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Dendrimers and nanomedicine: multivalency in action†

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What is the contribution of dendrimers to the field of nanomedicine? Rather than disclosing an exhaustive catalogue of their possible applications, this review article sets a putative answer by showing the basics and the underlying concepts that make dendrimeric systems so attractive to nanomedicine, emphasizing on the extraordinary possibilities offered by their multivalent and defined structure.

1. Introduction

The meaning of nanotechnology (and nanoscience) has been eroded as its use has become more and more popular, transforming it into a buzzword. Indeed, the risk is that nanotechnology becomes its own justification, as if, from an arbitrary principle, everything is nanotechnology and nanotechnology will undoubtedly turn out for the good. Far from this concept, this review investigates the unique size/properties relationship in nanomedicine: smallness is not in itself the prime goal; it is rather the expectation that new intrinsic properties can be created by manipulating matter at the molecular level.

Actually, the development of nanotechnologies is changing the foundation of disease prevention, diagnosis and treatment. The basics of these technological innovations referred to as nanomedicine can be explored in two main fields of application: (*i*) diagnosis and imaging, (*ii*) therapeutics and formulations. Nanomedicine is usually defined as the monitoring, repairing, construction and control of human biological systems at the cellular level by using materials and structures engineered at the molecular level.[1,2] In this broad definition, the intrinsic properties induced by the nanometre-length scale are not mentioned although they constitute the reason why nanomedicine is useful. In a more accurate definition, Wagner[3] describes nanomedicine as "the use of nanoscale or nanostructured materials in medicine that, according to their structure, have unique medical effects". Nanomedicine aims at the synthesis of biomedical nanodevices dedicated to a wide range of applications from the analysis of sub-cellular entities to disease diagnosis, prevention and treatment.[4] The ultimate goal would be to detect a disease in its earliest stage. This predictive medicine would allow to treat it rapidly, therefore, limiting risks and long-term damages.

This review surveys the particular role that dendrimers[5–8] might play in these fields of nanomedicine, which is undoubtedly speculated to be pivotal in next decades by dendrimers'

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† Recent reports[168–173] warn about toxicity of nanoparticles and other products issued from nanotechnologies. It is noteworthy that this concern is related to their use within mass market products and not as therapeutic agents, in which toxicity issues are always studied. This review dedicated to specific therapeutic applications of dendrimer-based nanodevices does not address this topic.

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experts. In fact, the multivalency of dendrimers is one of their most exciting features – along with their perfect definition and ease of functionalization – for the design of multi- and multipluri-functional nanodevices with a systemic approach. Dendrimers main features are shortly exposed in the first part of this review, and the role they might play in the service of nanomedicine is illustrated by representative examples in the fields of imaging, diagnosis, drug delivery and therapeutics.

2. Introducing dendrimers

2.1 Bridging the gap between polymeric and organic chemistries

The emergence of nanotechnology has pointed out the increasing need to prepare stable, well-defined sophisticated molecular devices at the nanometre-length scale. One of the most efficient ways to access such entities comprises covalent binding. This challenge relies on extending synthetic organic chemistry into the nanometre-length scale, which until recently was a world governed by polymer chemistry.[9]

Synthetic polymers have evolved in complexity over time, from linear to cross-linked, branched and hyperbranched polymers. Polymerisation processes used to prepare these architectures do not permit a fine control over the molecular architecture and lead to high polydispersity, although, there have been some recent advances using living anionic, cationic and radical polymerisations, which have led to polymers with well-defined blocks that vary in structure and function.[9,10] Control over the architecture and polydispersity were improved using organic synthetic methodologies; and synthesis of molecules with well-defined molecular structure was achieved, giving birth to a special class of hyperbranched polymers, including dendrigrafts (M_w/M_n = 1.1–1.5), dendrons and dendrimers. Unlike "traditional" polymers, dendrons and dendrimers are monodisperse (M_w/M_n = 1.00) owing to an organic step-by-step controlled synthesis. This expensive feature in terms of synthesis costs is a crucial issue to ensure the reproducibility of pharmacokinetic behaviour in all the stages of drug discovery and development.[10,11] Indeed, dendrimers are polymeric macromolecules constituted of a repetitive sequence of monomers, also called branching units, growing step by step from a multifunctional core in a radial iterative fashion and not by polymeric (statistic) reactions.

These molecules were first described in 1978 by the group of Vögtle and referred as "cascade" molecules.[12] In 1981, Denkewalter *et al.* described the synthesis of macromolecular compounds composed of at least four successive layers of lysine units, which will be referred to as polylysine dendrimer later.[13] In 1985, the group of Newkome proposed the synthesis of the same type of molecules, which they called "arborols",[14] while D. A. Tomalia finally gave the name of "dendrimers", based on two Greek words, "dendros" meaning tree and "meros" meaning part.[15] His group synthesised the now widely used Starburst polymers also called polyamidoamine (PAMAM) dendrimers. In the late 1980s, Tam and co-workers developed peptide-based dendrimers[16] or MAPs (multiple antigen peptides) that paved the way for further development of these dendrimers in the fields of medical and immunological applications.[17] In 1990, a new type of dendrimer based on polyethers was introduced by the group of Fréchet.[18] In 1993, the groups of Mülhaupt[19] and Meijer[20] simultaneously continued the work initiated by Vögtle and described the preparation of diaminobutane core dendrimers with a polypropylenenimine (PPI) structure. In 1994, the group of Majoral was the first to achieve the synthesis of a neutral phosphorus-containing poly(phosphorhydrazone) (PPH) dendrimer.[21] Research on dendrimers has kept going exponentially from this date leading to the preparation of new dendrimers, especially using heteroatoms such as silicon[22,23] or phosphorus.[24]

Dendrimers are differentiated according to their structural (and structure-related) parameters. As depicted in Fig. 1, six key parameters can be distinguished to describe the structure of dendrimers. The scaffold is based on a core, which is the focal point from where branching units (Fig. 2) and divergent points are associated in a layer by layer fashion to increase dendrimer generation number (number of layers). The number of terminal groups, located at the periphery of dendrimers, depends on the multivalency of both the core and divergent points (respectively denoted as N_c and N_d), and on the generation number (G) and is equal to $N_c N_d{}^G$. It is noteworthy that terminal groups are generally referred to as end-groups or surface groups; the latter has to be used with caution given that it becomes inaccurate when dendritic branches are folding back into the interior of dendrimers.[25] The outer shell is the last dendritic parameter and consists in the area between the last divergent point and the surface.

The theoretical number of possible dendrimers is infinite given that the dendritic structure depends on the assembly of different building blocks. Considering the core, various

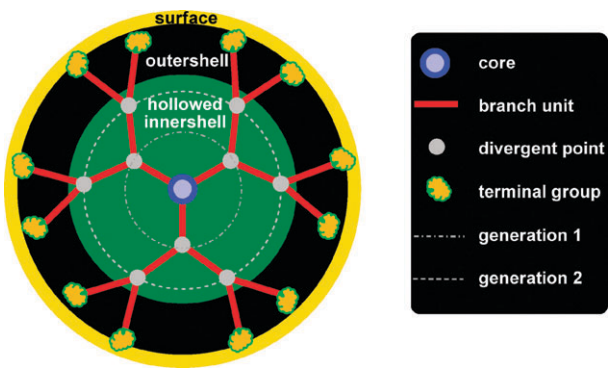


Fig. 1 Schematic representation of a generation 2 dendrimer.

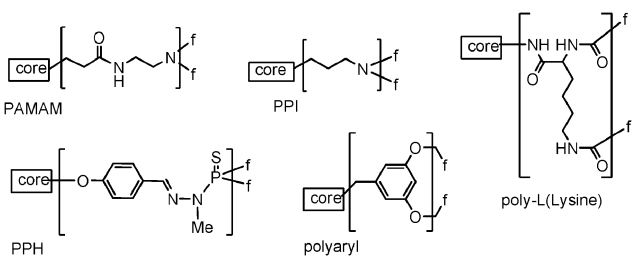


Fig. 2 Branching units of dendritic structures commonly used for biomedical purposes.

structures with different multifunctionalities have been used as the focal point for dendrimers. They can be based on atoms, small organic molecules or even large molecules from organic chemistry and biology. Despite this rich variety of building blocks, the number of possible dendrimers is limited. Actually, as depicted in Fig. 1, all the building blocks (core, branching units, divergent points and terminal functions) are connected, and even interconnected. For example, a change in the multivalency of the core impacts the total number of terminal groups. As well as the multivalency, the geometry of building blocks (*e.g.* the flexibility and length of branch unit) has an effect on the whole structure. Similarly, it can be difficult to increase the generation number because of steric issues at the surface. Therefore, it is crucial to consider a dendrimer as a complex structure, influenced by the nature and properties of its key parameters.

2.2 Physico-chemical properties

The conformational behaviour of dendrimers derivates from the dendritic growth and the inherent nature of building blocks, and depends on the external environment. Additionally, the ability of terminal groups to interact with each other is also relevant given that they can make the outer shell more rigid by non-covalent interactions such as hydrogen bonding or π–π stacking.

