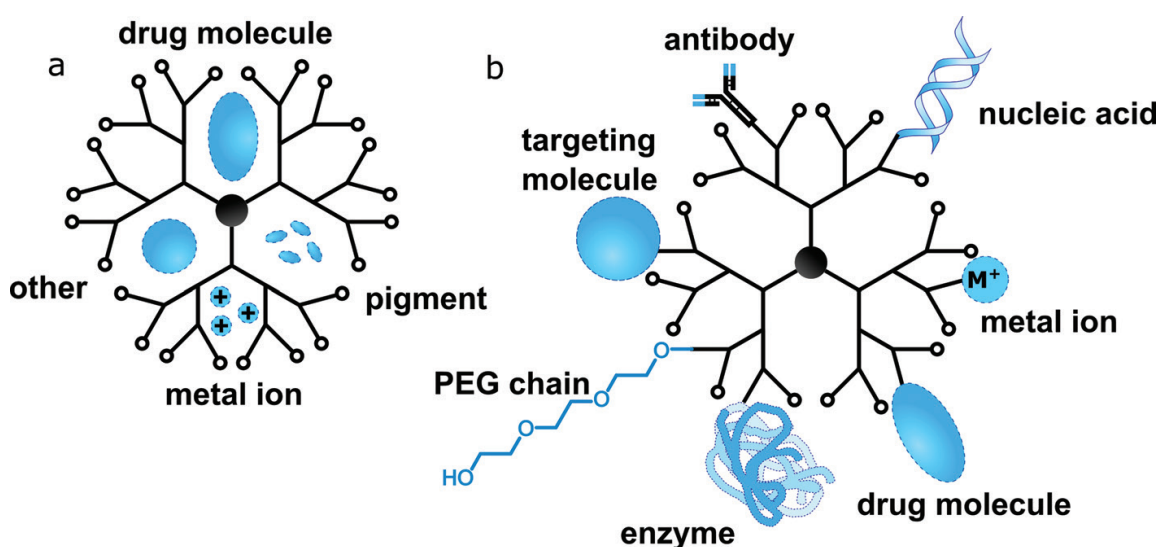

Figure 4. Dendrimer carrier abilities: (a) compound encapsulation and (b) covalent bonding of compound to the periphery.

3. Use of dendrimers against infectious diseases

3.1. Dendrimers as antibacterials and antifungals

Therapeutic efficiency of dendrimers as nanocarriers has been proved so far for, for example, potent anticancer, nonsteroidal and anti-inflammatory, antimicrobial and antiviral drugs. In this respect, two strategies have been applied for the application of dendrimers as drug carriers. The first one was encapsulation of drugs inside dendrimers or their binding to peripheral groups of dendrimers by electrostatic or ionic interactions. The second one concerned covalent bonding of drugs to the periphery of dendrimers [5]. The antibacterial activity of dendrimers has been already reviewed by Tülü and Ertürk [23] and Mintzer and co-workers [24]. The aim was to highlight the diversity of dendrimer structural modifications that led to an increased *per se* activity, as well as a decreased toxicity, thus prompting further applications in medical sciences.

Commercially available PAMAM dendrimers are effective antibacterial compounds (**Figure 5**). Amino-terminated G2 PAMAM dendrimer revealed differentiated minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) activities against various strains of *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella enterica*, *Klebsiella pneumoniae* and MDR-*Shigella flexneri*. Moreover, it exhibited little toxicity to human gastric epithelial cells and did not induce antibiotic resistance in bacteria [25]. Unfortunately, due to many amino end-groups, G3 and higher generation PAMAMs were found to be highly toxic to living organisms [25, 26]. To counter this, PAMAM dendrimers were modified in other studies with hydroxyl end-groups or the syntheses were stopped at so-called half-generations, which resulted in ester group end-capped dendrons. It was noticed that such end-group modifications affect the solubility of the desired dendrimers, while greatly decreasing their toxicities and enabling their assessment for potential use in biological systems. Good examples in this regard are the studies on PAMAM end-group modifications by Calabretta and co-workers and Lopez and co-workers [15, 27]. In these studies, highly toxic G3 and
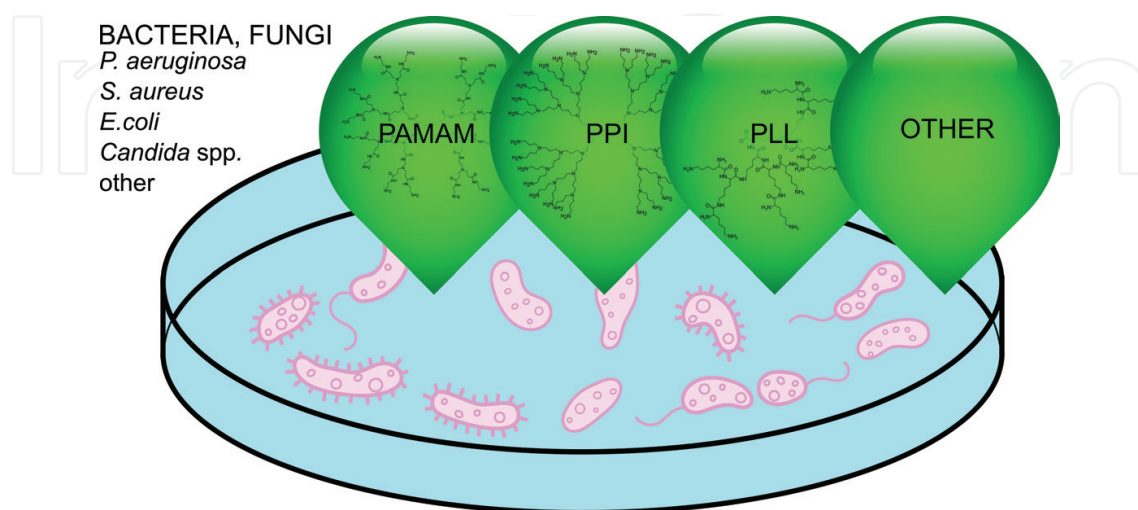
Figure 5. Schematic representation of dendrimer action on bacteria and fungi.

G5 PAMAM dendrimers with amino-terminated end-groups were partially substituted with poly(ethylene glycol) chains. Such PEGylated dendrimers expressed significantly lower toxicities, while antibacterial activities against *Pseudomonas aeruginosa* and *S. aureus* remained at a high level. In a similar manner, PAMAM dendrimers up to G4 were appended with aliphatic NO-releasing moieties [28, 29]. Such hybrids were tested on bacterial biofilms of *P. aeruginosa* [28, 29] and *S. aureus* [28] with positive results. It is worth noting that nitric oxide, which was additionally released, increased overall antibacterial activity. In addition, tested dendrimers revealed low-toxicity to mammalian cells.

A highly active but also toxic dendrimer is G4 PPI. Felczak *et al.* co-workers overcame its toxicity by attaching maltose to 25 or 100% of all the amino end-groups [16]. The modified maltose-appended PPI dendrimers were microbiologically assessed against *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa*, *Candida albicans* and found to be active against both bacteria and fungi with the lowered toxicity assessed against a Chinese hamster fibroblast cell line (B14), human liver hepatocellular carcinoma cell line (HepG2), mouse neuroblastoma cell line (N2a) and rat liver cell line (BRL-3A). The PPI G4 dendrimer modified with 25% of maltose was found superior to the 100% modified one in terms of antibacterial activity and demonstrated a striking selectivity towards *S. aureus* at the concentrations non-toxic for eukaryotic cell lines. As the continuation of this study, the G4 PPI dendrimer appended in 25% with maltose was used in combination with nadifloxacin against Gram-negative bacteria: *E. coli*, *P. aeruginosa*, *Proteus hauseri* revealing an increase in the antibacterial activity of the latter. Similar to the previous studies, the tested fluoroquinolone in dendrimer-complex was found to be less toxic than the drug alone [30]. In another study, unmodified G4 PPI complex with ciprofloxacin was found to increase quinolone activity at non-toxic concentrations to mammalian cells [31]. It is worth pointing out that other quinolones (nadifloxacin and prulifloxacin) were also complexed with dendrimers, like PAMAM (up to the 5th generation) [32]. Although, this combination did not enhance the potency of these drugs against *E. coli*, an increase in their solubility was observed.

Another type of dendrimers, poly(phosphorhydrazone) dendrimers appended with PEG chains were synthesized on a solid support provided by silica nanoparticles [33]. These composites were used for hosting silver-based nanoparticles and assessed on the basis of their antibacterial activity, which was found to reach Gram-negative (*E. coli*, *P. aeruginosa*) and Gram-positive (*S. aureus*, *Enterococcus hirae*) bacteria. Wang *et al.* conducted a similar experiment, although utilizing titanium dioxide-supported G5 PAMAMs that were also PEGylated [34]. However, no silver addition was needed as the titania-supported composite tested as the thin antibacterial film was found to inhibit growth of *S. aureus* and *P. aeruginosa*. Again, phosphorus-containing dendrimers were synthesized by Ciepluch *et al.* [35] who obtained viologen-phosphorus dendrons (viologen—4,4'-bipyridinium salts) built around a cyclotriphosphazene core, which were found to be active against Gram positive (*S. aureus*) and Gram negative (*E. coli*, *P. aeruginosa*, *Proteus vulgaris*) bacteria, as well as against *C. albicans*.

An amino acid-based dendrimer [36] was found to exhibit low toxicity and high antibacterial activity against usually resistant bacterial strains of *Acinetobacter baumanii* and *P. aeruginosa*, including multidrug-resistant and extensively drug-resistant strains. Abd-El-Aziz *et al.* published the synthesis of G0-G2 dendrimers built of melamine core, arene-cyclopentadienyliron-based

dendrons and piperazine end-groups [37]. These dendrimers were assessed against drug-resistant bacteria methicillin-resistant *S. aureus* (MRSA), *Staphylococcus warneri* and vancomycin-resistant *Enterococcus faecium* (VRE). It is noted that the G2 piperazine end-capped dendrimer was the most active. Interesting in its structure was polyanionic ether-based G1 dendrimer with lipophilic core, which was highly active against Gram-positive bacteria—*Bacillus subtillis* [38]. The noteworthy point is that this dendrimer was found to exhibit low toxicity to healthy human cells. Carbosilane dendrimers have also been found to be potent antimicrobials. Fuentes-Paniagua *et al.* synthesized and investigated microbiological evaluation of dendrimers up to the 2nd generation with different terminal groups, which revealed promising MIC and MBC values against *S. aureus* and *E. coli* [39].

Quite a lot of attention has also been given to so-called antimicrobial peptides. These are short, naturally occurring peptides that exhibit high antimicrobial activity. Problems associated with the use of these compounds are related to their susceptibility to bacterial enzymes and a not fully recognized mechanism of action. Reports published on this subject regarding antibacterial activity of synthetic short dendrimeric peptides suggest the high potential of such an approach. Lind *et al.* synthesized modified amino acid-based low-generation dendrimers [40]. The compounds obtained in this study were assessed for their action against *S. aureus*, *E. coli* and *P. aeruginosa* and proven active. At the same time, some hemotoxicity was observed. Structurally similar peptide appended PLL dendrimers were the subject of a study performed by Bruschi *et al.* [41] who synthesized a functionalized dendrimer that showed promising potential as an antibacterial agent interacting with bacterial lipopolysaccharide. It exhibited quite good activity against various Gram-positive strains and especially high activity against Gram-negative strains with potency similar to lipopeptide antibiotics like colistin and polymixin B. One of the most promising activities of dendrimers against pathogenic fungi was reported by Staniszewska *et al.* [42] who designed and synthesized an amino acid-based dendrimer end-capped with tyrosine moieties for specific action against fungi. This dendrimer was found not only to be active against reference and clinical strains but also affected the virulence factors of *Candida* species.

Another approach of utilizing dendrimers in the fight against bacteria is to combine them with other structures or compounds. In this way, PAMAMs were combined with multiwalled carbon nanotubes and CdS or Ag_2S quantum dots to form novel hybrid materials [43]. Nanohybrids were found to be highly active against bacteria and the activity was found to be just as high or higher than for each component alone. As a continuation of this study, authors functionalized the surface of multiwalled carbon nanotubes with polyamide dendrons [44]. This composite material was used for synthesis of silver nanoparticles and then applied as a carrier for these. Organic–inorganic hybrid was assessed and proven effective as antimicrobial against *E. coli*, *P. aeruginosa* and *S. aureus*. PAMAM dendrimers were also numerously tested with metals and their ions. The G2 and G3 PAMAMs were utilized as a site for synthesis of silver nanoparticles [45]. After grafting cotton with G2 PAMAM-nanosilver complex, the material exhibited bactericidal activity against *S. aureus* and *E. coli*. In other studies, utilizing polyester dendrimers for nano-silver formation, it was shown that the antibacterial activity of the resulting dendrimer-silver nanoparticles was strongly dependent on the dendrimer generation [46]. Noteworthy is that nanoparticles that were stabilized by dendrimers exhibited

no toxic effect on human epithelial cell line A549 as has been proven using 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Another nanosized composite was developed by Strydom *et al.* [47]. They synthesized silver sulfadiazine nanoparticles stabilized with the addition of G1, G3.5, G4 or G4.5 PAMAM dendrimers. Such nanoparticles in topical formulations were evaluated in terms of their antibacterial activity. The use of dendrimers improved the activity of resulting material against *Staphylococcus* spp., *E. coli* and *P. aeruginosa*. Another interesting work was published by Staneva and co-workers [48]. They managed to append G1 PPI dendrimer with naphthalimide derivative. This nanostructure was found to create complex with bivalent ions, such as zinc and copper. Both metal ion complexes exhibited activity against Gram-negative and Gram-positive bacteria, as well as pathogenic fungi. A further application of dendrimers was proposed by Zainul Abid and co-workers, who applied PAMAM for water disinfection [49]. The antibacterial activity (*E. coli*, *S. aureus*, *P. aeruginosa*, *Bacillus subtilis*) of the G1 dendrimer was increased by polymerizing it with ethyleneglycol dimethacrylate to form polydendrimeric network. The resultant polymer exhibited very high antibacterial activity at 10 mg/L in a matter of minutes. The amount of 100% bacteria was eradicated within 2 minutes of incubation. Gangadharan *et al*. utilized dendrimers for water disinfection synthesizing them on a solid support [50]. They applied a divergent synthetic pathway to dendrimeric poly-ethylene amine supported on polystyrene copolymer beads and assessed its potential activity against both Gram-positive (*S. aureus*, *B. subtilis*) and negative (*E. coli*, *P. aeruginosa*) bacteria. A decrease in bacterial cell viability was seen, yet the effect was strongly dependent on the generation of supported dendrimers and their end-group functionalization.

Almost all dendrimers described in this chapter derive their high antibacterial and antifungal activity from the so-called starburst effect. Dendrimers mentioned earlier are characterized by the exponential growth of the number of terminal groups. Such a rapid increase in the number of active sites of small molecules (by means of their volume) is the result of this phenomenon. In case of dendrimers as antimicrobial drug carriers, dendrimeric formulations are often just as effective or even more so on pathogens as a drug used alone. Use of dendrimers usually results in prolonged release of the drug with simultaneously decreased toxicity comparing to the parent compound. This can be clearly seen for a plethora of drug molecules. For example, such a study was performed for sulfamethoxazole, which is a poorly soluble sulfonamide. Its solubility increased in the formulations prepared with PAMAM dendrimers [51]. This increase was generation-dependent. As the result of this change, an increase of sulfamethoxazole antibacterial activity and sustained release of the drug were observed. Navath *et al.* carried out a study with amoxicillin in modified G4 PAMAM formulation, which was found to be non-toxic and enabled a sustained release of the drug *in vitro* [52]. The study was enhanced to *in vivo* assessment on pregnant guinea pigs. The intravaginal dendrimer-amoxicillin biodegradable formulation was found not to cause such adverse effects as necrosis or inflammatory response in deeper tissues. Also, no pH change in application site was observed nor was any transfer across the foetal membranes noticed. Amoxicillin was also the subject of the report by Wrońska *et al.* [53]. However, in this case the G3 PPI, either unmodified or maltose end-capped was studied. Because of toxicity related to unmodified PPI dendrimers, results obtained for maltose terminated PPIs are of significance. Antibacterial activity of drugs increased by the application of the above mentioned combination. Another drug molecule of antimicrobial activity that was