High-generation dendrimers have often been referred as possible synthetic compounds able to mimic proteins.[26,27] Considering PAMAM dendrimers, their sizes (1–10 nm) are in the same range as of small or medium-sized proteins such as cytochrome C (4 nm) or haemoglobin (5.5 nm), and their molecular weight is around half of that of a protein of comparable size. Nevertheless, a large difference resides in their ability to modulate their 3D structures. Actually, contrary to proteins that are flexible and can tightly fold back, being stabilised by hydrogen bonds, high generation dendrimers are rather rigid structures with a limited degree of flexibility and are hollow. Even if medium generation (4–6) dendrimers can be tailored to involve morphological trends, the most influent factor regarding their 3D arrangement is mainly the external environment. For instance, acid- and base-containing dendrimers are very sensitive to pH. It has been shown, by molecular modelling, that PAMAM dendrimers can shrink from low to high pH.[28] At low pH, all amino groups are protonated, which makes dendrimer adopt an extended conformation whereas at high pH, the charge of the dendrimer is neutral and repulsive interactions between the wedges and end-groups reach a minimum. Interestingly, this pH dependence does not affect high-generation PAMAM dendrimers because of their rigid outer shell. Each type of low and intermediate generation dendrimer is affected by pH in a unique fashion, depending on the distribution between basic and acidic groups in their scaffold.

It has also been shown that the conformation of dendrimers is modified according to the ability of the solvent to solvate them.[27] One striking example resides in the study realised by Leclaire *et al.* concerning water-soluble hydrophobic dendrimers.[29] The generation 5 of a series of phosphorus-containing dendrimers of interest has a hydrophilic ammonium-capped surface and a hydrophobic interior. By solubilising it in water and tuning the amount of THF, the dendrimer was found to swell and to increase dramatically its size (35% of the diameter) and volume (150%). Generally, "poor" solvation leads to packed structures whereas "good" solvation leads to extended structures.

Dendrimer conformation is also sensitive to ionic strength. For example, charged PPI dendrimers have their conformation contracted, with a high degree of back-folding when a high concentration of salt is set.[30] This is due to the limitation of charge repulsion between the salts present in the solvent and the charged ammonium groups from PPI dendrimers.

All these features can have a significant impact on the interactions of dendrimers and cells, as well as the intracellular trafficking of dendrimer-based nanodevices. For instance it has been shown recently that anionic dendrimers are mainly taken up by caveolae mediated endocytosis in A549 lung epithelial cells, while cationic and neutral dendrimers are taken in by a non-clathrin, non-caveolae mediated mechanism that may be by electrostatic interactions or other non-specific fluid-phase endocytosis.[31]

2.3 Multivalent character of dendrimer

Multivalent systems[32–34] are found widely in Nature, and especially in biology: adhesion of viruses or bacteria to cells' surface, cell to cell adhesion and cell to polyvalent molecule interactions. A good example resides in the defence process of the immune system involving bacteria, antibodies and macrophages. Antibodies have the ability to recognise "non-self" entities, such as bacteria, upon polyvalent binding with antigens, or other proteins, located at their surface. It is noteworthy that weak ligand–receptor interactions can be made much stronger simply by the simultaneous bonding of these ligands to these multiple receptors.

Multivalent ligands can interact with a wide range of receptors located at cells' surface using four main mechanisms.[35] They can bind oligomeric receptors; this process involves that second and further bindings are favoured by the first one and is called a "chelate" effect. Multivalent ligands can also recruit receptors to make them cluster. This is a usual process inducing signalling pathways. Quite similar to the "chelate" effect, a multivalent ligand can bind primary and secondary sites on the same receptor. Finally, taking advantage of the high concentration of ligands defined by the microenvironment of a multivalent ligand, high affinity can be achieved with one single receptor. In addition, other parameters influence the mechanisms by which ligands act: the structure of the scaffold, the number of binding groups and the density of binding elements.[36]

Dendrimers are inherent multivalent ligands that can present multiple recognition elements from a central scaffold. The scaffold plays a crucial role because it moulds the final architecture, in terms of shape, orientation of recognition elements, flexibility, size and valency. All the properties rapidly described previously influence the mechanism of action and the resulting biological activity. Among the variety of natural and synthetic scaffolds that have been

used as multivalent ligand carriers,[37] such as dendrimers, polymers, proteins and liposomes, dendritic scaffolds present the advantage that many features can be controlled during the synthesis. In addition, the perfect chemical definition of the scaffold affords a valuable versatility related to the possibility of grafting different chemical moities on the surface, at the core, or within the structure of the scaffold, in order to control several functions whose interactions can be controlled, either with external partners or inside the dendrimer structure. This systemic approach applied to dendrimers and dendritic architectures opens new perspectives in the design of "smart" dendritic nanodevices.[38]

2.4 Health issues

From this brief description of dendrimers, it is clear that dendrimers constitute a versatile platform whose parameters mentioned above, size, shape, flexibility and conformational behaviour, can be early tuned to reach a specific future application.[39,40] The ability to easily tune this structural arrangements and, consequently, the intrinsic properties makes medium-generation dendrimers the best dendritic candidates as nanodevices. Nevertheless, the expected success of dendrimers in nanomedicine is closely related to their biocompatibility.

This issue has been extensively reviewed recently by several authors.[41–44] *In vitro* studies have revealed that the surface groups of dendrimers interacting directly with cells' membrane play a major role.[27] The cytotoxicity of cationic dendrimers is proportional to their ability to expose a cationic surface, and to the degree of substitution of amines, tertiary amine-based surfaces rendering a dendrimer less toxic than primary amine-based ones.[27,45,46] On the contrary, dendrimers bearing a neutral or polyanionic surface provide low or even no cytotoxicity.[47] PEG chains[48] also reduce the cytotoxicity of dendrimers[49] by hiding the surface from possible ionic interactions with the cell membrane.[50] The cytotoxicity of polycationic dendrimers is also decreased when conjugated with DNA or RNA strands,[51–53] the overall charge of DNA–dendrimer complex being still positive but reduced so that DNA strands act as an anionic shield which masks partly the amino surface groups of dendrimers. It has also been demonstrated that *in vitro* cytotoxicity is dependant on the generation number (*i.e.* the size) for PAMAM and PEI dendrimers. This is in accordance with the fact that cytotoxicity of polycations is dependant on their molecular weight.[46,51] The general trend lying in the parallel increase of dendrimer size and cytotoxicity *in vitro* is confirmed *in vivo* with high-generation dendrimers being highly toxic;[54] the nature of surface functions is also a key parameter influencing the *in vivo* toxicity. Nevertheless, the chemical composition of the skeleton can also be determinant, according to the metabolic fate of the dendrimer. For instance, a fully hydrolysable polyester-based (see Fig. 3) dendrimer bearing hydroxy end-groups was found to be only slightly toxic *in vivo*, even at very high concentration in mice (LD_{50} = 1.3 $g.kg^{-1}$).[55]

The acute toxicity of dendrimers can be related to their surface function and size which are responsible for direct interactions with cell membranes, while long term

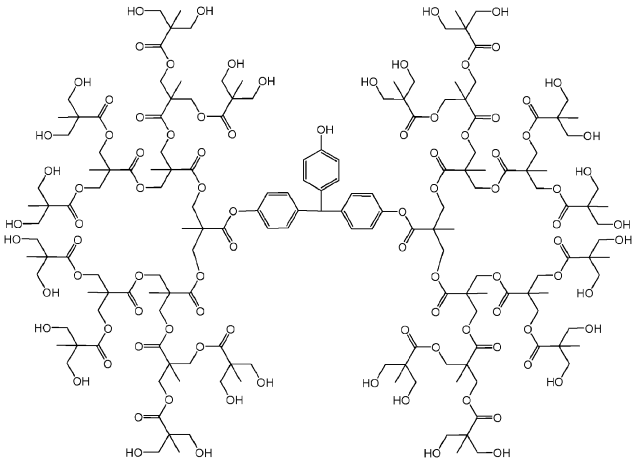


Fig. 3 Non-toxic polyester-based dendrimer.

biocompatibilty can be related to the ability of dendrimers to decompose to non-toxic products, to be fully cleared from the body, and to have a controlled immunogenicity. These issues have been studied *in vivo* for several dendrimer materials, and the collected results show that only a few general trends can be highlighted, as the terms biocompatible or non-toxic can not be used without qualification if the targeted tissue or means of administration is not specified.[42] For instance, most of the data concerning the biodistribution and the parenteral use of dendrimers *in vivo* are connected to dendritic MRI agents,[56] while the aspects of immungenicity have been studied for peptide and glycopeptide dendrimer based vaccine candidates.[16,57] A growing number of reports include biodistribution studies,[58] nevertheless further work is duly needed in this field to assay the pharmacokinetics of dendrimers that are candidates for biomedical applications as is the case for any other biomedical nanodevice.[59]

3. Expected fields of application of dendrimers in nanomedicine

3.1 Dendrimers for imaging

3.1.1 Dendrimeric MRI contrast agents. Magnetic resonance imaging (MRI) is a nuclear magnetic resonance-based technique that maps the proton density in the tissues, by measuring relaxation times gradients responsible for contrasts in the pictures. Most of these studied protons come from water located in the tissues; therefore, the contrast given by MRI depends on the amount of water. The variations in concentration of water between two close regions in the same tissue are often too weak to render a high contrast. This problem is solved by the use of contrast agents that, upon coordination with water, modify relaxation times of the corresponding proton.