successfully solubilized by PAMAMs was triclosan [54]. Although this formulation has not been tested on bacteria, the amount of drug solubilized was remarkable and—as found in this report—highly dependent on the pH of the analysed solution. Various interesting topical formulations were prepared by Wroblewska and Winnicka with the use of erythromycin and G2 or G3 PAMAM dendrimers [55]. The formulations were optimized and their utility for therapy proven by *in vitro* assessment against *Enterococcus faecalis* and different strains of *S. aureus*. The same group continued their research with erythromycin and PAMAMs by attempting to increase the antibiotic water-solubility [56]. In this study, PAMAM dendrimers were used to solubilize erythromycin and tobramycin to form stable water solutions. While this goal was achieved, a slight increase in antibacterial activity against Gram-positive bacteria (in this case, *S. aureus* and *E. faecalis*) by means of MBC of solubilized erythromycin was observed. However, tobramycin used with dendrimers exhibited unchanged or lower MICs and MBCs when compared to the drug alone. One of the latest reports describes the development of the G4 PAMAM containing lipid hybrid nanoparticle capable of transporting one of the most potent last-resort antibiotics—vancomycin [57]. The results clearly indicate that the use of dendrimer carrier increases vancomycin potency against MRSA. Moreover, Skwarczynski *et al.* developed a G1 triazole-based dendrimers, which were end-capped with B-cell epitope of *Streptococcus pyogenes* [19]. Such potential vaccine for *Straphyloccus* infection was found to be self-adjuvating by forming micelle-like assemblies with dendrimer core, branches in the centre and peptides on the periphery. Apart from of immunization properties, the authors suggest, that such approach may be applied to other peptides in the preparation of vaccines for various diseases.

3.2. Dendrimers for treatment and prevention of virus-related infection

Dendrimers have been applied for treatment and prevention of virus-related infections (**Figure 6**). The best-known dendrimer acting in an antiviral manner is probably SPL7013, discovered by Starpharma, which is the active ingredient of VivaGel [58]. It is a G4 PLL dendrimer with functionalized end-groups used in the form of a gel and marketed as condom lubrication. SPL7013 successfully underwent second-stage clinical trials and was found to prevent sexually transmitted diseases, most notably *Human immunodeficiency virus 1* (HIV-1), *Herpes simplex virus* (HSV) and *Human papilloma virus* (HPV). Recently, Starpharma claimed to obtain results indicating prevention of Zika virus infection. Sánchez-Rodríguez *et al.* synthesized second-generation carbosilane dendrimers, functionalized on the periphery with highly anionic sulfonate groups or carboxy groups [59]. Both synthesized dendrimers when applied to the vagina-derived human epithelial VK2/E6E7 cell lines and peripheral blood mononuclear cells before exposure to HIV decreased its infectivity. The test conditions also included physiological pH to prove their potential utility in protection against *in vivo* sexually transmitted HIV infection. Authors discussed the superior activity of carbosilane dendrimers as compared to PAMAMs, as carbosilane dendrimers do not induce inflammatory reaction, which influences the infectivity of HIV. Structurally related compounds were also the subject of investigation by Vacas-Córdoba and co-workers [60]. They synthesized the G1 carbosilane dendrimer appended with sulfonated naphthyl groups and G2 carbosilane dendrimer with sulphated end-groups and then assessed their action on HIV and its infectivity. The results suggested that developed dendrimers decrease the infectivity rate of pre-treated peripheral
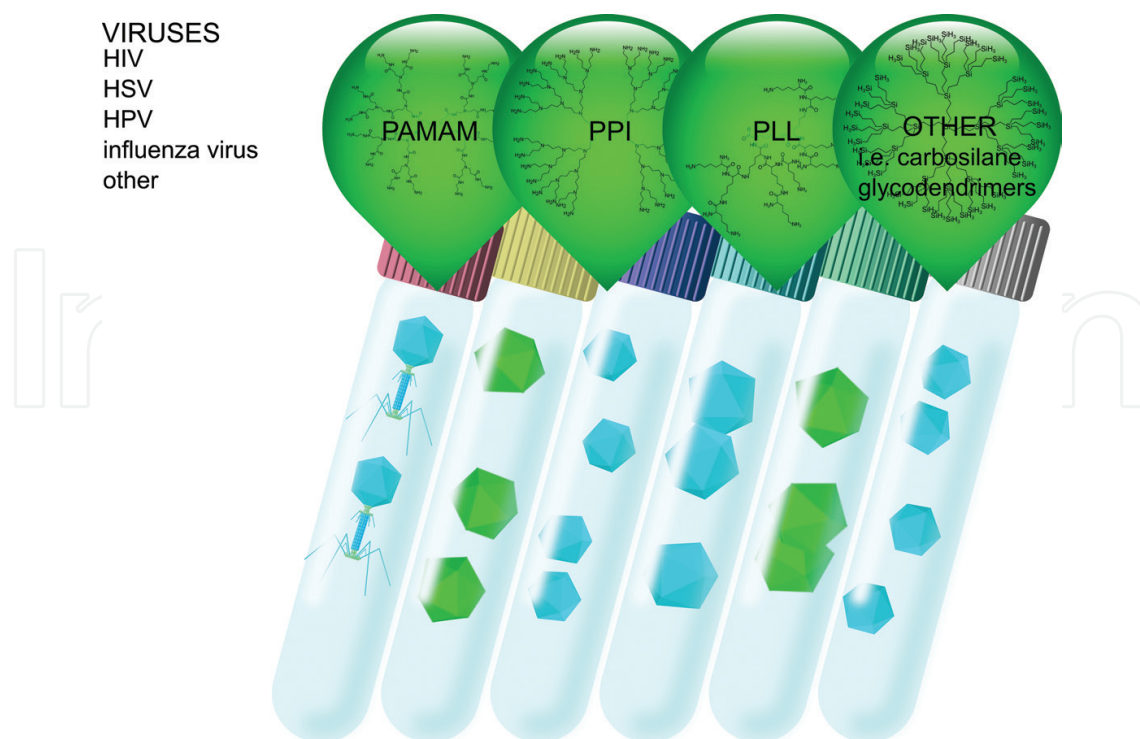
Figure 6. Schematic representation of dendrimer action on viruses.

blood mononuclear cells when exposed to the virus. Authors concluded that poly-anionic dendrimers act by electrostatic interaction with HIV proteins inhibiting the ability of the virus to bind to target cells and thus its ability to internalize. In addition, the cell-to-cell transmission was impaired, as assessed in a different test by co-culturing infected cells with healthy TZM. bl cells (modified HeLa cell line especially prone to HIV infection). High capability to prevent infection by the G1 dendrimer was also presented in the presence of seminal fluid [61].

Carbosilane dendrimers were also investigated in terms of other potential antiviral applications [62, 63]. Knowing that dendrimers are excellent non-viral transfection agents, two polycationic G1 carbosilane dendrimers with different cores were assessed for gene therapy in order to inhibit the development of ongoing HIV infection. Both dendrimers were found to exhibit properties making them suitable for their planned use. Their non-toxicity was confirmed (MTT assay). They were able to form complexes with nucleic acids and—as siRNA complexes—inhibit replication of HIV-1 and affect macrophage response, thus encouraging further study on this subject.

Antiviral PPI dendrimers were also assessed for potential anti-HIV treatment [64]. In this case, dendrimers up to their third generation were modified on the periphery with anionic groups such as carboxylate or sulfonate functional groups. Modified dendrimers were used to complex bivalent metal ions: Cu^{2+}, Ni^{2+}, Co^{2+} or Zn^{2+}. Metal complexes were assessed for their HIV infection potential applying *in vitro* models of first and second infection barriers. Complexes exhibited high inhibition rates and prevented internalization of HIV-1. Inhibition of the virus replication was also observed. Han *et al.* synthesized a G3 PLL built on the core of ammonia and functionalized with sulphated cellobiose as terminal groups [65]. The dendrimer was found to be non-toxic and

active against HIV, as revealed by *in vitro* MTT assay. Replacing ammonia core with long alkyl stearylamide chain resulted in obtaining dendrimer with comparable EC_{50} values [66]. Recently Ceña-Díez *et al.* published a review paper dealing with the topic of various dendrimers expressing antiviral activity against heparan sulphate-related viral diseases caused by HSV, HIV, HPV, *Hepatitis C virus* and *Human respiratory syncytial virus* [67]. The authors concluded that all the dendrimers analysed (i.e. amino acid-based dendrimers, glycodendrimers, PAMAMs, carbosilane dendrimers) were acting by means of preventing the virus entry to the cell.

Dendrimers have been also assessed as potential vaccine carriers. For an excellent review on this subject, the paper by Heegaard *et al.* is recommended [68]. A very interesting study on this subject was performed by Chahal *et al.* who modified a G1 PAMAM dendrimer with long aliphatic chains [69]. Such functionalized dendrimer among other vaccine components was used to prepare a formulation for transporting mRNA chains. In this way, a vaccine was developed that was capable of providing protection with a one-dose application from H1N1 influenza virus, *Toxoplasma gondii* and Ebola virus. This was confirmed by performing *in vivo* experiment and by comparing the results with bare mRNA vaccination. PAMAM formulation was proven to increase stability of the vaccine by protecting nucleic acids from RNAses. The vaccine was stable even after 30 days of storage. In another study, a vaccine against rabies was developed by Ullas *et al.* [70] who used G3 poly-ether imine dendrimer to prepare a formulation of rabies virus glycoprotein gene. The efficiency of the formulation was evaluated by measuring the rabies virus neutralizing antibody levels in treated mice. Addition of dendrimer resulted in 4.5-fold greater increase and thus all the mice that underwent rabies virus challenge survived, while in the group treated with formulation without dendrimer only 60% viability was observed. PLL dendrimers were the subject of research by Blanco *et al.* to create a suitable vaccination for swine from foot-and-mouth disease virus [71]. They prepared G0 and G1 PLL dendrimers with T-epitope as a core and B-epitopes as terminal groups. Such prepared functionalized dendrimer was found to offer 100% protection in virus challenge.

A quite different approach was undertaken by Yandrapu *et al.* [72] who exploited the drug delivering properties of dendrimers. The G3.5 PAMAM was modified in the periphery with cysteamine. Modified dendrimers were loaded with acyclovir, a known antiviral, and assessed *in vitro* by means of drug release and mucoadhesive properties of the formulation. As in most cases, use of dendrimer formulation resulted in sustained release of the drug. Although unmodified dendrimer was characterized by higher acyclovir loading, presence of thiol groups was crucial for increase of bio-adhesion. In a different study, G4 PPI dendrimers were modified in the periphery by mannosylation and conjugation with sialic acid [73]. Functionalized dendrimer was used as a carrier for zidovudine. This complex was found to be more effective than PPI functionalized with only sialic acid or mannose and was also found non-toxic as well as zidovudine being released in a prolonged manner. Dendrimers with antimicrobial activity and for vaccination are also of commercial value as can be seen by a number of patents on the subject [74].

3.3. Dendrimers in fighting off the parasitic/protozoa infection

The action of dendrimers on protozoa and parasitic infections is mostly unexplored areas in comparison with their antiviral and antibacterial activity (**Figure 7**). There is also only limited

data on the drug delivery of compounds for treating such infections using dendrimer carriers. Below is a summary and discussion of some reports on this subject.

Heredero-Bermejo *et al.* published a report regarding anti-trophozoite *Acanthamoeba castellanii* activity of G0 and G1 carbosilane dendrimers with ammonium salts as terminal groups [75]. It was found that G1 dendrimer had the best effect on *Acanthamoeba*, with LD_{50} at 0.193 μg/μL after 24 hours of exposure.

Sulfadiazine is one of the drugs used for treatment of *T. gondii* infections. For a more efficient method of this drug delivery, Prieto *et al.* assessed the possibility of using PAMAMs for its delivery [76]. They used commercially available G4 and G4.5 PAMAM dendrimers and loaded them with sulfadiazine. Such complexes were assessed by means of their drug loading capabilities, toxicity and *in vitro* activity. Each dendrimer was complexing up to 30 molecules of drug. Dendriplexes decreased the infection, with the highest decrease for G4 PAMAM-sulfadiazine at 30 nM concentration. In toxicity assessment, the G4.5 complex was found non-toxic, whilst G4 (amino end-capped) was proven toxic at low concentrations, as shown in MTT assay with Vero cells and murine macrophage-like cell line J774. In other studies, researchers focused on Amphotericin B (AmB), which is a drug that possesses anti-leishmanial activity. Jain *et al.* [77] evaluated the G5 PPI with muramyl dipeptide-modified terminal groups for its potential use as AmB carrier for treatment of leishmaniosis. Such formulation was compared to AmB commercial formulations: *Fungizone* and *AmBisome* assessing its toxicity and antiparasitic activity. The results indicated that this dendrimer significantly reduced the haemolytic toxicity of AmB to human erythrocytes, as well as cytotoxicity against J774A.1 macrophage cell line as shown
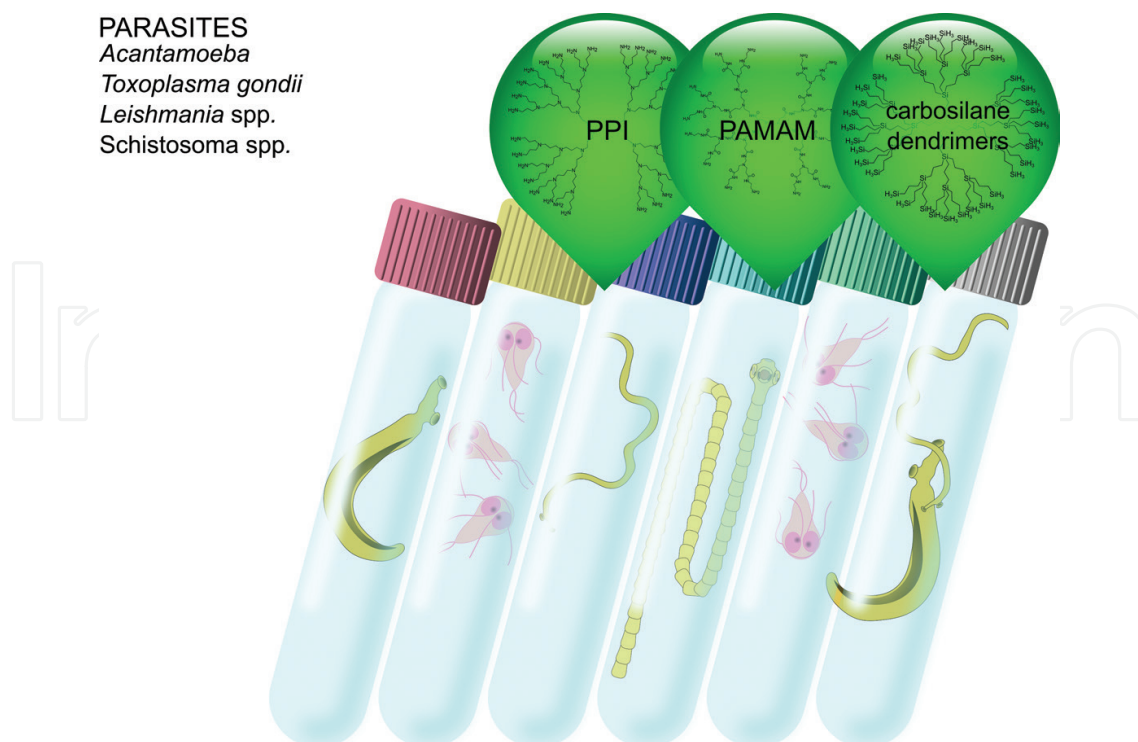
Figure 7. Schematic representation of dendrimer action on parasitic/protozoa infections.