Mostly used contrast agents are based on gadolinium ion Gd^{3+} and to a lesser extent on manganese ions Mn^{2+} and Mn^{3+}. Because of their toxicity as free ions for gadolinium or as corresponding oxides for manganese, they are bound to chelating ligands, usually from the diethylenetriaminepentaacetic acid (EDTA) family including, mainly, diethylenetriamine

pentaacetic acid (DTPA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) and 1,4,7-tris[carboxymethyl]-10-[2′-hydroxypropyl]-1,4,7,10-tetraazacyclododecane (DO3A). The most widely used low molecular weight contrast agents are gadolinium(III) complexes such as [Gd(DTPA)] known as Magnevist®, [Gd(DOTA)] known as Dotarem® and [Gd(DO3A)] known as Prohance®. These complexes present good biocompatibility of the chelating ligand, lower toxicity than the corresponding metal ion, good excretion of the complex, high stability, good solubility and high relaxivity. Nevertheless, a major drawback of these low molecular weight contrast agents is their high clearance rate; they are removed rapidly from the body, which necessitates high doses and injection rates.[60] Some attempts to solve this problem have been realised by grafting gadolinium complexes to high molecular weight macromolecules such as human serum albumin (HSA).[61] In these first attempts, an increase of the relaxivity from Magnevist® to the corresponding HSA covalently bound to 19 [Gd(DTPA)] could be observed. This phenomenon, related to proton relaxation enhancement,[62,63] was expected considering that larger molecules rotate more slowly than smaller molecules, decreasing the water exchange rate and causing longer *T*2 relaxation times. However, the use of a protein platform makes it difficult to prepare accurately with high reproducibility (problems of conformational changes, molecular shape and size) various MRI agents. An alternative resides in the use of polymers, instead of proteins, as cores to be conjugated with [Gd(DTPA)] or [Gd(DOTA)]. Several synthetic polymers, such as dextran[64] or polylysine,[65] have been conjugated to contrast agents, and showed good efficiency in imaging. However, their use was limited because of synthetic and purification difficulties (polydispersity), low level of characterisation and non-reproducibility of the final chemical entities. In addition, some toxicity problems were encountered, especially bad renal elimination.

An alternative to protein conjugates is dendrimer conjugates. The group of Wiener[62,63,66] was the first to describe a dendrimer-based contrast agent for MRI. Using a [Gd(DTPA)] complex covalently bound to polyamidoamine dendrimers, they managed to visualise the vascular structure of a rabbit (magnetic resonance angiography) and to increase the blood circulation half-lives to between 40 to 200 min (depending on the molecular weight of the dendrimers). Other groups are investigating the role of the dendritic skeleton.[67,68] Comparing PAMAM and polypropyleneimine (PPI) dendrimer-based agents carrying DTPA groups at the surface, it has been found that generation 3 of both dendrimers (with 16 end-groups) were the most suitable. Some differences appear in the clearance rate and in the measured relaxivity. Generation 3 PPI dendrimer-based agent was more rapidly excreted from the body; however, it has higher relaxivity than the corresponding PAMAM dendrimer.

Large efforts have been made to introduce a dendrimer-based MRI contrast agent on market. For example, a team of Schering AG developed a dendrimer-based MRI contrast agent called Gadomer 17, or SHL643A (see Fig. 4). This compound consists of a trimesic acid core on which second-generation lysine dendrons bearing a total of 24 [Gd(DO3A)] are anchored. Gadomer 17 has been used to

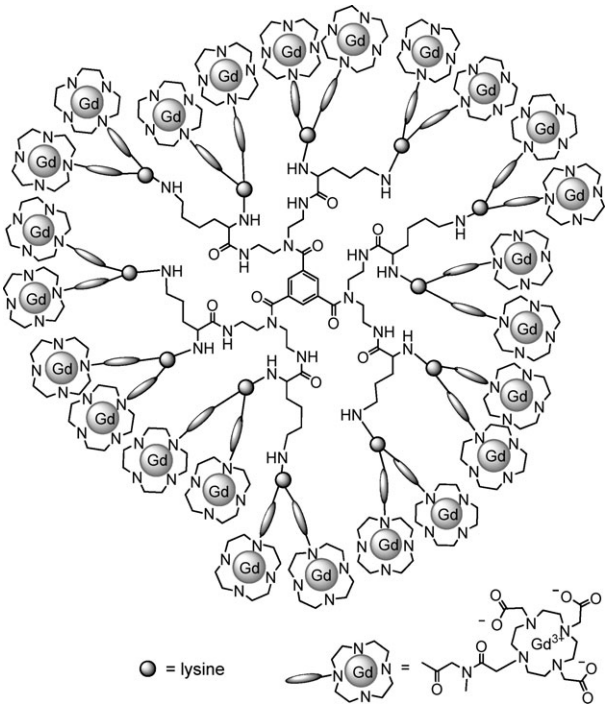


Fig. 4 Dendrimer-based MRI contrast agent Gadomer 17.

image the peripheral blood vessels of a dog.[69] It shows a good renal elimination rate and high intravascular retention time. For example, Roberts *et al*. have demonstrated that Gadomer 17 extends the temporal window of dynamic contrast-enhanced MRI when studying myocardium.[70] More recently, it has been used with success as contrast agent for coronary magnetic resonance angiography,[71] and it is currently under phase II clinical trials. This success shows the opportunity to use magnetic resonance angiography instead of the invasive coronary artery angiography, which is usually considered as the standard in the diagnosis of coronary artery diseases.

Dendrimers offer several advantages as contrast agents. The dendritic architecture is highly suitable for incorporation of many contrast agents, while remaining soluble. Moreover, taking advantage of enhanced permeability and retention effect (EPR), their size allows the accumulation in vascular structures, and subsequently their visualisation, that would not be possible with low molecular-weight contrast agent.[56] Actually, a high porosity is found typically in inflammation tissues or tumours; for example, although the cut-off size of a vascular pore in normal vasculature is between 2 to 4 nm, that in a solid tumour is between 380 to 780 nm.[72] It was observed that proteins and macromolecules accumulate more specifically at tumour sites than low-molecular weight therapeutic agents.[73,74] The EPR effect is directly related to the size (and molecular weight) of the injected compounds,[75] and therefore, is widely used in passive targeting of large nanodevices.

Recently, the group of J. R. Baker has developed dendrimer-functionalised shell-crosslinked iron oxide nanoparticles for tumor MRI.[76] These systems combine layer-by-layer (LbL) deposition of poly(L-lysine) and poly(glutamic acid) polymers onto Fe_3O_4 nanoparticles, with a subsequent covalent cross-linking, and a final layer consisting of a PAMAM

dendrimer bearing fluorescent probes and folic acid moities for tumor addressing. The author proved that these nanoparticles were stable, water-soluble and biocompatible, allowing *in vivo* imaging of a folic acid receptor expressing tumor model.

3.1.2 Dendrimeric two-photon tracers for *in vivo* imaging. Microscopic imaging based on two-photon excited fluorescence (TPEF) is attracting a growing interest owing to a high spatial resolution, and the possibility to image at an increased depth with limited photodamages and noise by means of near infrared fluorophores (NIRF). A first dendrimeric system bearing two-photon absorption (TPA) fluorophores was first developed by the group of Fréchet in 2000.[77,78] These systems having a high fluorophore density opened a new route to synthesize organic macromolecules able to compete with inorganic quantum dots (QDs).[79–81] Recent studies[82–85] published by the group of Majoral and Caminade and by the group of Blanchard-Desce describe the synthesis of various phosphorus-containing dendrimers bearing TPA chromophores. Different systems have been described by these authors, bearing either TPA chromophores at the core, within the branches or on the surface of phosphorus-containing dendrimers. The latter systems exhibit very high TPA cross-sections and high quantum yields, proving the relevance of the dendrimer approach to afford organic nanodots that would not suffer from the drawbacks of QDs (blinking, toxicity, insolubility, ...).

3.2 Dendrimer based DNA chips

The use of dendrimers for the elaboration of DNA chips or DNA arrays[86] has allowed striking improvements of the sensitivity and reliability of these devices devoted to genetic information sensing. This field of investigation has been thoroughly reviewed recently by Caminade *et al.*[87] and will not be addressed in detail herein. Most studies concern the use of dendrimers as linkers between the slide and the probe, in order to move the probe away from the solid surface. Since 1999, this field of research has evolved from simple protein attachment onto quartz or glass slides[88] to the first operating arrays obtained by PAMAM dendrimer cross-linking onto a glass surface by means of homobifunctional cross-linkers.[89] Nevertheless, these cross-linked systems offer a poor oligonucleotide loading, due to the high number of surface functions of the dendrimers involved in the cross-linking.