by results of MTT assay. Simultaneously, G5 PAMAM-AmB formulation outruns commercial formulations, as can be seen in tests against *Leishmania donovani*. Jain *et al.* continued their efforts for finding a dendrimer capable of delivering AmB [78]. They reported G5 PPI dendrimer partially end-capped with mannose groups. Dendrimer revealed good loading capacity and AmB release was pH-dependent, as faster release was observed in acidic conditions. Haemolytic toxicity (human erythrocytes) and cytotoxicity (J774A.1 macrophage cell lines in MTT assay) of sole dendrimer and AmB-loaded dendrimer were assessed and the obtained data revealed a decreased toxicity of AmB-dendriplexes, negligible haemotoxicity and no cytotoxicity of dendrimer. Experiments on BALB/c mice were then conducted to determine *in vivo* properties: formulation pharmacokinetics, biodistibution, haematological toxicity and nephrotoxicity. These studies demonstrated that G5 PPI-mannose conjugate is a low toxic AmB formulation, providing prolonged release of carried drug. AmB was also the object of interest for Daftarian *et al.* [79]. They prepared G5 PAMAM bound to a targeting peptide—Pan-DR-binding epitope. Subsequently, such modified dendrimer was complexed with liposomal AmB in different ratios. Formation of a desired complex was confirmed by DLS analysis and electron microscopy. Cytotoxicity was tested on Hep2 cells and it was proven to be low. An *in vitro* and later *in vivo* study on infected BALB/c and C57BL mice was performed to assess therapeutic efficacy against *Leishmania major* infection. In the anti-leishmanial activity assessment, a six-fold decrease in AmB dose for the same therapeutic effect was observed. Due to the designed peptide terminal groups, AmB was more likely to accumulate in targeted cells.

It is worth noting that Wang *et al.* prepared a vaccine for *Schistosoma japonicum* infection [80]. To achieve this, they have end-capped a G4 PAMAM dendrimer with lysine, which greatly decreased the cytotoxicity to human kidney transformed cells 293T. The G4 PAMAM-lysine was then used as a carrier for DNA vaccine, namely SjC23, and *in vivo* assessment on BALB/c mice was conducted as a *S. japonicum* challenge after three prior immunizations. Dendrimer-based vaccine was found to reduce worm levels up to 50% and liver eggs by up to 62%.

3.4. Dendrimers for sensing of infective microbes

Dendrimers were also considered as components of microbial sensing devices (**Figure 8**). Use thereof as bacterial and viral presence has been reviewed by Satija *et al.* [81]. There is still not much known regarding fungi sensing, as only few reports have been published to date. Castillo *et al.* developed a system of indirect detection of fungal presence by sensing aflatoxin B_1—a known fungal toxin [82]. The system was built of gold electrode coupled with sequential monolayers of cystamine, glutaraldehyde, G4-PAMAM, glutaraldehyde and aptamers (synthetic receptors). Dendrimers used to construct this sensor provided the signal amplification, enabling the detection of aflatoxin B_1 in the concentration range 0.1–10 nmol. Use of different mycotoxins proved that the detection system was specific only for aflatoxin B_1 as the response was concentration-dependent in this case only. The second and last report regarding fungi sensing with the help of dendrimers was published by Mejri-Omrani *et al.* [83]. The authors described a system prepared for detection of ochratoxin A. The sensor was prepared with the use of G4 PAMAM dendrimers and found to detect ochratoxin A in concentration up to 5 μg/L with detection limit 2 ng/L. Developed sensor utility was confirmed in real food products tests.
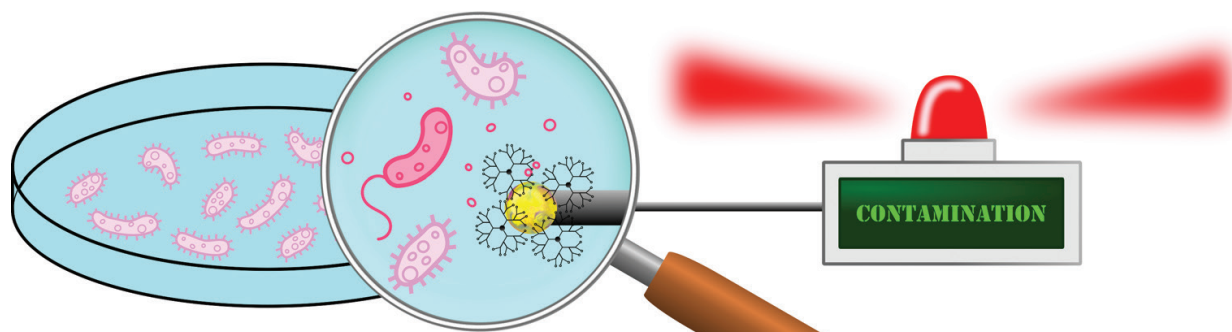
Figure 8. Schematic representation of dendrimer sensing.

Use of dendrimers for sensing parasitic presence was assessed by Perinoto *et al.* who developed a device suitable for sensing *Leishmania* infections in infected BALB/c mice serum samples [84]. The sensor was built by depositing in sequence several layers of G4 PAMAMs and liposomes containing antigenic proteins on a gold electrode. The device was able to detect *Leishmania amazonensis* antibodies in concentration as low as 10^{-5} mg/mL and the specificity of the sensor allowed it to distinguish between leishmaniasis and *Trypanosoma cruzi* infection.

4. Conclusions and perspectives

In recent years, dendrimers, which represent a distinct class of polymers, have gained considerable attention from medicinal chemists and pharmaceutical technologists, mostly due to their known and potential applications for medicine. Although there are still many unresolved issues on the topic of dendrimers, our goal in this review was to discuss selected modifications of dendrimers dedicated to the prevention and fighting of infections caused by bacteria, fungi, viruses and parasites.

A great increase of dendrimer-related research is to some extent bound to their commercial availability (mostly PAMAMs) as well as novel and efficient methods of their synthesis. In this regard, the development and commercial availability of various innovative building blocks for the synthesis of full-grown dendrimers is especially important. Dendrimer chemistry continues to develop year by year and many research groups and companies are interested in studying their properties and potential uses. In this chapter, many potential and practical applications of dendrimers in prevention of diseases, diagnostics of microbes have been discussed.

Based on the reviewed literature, dendrimers have proven to be useful in many ways. The starburst effect of nanopolymers obtained is magnified exponentially, resulting in unprecedented outcomes. A plethora of various studies on PAMAMs revealed how the dendrimers activity and toxicity changes upon a slight modification in their structure. Formation of dendriplexes quite often increases the biological activity of both dendrimer and encapsulated drug molecules. Furthermore, discoveries in the field of dendrimers encourage various studies in pharmaceutical technology on poor water-soluble APIs, which otherwise would not be considered useful for clinical practice. Moreover, there is still much to be done in regard

to Gram-negative bacteria. Because of differences in cell wall structure, they are not as susceptible to anti-bacterials and disinfectants as their Gram-positive counterparts. In addition, studies published on dendrimers do not point out their mechanism of action. Based on the non-specific action of dendrimers on bacteria and fungi, one cannot assume that dendrimers always exhibit an identical mechanism of action on these organisms.

Only a few reports deal with the subject of infective fungi and there is almost none regarding the parasitic and protozoan infections. Systemic fungal infections are on the rise in developed countries because of the increasing use of immunosuppressive drugs after transplantations and due to opportunistic infections associated with HIV infected people suffering from AIDS. In terms of viruses, the research on the application of dendrimers for the improvement of vaccines is very important. As for parasites, encouraging are studies aimed at developing diagnostic methods for the detection of these organisms. Generally, high possibilities of modifying dendrimer core, branches and terminal groups, as well as development of methods on combining them with other active moieties make them unique and highly promising molecules for future use.

Acknowledgements

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References

[1] Buhleier E, Wehner W, Vögtle F. "Cascade"- and "nonskid-chain-like" syntheses of molecular cavity topologies. Synthesis 1978; 1978:155-8. doi:10.1055/s-1978-24702

[2] Braine T. Race against time to develop new antibiotics. Bull World Health Organ 2011; 89: 88-9. doi:10.2471/BLT.11.030211

[3] Hof H. Will resistance in fungi emerge on a scale similar to that seen in bacteria? Eur J Clin Microbiol Infect Dis 2008; 27: 327-34. doi:10.1007/s10096-007-0451-9

[4]   Collins TL, Markus EA, Hassett DJ, Robinson JB. The effect of a cationic porphy-
      rin on *Pseudomonas aeruginosa* biofilms. Curr Microbiol 2010; 61: 411-6. doi:10.1007/
      s00284-010-9629-y

[5]   Astruc D, Boisselier E, Ornelas C. Dendrimers designed for functions: from physical,
      photophysical, and supramolecular properties to applications in sensing, catalysis,
      molecular electronics, photonics, and nanomedicine. Chem Rev 2010; 110: 1857-959.
      doi:10.1021/cr900327d

[6]   Campagna S, editor. Designing dendrimers. Hoboken, NJ: Wiley; 2012.

[7]   Fréchet JMJ, Tomalia DA, editors. Dendrimers and other dendritic polymers. John Wiley
      & Sons, Ltd; Baffins Lane, Chichester, West Sussex, 2001.

[8]   Setaro F, Ruiz-González R, Nonell S, Hahn U, Torres T. Synthesis, photophysical studies
      and $^1O_2$ generation of carboxylate-terminated zinc phthalocyanine dendrimers. J Inorg
      Biochem 2014; 136: 170-6. doi:10.1016/j.jinorgbio.2014.02.007

[9]   Wieczorek E, Piskorz J, Popenda L, Jurga S, Mielcarek J, Goslinski T. First example of
      a diazepinoporphyrazine with dendrimeric substituents. Tetrahedron Lett 2017; 58:
      758-761. doi:10.1016/j.tetlet.2017.01.027

[10] Mlynarczyk DT, Lijewski S, Falkowski M, Piskorz J, Szczolko W, Sobotta L, et al.
      Dendrimeric sulfanyl porphyrazines: synthesis, physico-chemical characterization, and
      biological activity for potential applications in photodynamic therapy. ChemPlusChem
      2016; 81: 460-70. doi:10.1002/cplu.201600051

[11] Falkowski M, Rebis T, Piskorz J, Popenda L, Jurga S, Mielcarek J, et al. Improved electro-
      catalytic response toward hydrogen peroxide reduction of sulfanyl porphyrazine/multi-
      walled carbon nanotube hybrids deposited on glassy carbon electrodes. Dyes Pigm 2016;
      134: 569-79. doi:10.1016/j.dyepig.2016.08.014

[12] Tillo A, Stolarska M, Kryjewski M, Popenda L, Sobotta L, Jurga S, et al. Phthalocyanines
      with bulky substituents at non-peripheral positions: synthesis and physico-chemical
      properties. Dyes Pigm 2016; 127: 110-5. doi:10.1016/j.dyepig.2015.12.017

[13] Wang M, Wang Y, Hu K, Shao N, Cheng Y. Tumor extracellular acidity activated "off–
      on" release of bortezomib from a biocompatible dendrimer. Biomater Sci 2015; 3: 480-9.
      doi:10.1039/C4BM00365A

[14] Maiti PK, Çagın T, Wang G, Goddard WA. Structure of PAMAM dendrimers: genera-
      tions 1 through 11. Macromolecules 2004; 37: 6236-54. doi:10.1021/ma035629b

[15] Calabretta MK, Kumar A, McDermott AM, Cai C. Antibacterial activities of
      poly(amidoamine) dendrimers terminated with amino and poly(ethylene glycol)
      groups. Biomacromolecules 2007; 8: 1807-11. doi:10.1021/bm0701088

[16] Felczak A, Wrońska N, Janaszewska A, Klajnert B, Bryszewska M, Appelhans D, et al.
      Antimicrobial activity of poly(propylene imine) dendrimers. New J Chem 2012; 36: 2215.
      doi:10.1039/c2nj40421d

[17] Hamilton PD, Jacobs DZ, Rapp B, Ravi N. Surface hydrophobic modification of fifth-generation hydroxyl-terminated poly(amidoamine) dendrimers and its effect on biocompatibility and rheology. Materials 2009; 2: 883-902. doi:10.3390/ma2030883

[18] Najlah M, D'Emanuele A. Synthesis of dendrimers and drug-dendrimer conjugates for drug delivery. Curr Opin Drug Discov Devel 2007; 10: 756-67.

[19] Skwarczynski M, Zaman M, Urbani CN, Lin I-C, Jia Z, Batzloff MR, et al. Polyacrylate dendrimer nanoparticles: a self-adjuvanting vaccine delivery system. Angew Chem Int Ed 2010; 49: 5742-5. doi:10.1002/anie.201002221

[20] Majoros IJ, Myc A, Thomas T, Mehta CB, Baker JR. PAMAM dendrimer-based multifunctional conjugate for cancer therapy: synthesis, characterization, and functionality. Biomacromolecules 2006; 7: 572-9. doi:10.1021/bm0506142

[21] Albertazzi L, Mickler FM, Pavan GM, Salomone F, Bardi G, Panniello M, et al. Enhanced bioactivity of internally functionalized cationic dendrimers with PEG cores. Biomacromolecules 2012; 13: 4089-97. doi:10.1021/bm301384y

[22] Klajnert B, Peng L, Cena V, editors. Dendrimers in biomedical applications; RSC Publishing, Cambridge, 2013.

[23] Tülü M, Ertürk AS. Dendrimers as antibacterial agents 2012. In: Bobbarala V, editor. A search for antibacterial agents. 1st ed. Rijeka: Intech. p. 89-106. doi:10.5772/46051

[24] Mintzer MA, Dane EL, O'Toole GA, Grinstaff MW. Exploiting dendrimer multivalency to combat emerging and re-emerging infectious diseases. Mol Pharm 2012; 9: 342-54. doi:10.1021/mp2005033

[25] Xue X, Chen X, Mao X, Hou Z, Zhou Y, Bai H, et al. Amino-terminated generation 2 poly(amidoamine) dendrimer as a potential broad-spectrum, nonresistance-inducing antibacterial agent. AAPS J 2013; 15: 132-42. doi:10.1208/s12248-012-9416-8

[26] Pryor JB, Harper BJ, Harper SL. Comparative toxicological assessment of PAMAM and thiophosphoryl dendrimers using embryonic zebrafish. Int J Nanomed 2014; 9: 1947-56. doi:10.2147/IJN.S60220

[27] Lopez AI, Reins RY, McDermott AM, Trautner BW, Cai C. Antibacterial activity and cytotoxicity of PEGylated poly(amidoamine) dendrimers. Mol Biosyst 2009; 5: 1148. doi:10.1039/b904746h

[28] Worley BV, Schilly KM, Schoenfisch MH. Anti-biofilm efficacy of dual-action nitric oxide-releasing alkyl chain modified poly(amidoamine) dendrimers. Mol Pharm 2015; 12: 1573-83. doi:10.1021/acs.molpharmaceut.5b00006

[29] Lu Y, Slomberg DL, Shah A, Schoenfisch MH. Nitric oxide-releasing amphiphilic poly(amidoamine) (PAMAM) dendrimers as antibacterial agents. Biomacromolecules 2013; 14: 3589-98. doi:10.1021/bm400961r

[30]   Felczak A, Zawadzka K, Wrońska N, Janaszewska A, Klajnert B, Bryszewska M, et al. Enhancement of antimicrobial activity by co-administration of poly(propylene imine) dendrimers and nadifloxacin. New J Chem 2013; 37: 4156. doi:10.1039/c3nj00760j

[31]   Wrońska N, Felczak A, Zawadzka K, Janaszewska A, Klajnert B, Bryszewska M, et al. The antibacterial effect of the co-administration of poly(propylene imine) dendrimers and ciprofloxacin. New J Chem 2014; 38: 2987. doi:10.1039/c3nj01338c