A great improvement was proposed latter with the use of phosphorus dendrimers equipped with aldehyde end groups that allow both the direct coupling onto an amine-modified glass surface and the coupling of amine terminated oligonucleotides, in the absence of a specific linker.[90] This strategy proved to be highly efficient in terms of sensibility and stability, the resulting dendrislide devices being re-usable after ten or so hybridization-stripping cycles with a picomolar sensitivity (Fig. 5).[91]

3.3 Dendritic nanovectors for drug delivery

In a recent review, Koo *et al.* restricted the field of application of nanomedicine to drug delivery and imaging and evoked nanovectors as "delivery systems in the nanometre size range (preferably 1 to 100 nm) containing encapsulated, dispersed, adsorbed, or conjugated drugs and imaging agents".[72] Actually, drug delivery, whose share in pharmaceutical sales has been continuously increasing over the past few years, represents the main field of research (around 75%) in nanomedicine.[3,92] Nanovectors[72,93,94] can be classified according to their nature. Sahoo *et al.* distinguish six families of nanotechnology-based drug delivery systems:[95] (*i*) nanoparticles that are small polymeric colloids with therapeutic agent either dispersed in the matrix or encapsulated; (*ii*) liposomes that are made of lipids, encapsulating therapeutic agents; (*iii*) polymeric micelles resulting of self-assembled block-polymers that can protect hydrophobic therapeutic agents located at their core from the outer medium; (*iv*) dendrimers that are organic monodisperse macromolecules that can be loaded with therapeutic agents; (*v*) quantum dots that are fluorescent nanocrystals and (*vi*) ferrofluids that are colloidal solutions of iron oxide magnetic particles surrounded by a polymeric layer.

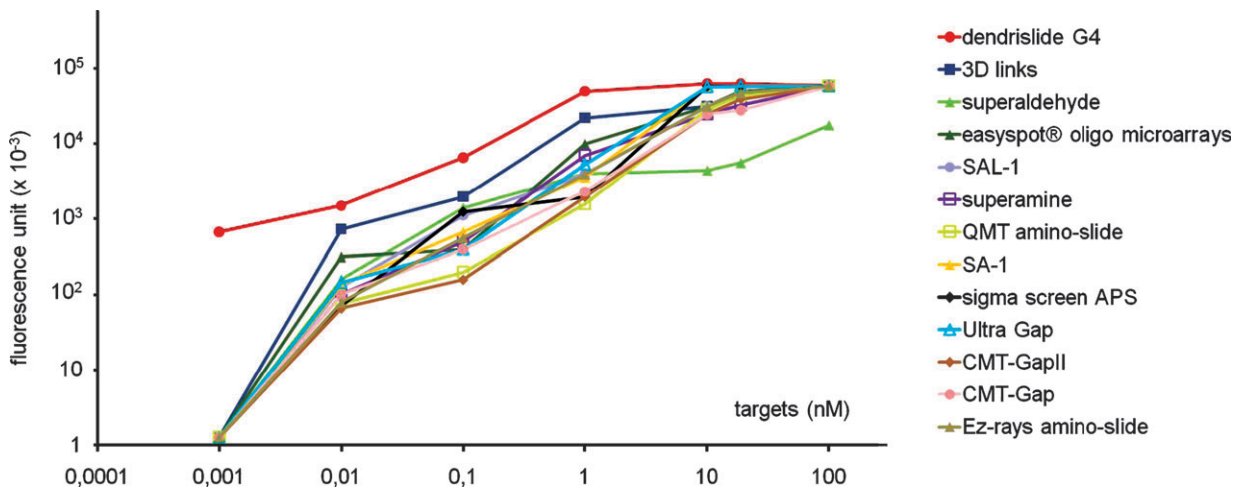


Fig. 5 Detection sensitivity of dendrislide compared with commercially available activated glass slides. A single-stranded 35 mer amino-oligonucleotide at a concentration of 10 mM was spotted on the different functionalised glass slides. The target Cy5-labelled 15mer oligonucleotides were hybridised at concentrations ranging from 0.001 to 100 nM.

The microenvironment of the inner shell of a dendrimer may differ significantly from that of its outer shell. This phenomenon appears, for example, using dendrimers with a hydrophilic periphery and hydrophobic interior, and becomes stronger while the generation increases. This unimolecular micelle-like behavior[14] allows the simple, and yet efficient, entrapment of drugs. This cargo loading of dendrimers is an alternative approach to the covalent bonding of drugs onto the structure of dendrimers to afford multivalent prodrugs. This field of investigation has evolved recently to more sophisticated systems approaching the ideal ''smart'' nano-delivery system. The latter has been described as a multifunctional nanoparticle[96] that should have the ability to carry one or more therapeutic agent, to target specific locations (conjugation with recognition agent), to avoid barriers (conjugation with permeation enhancer) and to allow imaging (imaging agents).

3.3.1 Dendrimers for gene delivery. The growing demand for safe, efficient, non-viral transfecting agents has led to strong efforts for the elaboration of cationic dendrimer-based transfection agents very early.[97] We will not focus on this prolific field of investigation that has been extensively reviewed.[98–102] It relies on the interaction of negatively charged plasmid DNA, siRNA or antisense oligonucleotides with positively charged dendrimers. The resulting positively charged supramolecular arrangement denoted as a dendriplexe can interact with the negatively charged membrane of cells, to further allow the entrance of the dendriplexe through endosomal uptake. Once the dendriplexe is released in the cytoplasm, its access to the nucleus and the fate of the dendrimer is still unknown. A generation effect, or dendritic effect, is often observed, medium sized dendrimers being generally the most efficient ones.[103,104] Recent advances concern mainly the PEGylation of cationic dendrimers to increase their transfection potential and to lower their intrinsic toxicity.[50] Another strategy to reduce toxicity relies on the acetylation of the surface of PAMAM dendrimers, the positive charges being then on the amino groups located within the structure.[105]

3.3.2 Encapsulation of drugs inside dendrimers. Several dendrimers have been used for drug delivery purposes[27,106,107] assuming a core–shell structure with a hollow core and a crowded outer-shell.[108] However, several studies[109,110] have shown that this model is not relevant for flexible dendritic structures subject to branch backfolding, or to collapsed dendritic structures having a lipophilic interior.[29] In addition, a study on the solubility enhancement of model drugs by cationic PAMAM dendrimers suggests that the electrostatic external interaction contributes more to solubility than internal encapsulation.[111] Although these observations are rather specific to a class of dendrimers, it emphasizes on the importance of the outer shell and the surface functions in both drug–dendrimer and dendrimer–target cell interactions.

The group of M. W. Grinstaff has described a generation 4 dendrimer based on succinic acid and glycerol with hydroxyl or carboxylic acid terminations for the delivery of hydrophobic camptothecin analogues.[112] *In vitro* studies showed that the acid terminated G4 dendrimer was able to solubilize the drugs and to deliver them with a faster internalization and a longer retention time, traduced by improved TI (Therapeutic Index) when compared with the free drugs. The solubility in water was even improved by a 3400 Da PEG chain located at the core of the dendrimer. In this regard, recent dendritic systems have also been improved by PEGylation of the dendrimer surface, in order to increase biocompatibility and to protect the delivery system from opsonins.[50,113,114]

3.3.3 Dendrimer–drug conjugates for anticancer chemotherapy. An alternative approach to non-covalent encapsulation of molecules is to use their multivalency in order to conjugate cargo molecules as end-groups. In a drug delivery purpose, drug loading can be tuned easily by controlling the generation number and its release can be designed using a degradable spacer between the drug and the dendrimer, transforming the drug into a prodrug. In the past few years, the cargo loading ability of dendrimers has been investigated exponentially. It is one of the most flourishing fields of research in dendrimer-based anticancer therapy,[115] because dendrimers offer a monodisperse polymeric scaffold, tuneable at will. This strategy often requires highly sophisticated syntheses affording dendritic platforms that offer several functions or chemical anchors to ensure different functions: biocompatibility, drug conjugation, imaging tracers, ... Nevertheless, the ideal drug delivery nanovector imagined by Ferrari[96] seems within reach using dendrimers. Actually, the group of J. R. Baker has developed a versatile nanoscale platform that has proved its efficiency for monitored and targeted drug delivery. In first reports,[116–118] a nanovector (see Fig. 6) has been synthesised on the basis of a generation 5 acylated PAMAM dendrimer conjugated to glycidol, folic acid as targeting moiety, fluorescein isothiocyanate (FITC) as fluorescent probe and methotrexate, an antimetabolite and antifolate drug used in treatment of cancer and autoimmune diseases.