[32]   Cheng Y, Qu H, Ma M, Xu Z, Xu P, Fang Y, et al. Polyamidoamine (PAMAM) dendrimers as biocompatible carriers of quinolone antimicrobials: an in vitro study. Eur J Med Chem 2007; 42: 1032-8. doi:10.1016/j.ejmech.2006.12.035

[33]   Hameau A, Collière V, Grimoud J, Fau P, Roques C, Caminade A-M, et al. PPH dendrimers grafted on silica nanoparticles: surface chemistry, characterization, silver colloids hosting and antibacterial activity. RSC Adv 2013; 3: 19015. doi:10.1039/c3ra43348j

[34]   Wang L, Erasquin UJ, Zhao M, Ren L, Zhang MY, Cheng GJ, et al. Stability, antimicrobial activity, and cytotoxicity of poly(amidoamine) dendrimers on titanium substrates. ACS Appl Mater Interfaces 2011; 3: 2885-94. doi:10.1021/am2004398

[35]   Ciepluch K, Katir N, Kadib A El, Felczak A, Zawadzka K, Weber M, et al. Biological properties of new viologen-phosphorus dendrimers. Mol Pharm 2012; 9: 448-57. doi:10.1021/mp200549c

[36]   Pires J, Siriwardena TN, Stach M, Tinguely R, Kasraian S, Luzzaro F, et al. *In vitro* activity of the novel antimicrobial peptide dendrimer G3KL against multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2015; 59: 7915-8. doi:10.1128/AAC.01853-15

[37]   Abd-El-Aziz AS, Abdelghani AA, El-Sadany SK, Overy DP, Kerr RG. Antimicrobial and anticancer activities of organoiron melamine dendrimers capped with piperazine moieties. Eur Polym J 2016; 82: 307-23. doi:10.1016/j.eurpolymj.2016.04.002

[38]   Meyers SR, Juhn FS, Griset AP, Luman NR, Grinstaff MW. Anionic amphiphilic dendrimers as antibacterial agents. J Am Chem Soc 2008; 130: 14444-5. doi:10.1021/ja806912a

[39]   Fuentes-Paniagua E, Hernández-Ros JM, Sánchez-Milla M, Camero MA, Maly M, Pérez-Serrano J, et al. Carbosilane cationic dendrimers synthesized by thiol-ene click chemistry and their use as antibacterial agents. RSC Adv 2014; 4: 1256-65. doi:10.1039/C3RA45408H

[40]   Lind T, Polcyn P, Zielinska P, Cárdenas M, Urbanczyk-Lipkowska Z. On the antimicrobial activity of various peptide-based dendrimers of similar architecture. Molecules 2015; 20: 738-53. doi:10.3390/molecules20010738

[41]   Bruschi M, Pirri G, Giuliani A, Nicoletto SF, Baster I, Scorciapino MA, et al. Synthesis, characterization, antimicrobial activity and LPS-interaction properties of SB041, a novel dendrimeric peptide with antimicrobial properties. Peptides 2010; 31: 1459-67. doi:10.1016/j.peptides.2010.04.022

[42] Staniszewska M, Bondaryk M, Zielińska P, Urbańczyk-Lipkowska Z. The in vitro effects of new D186 dendrimer on virulence factors of *Candida albicans*. J Antibiot (Tokyo) 2014; 67: 425-32.

[43] Neelgund GM, Oki A, Luo Z. Antimicrobial activity of CdS and Ag$_2$S quantum dots immobilized on poly(amidoamine) grafted carbon nanotubes. Colloids Surf B Biointerfaces 2012; 100: 215-21. doi:10.1016/j.colsurfb.2012.05.012

[44] Neelgund GM, Oki A. Deposition of silver nanoparticles on dendrimer functionalized multiwalled carbon nanotubes: synthesis, characterization and antimicrobial activity. J Nanosci Nanotechnol 2011; 11: 3621-9. doi:10.1166/jnn.2011.3756

[45] Tang J, Chen W, Su W, Li W, Deng J. Dendrimer-encapsulated silver nanoparticles and antibacterial activity on cotton fabric. J Nanosci Nanotechnol 2013; 13: 2128-35. doi:10.1166/jnn.2013.6883

[46] Mahltig B, Cheval N, Astachov V, Malkoch M, Montanez MI, Haase H, et al. Hydroxyl functional polyester dendrimers as stabilizing agent for preparation of colloidal silver particles—a study in respect to antimicrobial properties and toxicity against human cells. Colloid Polym Sci 2012; 290: 1413-21. doi:10.1007/s00396-012-2650-x

[47] Strydom SJ, Rose WE, Otto DP, Liebenberg W, de Villiers MM. Poly(amidoamine) dendrimer-mediated synthesis and stabilization of silver sulfonamide nanoparticles with increased antibacterial activity. Nanomed Nanotechnol Biol Med 2013; 9: 85-93. doi:10.1016/j.nano.2012.03.006

[48] Staneva D, Vasileva-Tonkova E, Makki MSI, Sobahi TR, Abdel-Rahman RM, Boyaci IH, et al. Synthesis and spectral characterization of a new PPA dendrimer modified with 4-bromo-1,8-naphthalimide and in vitro antimicrobial activity of its Cu(II) and Zn(II) metal complexes. Tetrahedron 2015; 71: 1080-7. doi:10.1016/j.tet.2014.12.083

[49] Zainul Abid CKV, Jackeray R, Jain S, Chattopadhyay S, Asif S, Singh H. Antimicrobial efficacy of synthesized quaternary ammonium polyamidoamine dendrimers and dendritic polymer network. J Nanosci Nanotechnol 2016; 16: 998-1007. doi:10.1166/jnn.2016.10656

[50] Gangadharan D, Dhandhala N, Dixit D, Thakur RS, Popat KM, Anand PS. Investigation of solid supported dendrimers for water disinfection. J Appl Polym Sci 2012; 124: 1384-91. doi:10.1002/app.34967

[51] Ma M, Cheng Y, Xu Z, Xu P, Qu H, Fang Y, et al. Evaluation of polyamidoamine (PAMAM) dendrimers as drug carriers of anti-bacterial drugs using sulfamethoxazole (SMZ) as a model drug. Eur J Med Chem 2007; 42: 93-8. doi:10.1016/j.ejmech.2006.07.015

[52] Navath RS, Menjoge AR, Dai H, Romero R, Kannan S, Kannan RM. Injectable PAMAM dendrimer–PEG hydrogels for the treatment of genital infections: formulation and *in vitro* and *in vivo* evaluation. Mol Pharm 2011; 8: 1209-23. doi:10.1021/mp200027z

[53] Wrońska N, Felczak A, Zawadzka K, Poszepczyńska M, Różalska S, Bryszewska M, et al. Poly(propylene imine) dendrimers and amoxicillin as dual-action antibacterial agents. Molecules 2015; 20: 19330-42. doi:10.3390/molecules201019330

[54] Gardiner J, Freeman S, Leach M, Green A, Alcock J, D'Emanuele A. PAMAM dendrimers for the delivery of the antibacterial triclosan. J Enzyme Inhib Med Chem 2008; 23: 623-8. doi:10.1080/14756360802205257

[55] Wróblewska M, Winnicka K. The effect of cationic polyamidoamine dendrimers on physicochemical characteristics of hydrogels with erythromycin. Int J Mol Sci 2015; 16: 20277-89. doi:10.3390/ijms160920277

[56] Winnicka K, Wroblewska M, Wieczorek P, Sacha P, Tryniszewska E. The effect of PAMAM dendrimers on the antibacterial activity of antibiotics with different water solubility. Molecules 2013; 18: 8607-17. doi:10.3390/molecules18078607

[57] Sonawane SJ, Kalhapure RS, Rambharose S, Mocktar C, Vepuri SB, Soliman M, et al. Ultra-small lipid-dendrimer hybrid nanoparticles as a promising strategy for antibiotic delivery: in vitro and in silico studies. Int J Pharm 2016; 504: 1-10. doi:10.1016/j. ijpharm.2016.03.021

[58] ©Starpharma Holdings Limited. Accessed January 8, 2017 from http://www.starpharma. com/vivagel