It is noteworthy that all the related dendritic structures are statistical and thus no longer monodisperse. Consequently, the given numbers of functionalities grafted at the dendrimer surface refer to an ideal representation. The synthesis was designed according to molecular modelling that determined the optimal modifications to obtain the best active

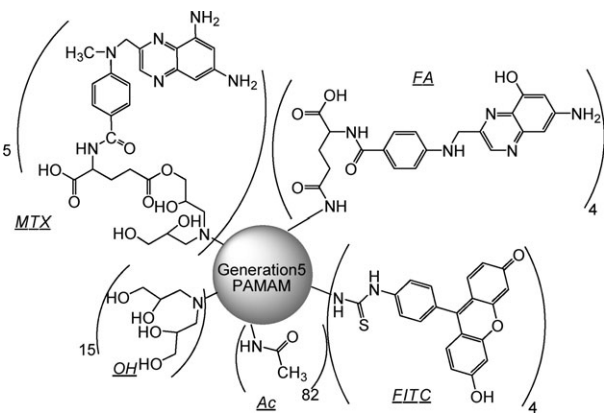


Fig. 6 Multifunctional dendrimer conjugated to methotrexate (MTX), folic acid (FA), fluorescein (FITC) and glycidol (OH).

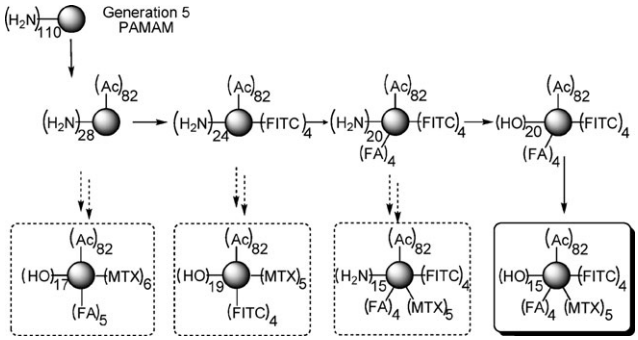


Fig. 7 Versatile synthetic methodology for multifunctional dendritic platform: methotrexate (MTX), folic acid (FA), fluorescein (FITC) and glycidol (OH).

multifunctional dendrimer.[116] Generation 5 dendrimer was first partially acetylated leaving 28 terminal primary amines to be readily functionalised. Partial acetylation was mainly used to neutralize the dendrimer surface, preventing toxicity, side-reactions and non-specific targeting from interfering with the "normal" drug delivery process. Four fluorescein groups were then conjugated to the dendrimer, followed by the attachment of four folic acid moieties. The 20 remaining amino groups were then reacted with glycidol to provide the surface with alcohol groups that would be able to form readily an ester linkage. In this regard, five methotrexate moieties were conjugated *via* an ester linkage to the dendrimer. Of note is the fact that this versatile synthesis was designed so that each intermediate can be functionalised on demand (see dashed lines on Fig. 7).

Methotrexate is bound to the bioconjugate *via* a pH-sensitive linked ester bond that can be hydrolysed in lysosomes, where physiological pH becomes acid, allowing the release of the anticancer drug. *In vitro* studies on KB cells (a human epidermal carcinoma) over-expressing folic acid receptor displayed good efficiency of this trifunctional dendrimer-drug conjugate.[116] It was proved that it bound specifically and highly selectively to KB cells and was then internalised (this step was monitored by confocal microscopy). Moreover, the cytotoxic response of the cells to this conjugate was 100-fold higher compared to that provided by free methotrexate.

Folate-mediated delivery constitutes a remarkable strategy when considering tumour-specific drug delivery, and especially using macromolecules as nanovectors.[119,120] In fact, folic acid receptor is over-expressed on cell surfaces in many human cancers such as ovary, brain, kidney, breast, lung and myeloid cancers. Moreover, its density increases hugely as the stage of cancer worsens (up to a 100-fold). Finally, when expressed on healthy cells, its access is difficult. Therefore, targeting folic acid moieties may be a good way to target cancer cells and not normal cells. Another interesting advantage resides in the non-destructive uptake of folate conjugates by mammalian cells *via* receptor-mediated endocytosis. As depicted on Fig. 8, after binding to folate receptors located at the cancer cell surface, folate conjugates are internalised, regardless of size, into endosomes that are subsequently acidified. This drop of pH makes some folate conjugates dissociate from their receptors and be released into the cytoplasm. The remaining folate

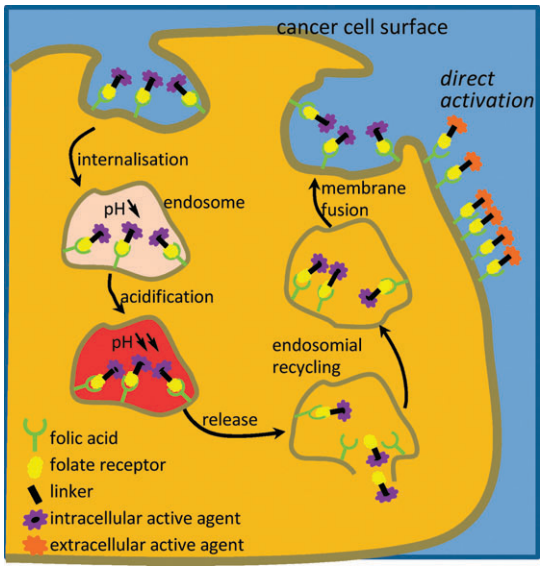


Fig. 8 Folate-mediated delivery of therapeutic agents to cancer cells (adapted from ref. 120).

conjugates still bound to their receptors are then redirected towards the outer cell membrane. Therefore, there are two ways to benefit from folate-mediated delivery using either an intracellularly or extracellularly active agent.

The potential of the dendritic conjugate developed by the group of Baker to target and induce apoptosis of tumour cells was not only demonstrated *in vitro* but also *in vivo*.[121] These conjugates, and a radiolabelled analogue, were injected intraveneously in mice bearing human KB tumour cells over-expressing folic acid receptors. Folate targeted radioactive conjugates were found to accumulate during the first four days as expected in the tumours, but also in the liver and kidneys. The accumulation in the two latter organs is not surprising given that these tissues express a large amount of folic acid receptors. Moreover, the internalisation of the conjugate into the cytoplasm of targeted tumour cells was demonstrated by monitoring using confocal microscopy. The efficacy of this conjugate was 10-fold higher compared with free methotrexate. It was also demonstrated that the four functions of the dendrimer (fluorescence, targeting, radiolabeling and cytotoxicity) were operating independently. Recently, the group of Baker has shown a batch-to-batch consistency in a preclinical antitumor efficacy assay.[122]

This dendrimer based platform was found advantageously versatile, and the authors modified the drug switching from methotrexate to paclitaxel.[123] The methodology to synthesize the new taxol-based conjugate (see Fig. 9) was similar to the previous one. Its targeting, internalisation and cytotoxic behaviours were tested *in vitro* on KB cells and were found promising at relatively low concentration (50 nM).

They also replaced FITC by Alexa Fluor 488, which is significantly brighter and more photostable, and succeeded in using other targeting moieties than folate, such as anti-human growth factor-2 monoclonal antibody[124] or RGD ligands,[125] which are tripeptides based on arginine-glycine-aspartate.[126] The latter are particularly interesting because they enable a flourishing anticancer technique, called antiangiogenic

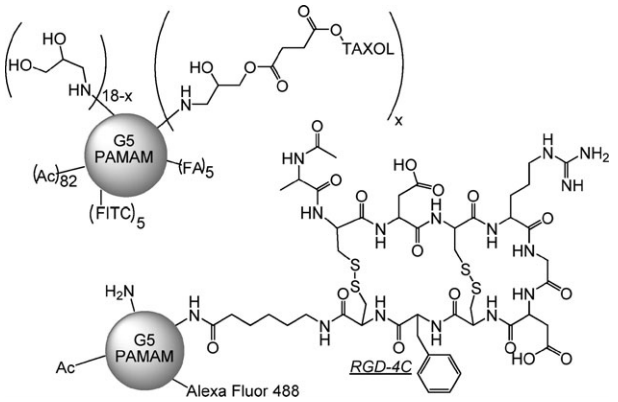
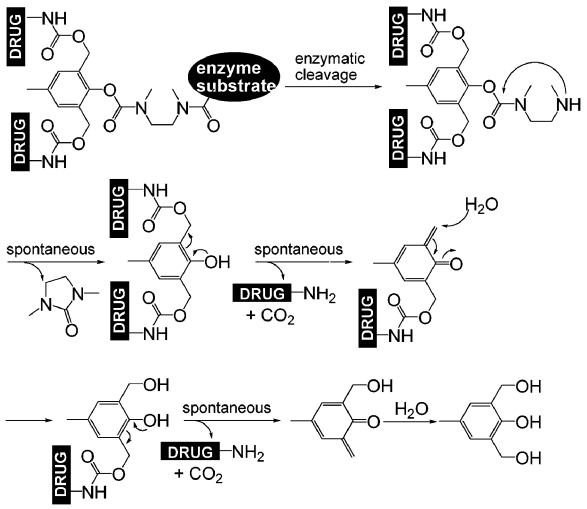


Fig. 9 (Top) Taxol-based conjugate ($x < 18$). (bottom) RGD-4C-based conjugate (statistical numbers of each group grafted at the dendrimer surface are not given).