[59] Sánchez-Rodríguez J, Díaz L, Galán M, Maly M, Gómez R, la Mata FJ de, et al. Anti-human immunodeficiency virus activity of thiol-ene carbosilane dendrimers and their potential development as a topical microbicide. J Biomed Nanotechnol 2015; 11: 1783-98. doi:10.1166/jbn.2015.2109

[60] Vacas-Córdoba E, Maly M, la Mata FJ de, Gómez R, Pion M, Munoz-Fernandez MA. Antiviral mechanism of polyanionic carbosilane dendrimers against HIV-1. Int J Nanomed 2016;11: 1281-1294. doi:10.2147/IJN.S96352

[61] Ceña Diez R, García Broncano P, la Mata FJ de, Gómez R, Munoz-Fernandez MA. Efficacy of HIV antiviral polyanionic carbosilane dendrimer G2-S16 in the presence of semen. Int J Nanomed 2016;11: 2443-2450. doi:10.2147/IJN.S104292

[62] Weber N, Ortega P, Clemente MI, Shcharbin D, Bryszewska M, la Mata FJ de, et al. Characterization of carbosilane dendrimers as effective carriers of siRNA to HIV-infected lymphocytes. J Control Release 2008; 132: 55-64. doi:10.1016/j.jconrel.2008.07.035

[63] Perisé-Barrios AJ, Jiménez JL, Domínguez-Soto A, la Mata FJ de, Corbí AL, Gomez R, et al. Carbosilane dendrimers as gene delivery agents for the treatment of HIV infection. J Control Release 2014; 184: 51-7. doi:10.1016/j.jconrel.2014.03.048

[64] García-Gallego S, Díaz L, Jiménez JL, Gómez R, la Mata FJ de, Muñoz-Fernández MÁ. HIV-1 antiviral behavior of anionic PPI metallo-dendrimers with EDA core. Eur J Med Chem 2015; 98: 139-48. doi:10.1016/j.ejmech.2015.05.026

[65] Han S, Yoshida D, Kanamoto T, Nakashima H, Uryu T, Yoshida T. Sulfated oligosaccharide cluster with polylysine core scaffold as a new anti-HIV dendrimer. Carbohydr Polym 2010; 80: 1111-5. doi:10.1016/j.carbpol.2010.01.031

[66] Han S, Kanamoto T, Nakashima H, Yoshida T. Synthesis of a new amphiphilic glycodendrimer with antiviral functionality. Carbohydr Polym 2012; 90: 1061-8. doi:10.1016/j.carbpol.2012.06.044

[67] Ceña-Díez R, Sepúlveda-Crespo D, Maly M, Muñoz-Fernández MA. Dendrimeric based microbicides against sexual transmitted infections associated to heparan sulfate. RSC Adv 2016; 6: 46755-64. doi:10.1039/C6RA06969J

[68] Heegaard PMH, Boas U, Sorensen NS. Dendrimers for vaccine and immunostimulatory uses. A review. Bioconjug Chem 2010; 21: 405-18. doi:10.1021/bc900290d

[69] Chahal JS, Khan OF, Cooper CL, McPartlan JS, Tsosie JK, Tilley LD, et al. Dendrimer-RNA nanoparticles generate protective immunity against lethal Ebola, H_1N_1 influenza, and *Toxoplasma gondii* challenges with a single dose. Proc Natl Acad Sci 2016; 113: E4133-42.

[70] Ullas PT, Madhusudana SN, Desai A, Sagar BKC, Jayamurugan G, Rajesh YRD, et al. Enhancement of immunogenicity and efficacy of a plasmid DNA rabies vaccine by nanoformulation with a fourth-generation amine-terminated poly(ether imine) dendrimer. Int J Nanomed 2014; 9: 627. doi:10.2147/IJN.S53415

[71] Blanco E, Guerra B, la Torre BG de, Defaus S, Dekker A, Andreu D, et al. Full protection of swine against foot-and-mouth disease by a bivalent B-cell epitope dendrimer peptide. Antiviral Res 2016; 129: 74-80. doi:10.1016/j.antiviral.2016.03.005

[72] Yandrapu SK, Kanujia P, Chalasani KB, Mangamoori L, Kolapalli RV, Chauhan A. Development and optimization of thiolated dendrimer as a viable mucoadhesive excipient for the controlled drug delivery: an acyclovir model formulation. Nanomed Nanotechnol Biol Med 2013; 9: 514-22. doi:10.1016/j.nano.2012.10.005

[73] Gajbhiye V, Ganesh N, Barve J, Jain NK. Synthesis, characterization and targeting potential of zidovudine loaded sialic acid conjugated-mannosylated poly(propyleneimine) dendrimers. Eur J Pharm Sci 2013; 48: 668-79. doi:10.1016/j.ejps.2012.12.027

[74] Heegaard PM, Boas U. Dendrimer based anti-infective and anti-inflammatory drugs. Recent Patents Anti-Infect Drug Disc 2006; 1: 333-51.

[75] Heredero-Bermejo I, Copa-Patiño JL, Soliveri J, García-Gallego S, Rasines B, Gómez R, et al. In vitro evaluation of the effectiveness of new water-stable cationic carbosilane dendrimers against *Acanthamoeba castellanii* UAH-T17c3 trophozoites. Parasitol Res 2013; 112: 961-9. doi:10.1007/s00436-012-3216-z

[76] Prieto MJ, Bacigalupe D, Pardini O, Amalvy JI, Venturini C, Morilla MJ, et al. Nanomolar cationic dendrimeric sulfadiazine as potential antitoxoplasmic agent. Int J Pharm 2006; 326: 160-8. doi:10.1016/j.ijpharm.2006.05.068

[77] Jain K, Verma AK, Mishra PR, Jain NK. Characterization and evaluation of amphotericin B loaded MDP conjugated poly(propylene imine) dendrimers. Nanomed Nanotechnol Biol Med 2015; 11: 705-13. doi:10.1016/j.nano.2014.11.008

[78] Jain K, Verma AK, Mishra PR, Jain NK. Surface-engineered dendrimeric nanoconjugates for macrophage-targeted delivery of amphotericin B: formulation development and *in vitro* and *in vivo* evaluation. Antimicrob Agents Chemother 2015; 59: 2479-87. doi:10.1128/AAC.04213-14

[79] Daftarian PM, Stone GW, Kovalski L, Kumar M, Vosoughi A, Urbieta M, et al. A targeted and adjuvanted nanocarrier lowers the effective dose of liposomal amphotericin B and enhances adaptive immunity in murine cutaneous leishmaniasis. J Infect Dis 2013; 208: 1914-22. doi:10.1093/infdis/jit378

[80] Wang X, Dai Y, Zhao S, Tang J, Li H, Xing Y, et al. PAMAM-Lys, a novel vaccine delivery vector, enhances the protective effects of the SjC23 DNA vaccine against *Schistosoma japonicum* infection. PLoS One 2014; 9: e86578. doi:10.1371/journal.pone.0086578

[81] Satija J, Sai VVR, Mukherji S. Dendrimers in biosensors: concept and applications. J Mater Chem 2011; 21: 14367. doi:10.1039/c1jm10527b

[82] Castillo G, Spinella K, Poturnayová A, Šnejdárková M, Mosiello L, Hianik T. Detection of aflatoxin B1 by aptamer-based biosensor using PAMAM dendrimers as immobilization platform. Food Control 2015; 52: 9-18. doi:10.1016/j.foodcont.2014.12.008

[83] Mejri-Omrani N, Miodek A, Zribi B, Marrakchi M, Hamdi M, Marty J-L, et al. Direct detection of OTA by impedimetric aptasensor based on modified polypyrrole-dendrimers. Anal Chim Acta 2016; 920: 37-46. doi:10.1016/j.aca.2016.03.038

[84] Perinoto ÂC, Maki RM, Colhone MC, Santos FR, Migliaccio V, Daghastanli KR, et al. Biosensors for efficient diagnosis of leishmaniasis: innovations in bioanalytics for a neglected disease. Anal Chem 2010; 82: 9763-8. doi:10.1021/ac101920t