Scheme 1 Degradation of Shabat's self-immolative dendrimers.

therapy, based on the detection, targeting and prevention of neovascularisation.[127] Actually, as tumour growth is achieved by a dramatic and unordered expansion of vascularisation, it is of great interest to target these new-born vessels to deliver cytotoxic drugs. Interestingly, $\alpha_V\beta_3$ integrin is a protein found at the surface of the endothelial cells only during angiogenesis. It presents a high affinity with RGD ligands, and even a better affinity with the doubly cyclised form RGD-4C. The RGD-4C based dendrimer provides a good affinity towards human umbilical vein endothelial cells and was highly internalised. Moreover, it was demonstrated that the great affinity was due to the multivalent presentation of RGD-4C by the dendrimer conjugate. In this regard, Hong *et al.*[128] proved, very recently, that previous folate based dendrimers bind to targeted tumour cells through multivalent interactions with folic acid receptors. They showed that the dissociation constants (K_D) were dramatically enhanced from 2500- to 170 000-fold. This huge dendritic effect may explain the tremendous targeting of these nanocarriers.

The group of J. M. J. Fréchet also contributed to the elaboration of dendrimeric prodrugs with the elaboration of "bow-tie" dendrimer systems based on polyester linkages.[129,130] Recently, *in vivo* experiments on mice have shown that a radiolabelled series of these hybrid macromolecules based on a polyester dendritic structure capped with doxorubicin and PEG residues are prone to accumulate more into cancer cells and less in healthy organs than a clinically used liposomal formulation of doxorubicin. However, biodegradability, related to the accessibility of the dendritic core and thus its ability to be degraded by enzymes, could not be fully assessed.[131]

As shown above, dendrimer–drug conjugates are designed so that drugs are released using the external environment, such as changes in pH conditions. The main limitation of such a method is that one chemical trigger induces the release of one drug molecule. Recently, the structural versatility of dendrimers has been exploited to overcome this problem, leading to the release of the payload following one biological stimulus.[132,133]

Initially, three groups[134–136] have reported, independently and almost at the same time, an advanced concept, consisting in the simultaneous release of all the conjugated molecules by a single chemical trigger. All three exploit the fact that a dendrimer skeleton can be built in such a way that it can be made to "self-destruct" and decompose into small known fragments upon biological or chemical stimuli. As the disintegration process is achieved in a cascade fashion, they gave related names to this new platform: "cascade-release" dendrimers by de Groot,[134] "disassembly" dendrimer by McGrath,[136] and finally, "self-immolative" dendrimers by Shabat.[135]

The first dendritic model[134] relies on the chemical reduction of an aromatic nitro group, which triggers a cascade of reactions, where all dendritic moieties fall apart leading to the release of the conjugated molecule. This principle was applied successfully to generation 1 and 2 dendrimers conjugated to the anticancer drug, paclitaxel. Although the main advantage of this methodology is the non-toxicity of resulting dendritic fragments, a major drawback resides in the triggering (reduction under non-physiological conditions: Zn, acetic acid).

The group of Shabat reported an alternative dendritic system cleaved by the activation with a catalytic antibody.[137] The dendrimers of interest are based on 2,6-bis(hydroxymethyl)-*p*-cresol whose phenol functionality is linked to a trigger through a short spacer, *N*,*N*′-dimethylethylenediamine. In early attempts,[135] they have demonstrated that the trigger cleavage initiated a self-immolative reaction, involving, first, a spontaneous cyclization to form an urea derivative, followed by a 1,4-quinone methide rearrangement and decarboxylation, releasing one drug molecule. The reiteration of this process allows the release of other drug molecules conjugated to the dendron (see Scheme 1). In this case, they used antibody 38C2 as the activating enzyme that catalyses retro-aldol retro-Michael cleavage reactions. Using doxorubicin and camptothecin anticancer molecules, they produced two homodimeric and one heterodimeric prodrug and tested them successfully in a cell-growth assay with leukaemia cells. They further explored and improved this dendritic prodrug platform by the synthesis of trimeric prodrugs, which allows the grafting of three

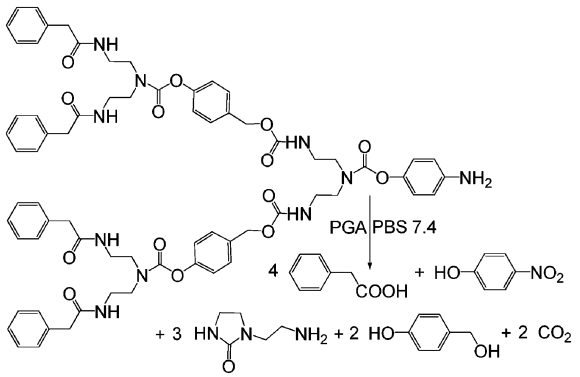


Fig. 10 PGA (penicillin-G-amidase) catalysed fragmentation of a generation 2 dendron to its building blocks.

different drugs on the same platform, and therefore their eventual release.[138]

This group also designed and synthesized fully biodegradable dendrimers disassembled through multienzymatic triggering followed by self-immolative chain fragmentation.[139] Extending the use of the cyclization process described above, with diethylenetriamine, they synthesised a dendrimer based on this triamine with phenylacetamide as the trigger for penicillin-G-amidase. Generation 1 to 3 dendrimers were completely biodegraded into their building blocks in aqueous medium (see Fig. 10). The use of different substrates at the core should allow the use of varying triggering enzymes.

These pioneering studies constitute a proof of concept and allow to envision promising applications of self-immolative dendrimers, especially as prodrugs. Their "self-destruction" activation through a single catalytic reaction by a specific enzyme can be of great advantage provided they are located at the site where the enzyme is expressed.

3.3.4 Dendrimer–boron conjugates for boron neutron capture therapy. Boron neutron capture therapy (BNCT) of cancer is based on nuclear capture and fission reactions that undergo boron-10 (^{10}B, a non-radioactive isotope of natural element boron) upon its irradiation with low-energy or thermal neutrons, which yields high linear energy transfer (LET) α particles (^{4}He) and recoiling lithium-7 (^{7}Li) nuclei.[140,141] LET is used to quantify the effects of ionizing radiations, through the energy lost per unit distance as an ionizing particle travels through a material. The death of tumour cells is induced by damages to their mitotic potential: ^{10}B(n, α)^{7}Li^{3+} reactions have the ability to induce double-stranded DNA breaks.

High LET particles have limited path length in tissues (they can travel only 5 to 10 μm from their sites of origin), therefore, the potential of spreading their radiant energy is confined to the cell from which they arise.[140] Consequently, ^{10}B atoms have to be at the targeted location before the irradiations. Moreover, the efficiency of BNCT depends on the amount of ^{10}B atoms delivered per cell. To ensure the desired effect, it is necessary to deliver approximately 10^{9} ^{10}B atoms per tumour cell.

Most important requirements for successful BNCT agents are (*i*) low toxicity and high uptake by tumour cells, (*ii*) a maximum BNCT concentration in tumour cells with a high tumour/blood partition ratio (>4 : 1) and (*iii*) rapid clearance from blood and healthy tissues with a persistence in tumour cells.[141] At the present time, no BNCT agent fulfils all these criteria. BNCT has improved by the emergence of two boron compounds, L-4-dihydroxyborylphenylalanine (BPA) and sodium mercaptoundecahydro-*closo*-dodecaborate (BSH), which have been used in clinical trials. The latter opened a new range of research using caged boron-containing clusters such as polyhedral boranes and icosahedral carboranes, which were found to be good candidates as they showed high boron content, chemical and hydrolytic stability and low toxicity.[141] Generally, their tumour-targeting ability and persistence in tumour cells were, however, not enough developed. This problem may be overcome by the design and preparation of tumour-targeting macromolecules with a high payload of boron-containing clusters. Earlier attempts using polymers, such as polylysine, conjugated to an isocyanatopolyhedral borane, $Na(CH_3)_3NB_{10}H_8NCO$, and antibodies show good properties *in vitro* but lost their targeting-ability *in vivo*.[142] This change in behaviour may be explained by the poly-dispersity of the original polylysine batch, which was increased following boronation.

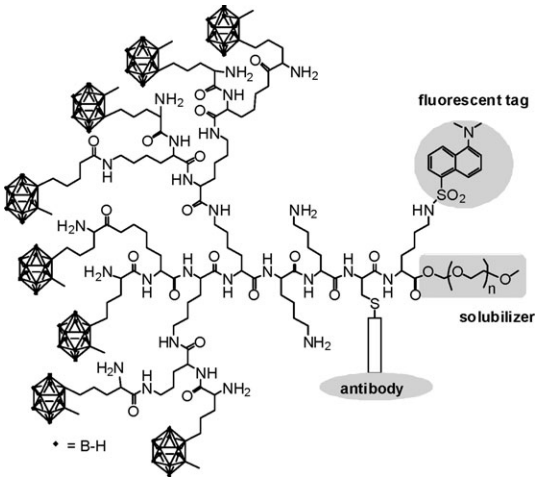


Fig. 11 Multifunctional BNCT dendritic agents.

Poly-L-lysine dendrimers have been used to circumvent this problem.[143] Actually, polylysine dendrons bearing a high boron payload (using eight dodecaborane groups), a dansyl-based fluorescent probe to allow the monitoring, a PEG tail to improve the solubility in water and an antibody for the targeting (see Fig. 11) have proven to be good candidates for this purpose, each function of the nanosystem operating correctly.

The use of G2 and G4 PAMAM dendrimers as scaffolds[144] for the grafting of $Na(CH_3)_3NB_{10}H_8NCO$ on their surface has also been described. A monoclonal antibody, directed to the murine B16 melanoma, was then conjugated to boronated dendrimers. These immunoconjugates had, however, a strong propensity to accumulate in the liver and spleen. In subsequent studies, epidermal growth factor (EGF) was used to target EGF receptors, which are cell surface receptors over-expressed in brain tumours such as gliomas.[145] *In vitro* studies showed that this immunoconjugate bound to cell surface membrane

and were endocytosed to accumulate in lysosomes. These observations were confirmed by *in vivo* intratumoral injections of this bioconjugate in rats, bearing cells of EGF receptor gene transfected C6 glioma.[146] Nevertheless, intravenous injections resulted in low amounts of dendrimers in tumour, because of its major uptake by the liver. *In vivo* studies proved that this new bioconjugate accumulated in tumour vasculature and was taken up by tumour cells. In this regard, a recent *in vitro* study reported by the group of Baker, warns on the inherent unwanted superagonist activity displayed by epidermal growth factor dendrimer conjugates, although this activity can be overcome by the drug transported on the scaffold bearing the EGF.[147] Another approach consisting in the targeting of endothelial cells of the tumour vasculature rather than the tumour itself has also been reported by the group of Barth.[148] They prepared a generation 5 PAMAM dendrimer conjugated to $Na(CH_3)_3NB_{10}H_8NCO$, a vascular endothelial growth factor and labelled with a near-infrared (IR) dye (Cy5).[149]

Shukla *et al.*[150] investigated the effect of the grafting of PEG chains on boronated dendrimers to prevent them from being uptaken by the liver. As mentioned above, PEG provides a usually good solution to enhance EPR and protect from RES cells uptake. Considering immunoconjugates based on generation 3 PAMAM dendrimers, they described that those with 1 to 1.5 PEG units, of 2000 g mol^{-1} each, exhibited the lowest uptake by the liver. In contrast, they also demonstrated that this improvement was lost when using 11 PEG units, of 500 g mol^{-1} each.

3.4 Drug-free dendrimers as potential therapeutic agents

Most of the bioactive dendrimers can be considered as nanovectors that carry on their surface suitable agents for a specific purpose such as targeting, monitoring or drug delivery. In these cases, dendrimers constitute the most versatile platform, taking advantage of their intrinsic properties such as monodispersity, multivalency, shape and so forth. Nevertheless, these unique properties can also make dendrimers act as drugs themselves. This issue is illustrated hereafter with the dendrimer based microbicide VivaGel™ to be marketed soon and a series of immunostimulating phosphorus-containing dendrimers.

3.4.1 Dendrimers for anti-STI therapies. The spread of sexually transmitted infections (STIs) due to the human immunodeficiency virus (HIV) and herpes simplex virus (HSV) type[151] is growing at an alarming rate. Alternatively to vaccine development, strong efforts are made to improve the current antiviral therapies. Particularly, several studies were devoted to the elaboration of multivalent scaffold mimicking the surface of the cells that are targeted by the virus. In 2000, Witvrouw *et al.*[152] reported the inhibition of HIV replication using sulfonated PAMAM dendrimers, which proved to inhibit HIV adsorption by binding gp120 glycoprotein located at the HIV surface. They also proved the internalisation of the dendrimer and its subsequent action to interfere with later steps of HIV replication. In parallel, Blanzat *et al.* evaluated the *in vitro* anti-HIV activity of dendrimeric galactosylceramide analogs. These original catanionic systems[153,154] have shown a good activity, with a clear dendritic shape dependent effect. At the same time, Bourne *et al.*[155] studied the activity of polyanionic dendrimers against HSV infection *in vitro* and introduced their use as topical microbicides *in vivo*. Whereas *in vitro* studies showed that all tested dendrimers managed to prevent HSV adhesion, *in vivo* studies against genital HSV infection in mice demonstrated the high efficiency of only polylysine dendrimers bearing naphthyl disodium disulfonate (SPL2999). This sulfonated dendrimer provided an efficient protection to mice from 20 s to 30 min before the introduction of HSV. Similarly to its PAMAM analogue, it affords dual sites of action by inhibiting not only HSV cell adhesion but also later stages of HSV replications.[156] Therefore, two polyanionic dendrimers analogues bearing sulfonated naphthalene were proved efficient against both HIV and HSV, and as importantly, they show no toxicity issues.

A structure–activity study from SPL2999 as a starting point was then led to identify the best lead molecule to take forward preclinical development.[151,157] In this regard, a major issue lies in the suitability of the chosen compound with Good Laboratory Practice/Current Good Manufacturing Practice requirements that demand stability and cost-of-production profile. In that sense, the linkage between the dendrimer and sulfonated naphthalene moieties was investigated and the initial thiourea bond was replaced by a more stable amide bond.[151] The influence of skeleton was also studied. They compared HIV antiviral activity of generation 3 polylysine, PAMAM and PPI dendrimers conjugated to sulfonated end

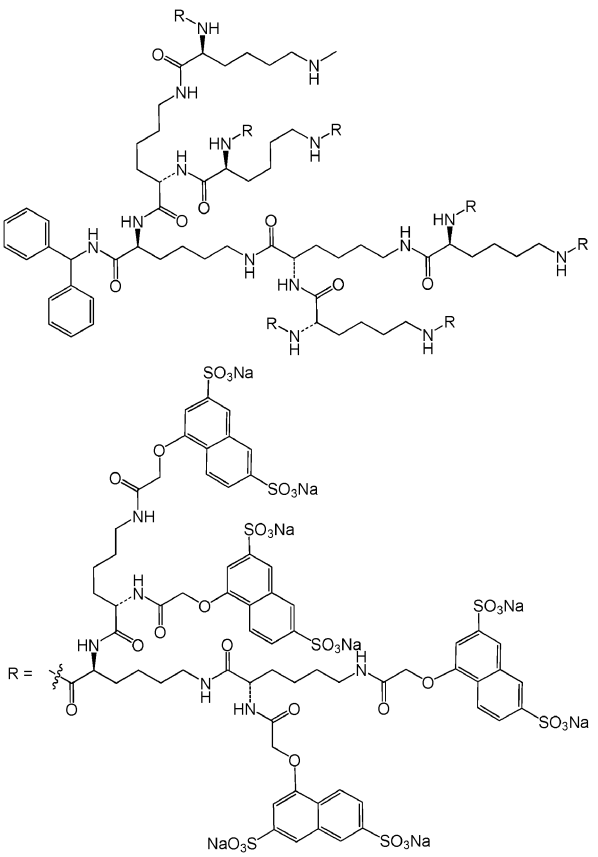


Fig. 12 SPL7013, the active dendrimeric compound of VIVAGEL™.

groups through an amide bond.[157] As the biological activities were not differing much, the synthetic methodology prevailed in the choice of the dendrimer. The use of cobalt during the synthesis of PPI dendrimer and the possible reverse Michael additions in PAMAM synthesis "disqualified" them in favour of the polylysine based dendrimer, referred to as SPL7013 (see Fig. 12).

This dendrimer is grown from a divalent core, the benzylhydrylamine amide of L-lysine. Upon successive conjugation of L-lysine amino acids, 32 sodium 1-(carboxymethoxy)-naphthalene-3,6-disulfonate groups are attached to amino groups through amide bonds, to produce SPL7013. Key intermediaries of this synthesis have been scaled up to 5–100 kg, and the final monodisperse product has been controlled by high performance liquid chromatography (HPLC), capillary electrophoresis, and electrospray mass spectral analysis.[157]

This compound was later formulated with a water-based Carbopol gel buffered to a physiological compatible pH, and dubbed VivaGel™. It is expected to be marketed either alone or coated on condoms, and it is the first and, to the best of our knowledge, the only dendrimer-based therapeutic agent that is under clinical phase 3 trials to date.

3.4.2 Phosphorus-containing dendrimers for cell-based therapies. In 2004, Solassol *et al.* reported that cationic phosphorus-containing dendrimers had a strong anti-prion activity, reducing prion replication both *in vitro* and *in vivo* (in mice infected with the abnormal scrapie isoform of the prion protein).[158] Similarly to SPL7013, these dendrimers did not bear any specific therapeutic agents, given that they were only terminated with tertiary amines, under their ammonium chloride form, and the results obtained by the group of Majoral and Caminade confirmed the preliminary observations made by the group of Prusiner on PEI and PAMAM dendrimers.[159,160]

Of note was the fact that a similar type of "natural" bioactivity of phosphorus-containing dendrimers was recently observed in immunology. In fact, Griffe *et al.* demonstrated that phosphorus-containing dendrimers capped with aminobis-(methylenephosphonic acid) induce a remarkable bioactivity towards specific cells of the immune system. In fact, they have the intriguing ability to activate human monocytes, and dramatically and selectively promote the multiplication of human Natural Killer (NK) cells.[161,162]

Both sub-populations targeted by the dendrimers described in these studies play a key role in the primary steps of immune response. The NK cells belong to the innate immune system, and behave like the first line of defence against all non-self pathogens (including foreign organisms such as viruses, bacteria, fungi and parasites, as well as tumour cells). Among the phagocitic cells of the innate immune system, monocytes are precursors of macrophages and play a key role in the initiation of the response against infection and in the controlling of the latter before cells of the adaptative immune system proceed.

The group of Majoral and Caminade in collaboration with the group of Fournié and Poupot showed that a generation 1 dendrimer having 12 aminobis(methylenephosphonic acid) end-groups derived from tyramine (G1-TamBP, Fig. 13), originally prepared for metal surface treatment, activated selectively monocytes after their *ex vivo* incubation with peripheral blood mononuclear cells (PBMC) during three days.[161,162]

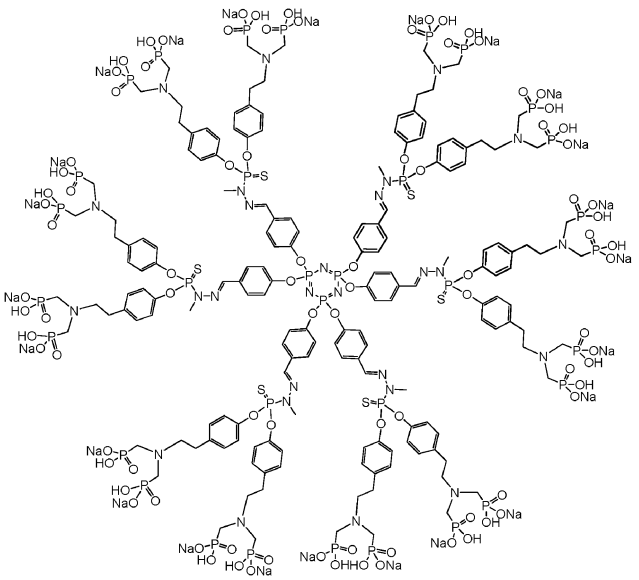


Fig. 13 Structure of tyramine-derived aminobis(methylenephosphonic acid) terminated generation 1 dendrimer (G1-TamBP).

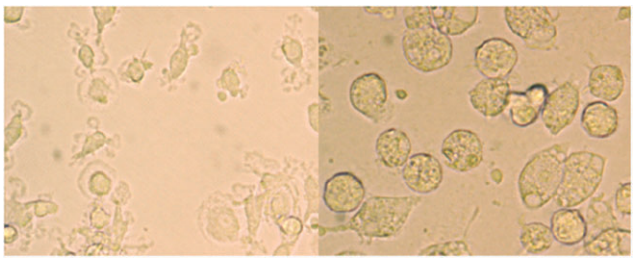


Fig. 14 Untreated monocytes (left) and G1-TamBP treated monocytes (right).

Within three to six days, monocytes in culture with the dendrimer G1-TamBP underwent morphological changes (Fig. 14) and down-regulation (decrease in number) of specific surface markers. Monocytes were found bigger under optical microscopy, and flow cytometry confirmed the increase of both size and granulosity. Moreover, the activation of monocytes by dendrimer G1-TamBP results in the down-regulation of two surface proteins, CD14 and HLA-DR. Dendrimer activated monocytes were also found to exhibit longer lifetimes in culture.

In order to monitor this process, monocytes are tagged with fluorescent monoclonal antibodies bound to these proteins, and another one bound to a "control" protein that will not be affected upon the activation. Flow-cytometry was used to monitor the down-regulation of a protein by calculating the mean fluorescence intensity ratio (mfi-R), which is the ratio between the mfi of cells stained with the antibody against the observed protein and that of cells stained with the control antibody only.[163]

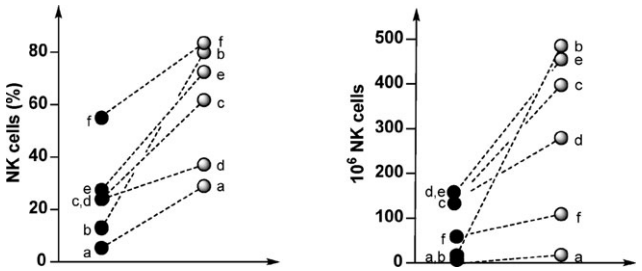


Fig. 15 Percentage and number of NK cells after four weeks of culture with IL-2 alone (black dots) and with IL-2 and G1-TamBP at 20 μM (grey dots). Data a–f represent six healthy donors.

Furthermore, dendrimer G1-TamBP, has also an intriguing bioactivity *versus* NK cells. In early *ex vivo* attempts, upon the addition of G1-TamBP to cultures of peripheral blood mononuclear cells (PBMC) supplemented with a growth factor, interleukin-2 (IL2), both percentage and number of NK cells were highly increased (see Fig. 15). In fact, after three weeks in culture, a mean multiplication of NK cells number of 105-fold was achieved in PBMC medium supplemented with G1-TamBP and IL2, whereas a mean multiplication of 7.5-fold was achieved in medium supplemented only with IL2. The multiplication of NK cells is blood-donor dependant and multiplications of 500-fold were obtained with some donors.

The bioactivity of the new generated NK cells with the dendrimer G1-TamBP is not altered, which is in contrast with reports on the activation of the same cells using reported activating ligands.[164] In addition, newly generated NK cells are fully functional and mature as normal NK cells are. For example, their ability to kill tumour cells remained the same, and, more importantly, they are still capable to discriminate between the self and non-self. Afterwards, a structure–activity relationship study has held with the view to identifying the dendrimeric key parameters responsible for the immuno-stimulating properties. A first part of these long studies has been published recently,[161,165] confirming the leading position of G1-TamBP for the activation of monocytes. Further investigations are in progress to determine the mechanism by which this phosphorus-containing dendrimer achieves the activation and multiplication of NK cells and triggers the monocytes.

These pioneering studies are very promising and allow envisioning, in a long term, future therapeutic applications, which may necessitate a large number of NK cells, such as immunotherapy for anti-viral and anti-cancer purposes.[166,167] Such cell therapies are tedious to achieve because they necessitate a large number of NK cells to succeed; therefore, in a preliminary step, NK cells have to be expanded and activated *ex vivo*. However, from the best of our knowledge, the amplification of NK cells, which has been realised with mainly IL2, have never been higher than 10-fold, which is not sufficient. Therefore, the use of dendrimer G1-TamBP may be an attractive alternative to increase the amplification rate of NK cells.

4. Conclusions

Nanoscience constitutes a unique scientific field whose specificity resides in the following statement: nanometre-length size matters. The ability to visualise and manipulate the matter at the nanoscale allows to understand the nanostructure–properties relationship and to prepare objects whose intrinsic properties depend directly on their size. These findings have been useful to application fields seeking devices bearing new and tuneable properties, such as electronics and medicine. Besides, the latter area has been deeply impacted by nanotechnology through new developments that were previously out of reach. In fact, the emergence of the recently recognised field of nanomedicine has allowed to access the elusive perfect therapeutic nanodevice capable of, upon its delivery to the human body, targeting the site of the disease, diagnosing the latter, treating it and then being removed harmlessly. Among the eligible devices that have already operated in this field, dendrimers present the required properties for this purpose.

These large organic monodisperse molecules based on repetitive units and whose architecture consists in surface groups spread at the periphery by branches anchored to a core, are in between polymer and organic chemistries. Their unique structure provides them with intrinsic properties that can be tuned quite easily during the synthesis. Among the properties already tested for therapeutic purposes, we can cite their stability, spherical shape, size, hollow structure, and multivalency. Actually, they constitute a versatile platform whose inherent parameters can be controlled and set on demand. In this regard, tissue targeting for drug delivery and imaging constitutes their main field of application in nanomedicine.

Dendrimers contribute also to nanomedicine, in a lesser extent, as therapeutic agents that induce a biological activity without presenting any molecular template known for its bioactivity when free. Such types of activities, which are limited in number, are mainly due to the dendritic multivalency, which non-specifically induces biological interactions. One of the most advanced dendrimer based therapeutic agent of this kind is VivaGel™, which is expected to be marketed as a barrier gel for the prevention of viral STDs.

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