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Dendrimers and DNA: Combinations of Two Special Topologies for Nanomaterials and Biology

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Abstract: Interactions between two precisely defined three-dimensional architectures (DNA and dendrimers) are described. Highly synergetic effects occur, as illustrated in two cases: dendrimers can be used as three-dimensional linkers for oligonucleotides, affording highly sensitive microarrays (biochips), and positively charged dendrimers strongly interact with DNA, allowing penetration inside cells (genetic transfection).

Keywords: biology · dendrimers · DNA · nanomaterials · self-assembly

Introduction

Molecular design is more and more able to produce fascinating architectures, and dendrimers are certainly one of the best illustrations of this concept. Dendrimers are regular tree-like macromolecules, constituted of a series of branches linked to a central core. Their step-by-step repetitive synthesis (generation after generation, see Scheme 1) affords structurally well-defined compounds, generally of globular shape, with a very low polydispersity compared to traditional polymers despite their large molecular weight. The structure of a dendrimer can be divided into three different regions: the core, the branches, and the periphery. Most of the properties of dendrimers are due to their numerous terminal groups, which can be varied at will. Functional dendrimers in preparatively useful amounts can be synthesized from a variety of building blocks and coupling chemistries. However, an important limitation to the synthesis of large dendrimers is that quantitative reactions are required, thus the types of dendritic structures that are/were commercially available (and thus widely used) are extremely limited. Poly(amidoamine) (PAMAM) dendrimers[1] are certainly the most popular type of dendrimers, but poly(propyleneimine) (PPI) dendrimers,[2] and phosphorus dendrimers[3] (see Scheme 1 for their structures) are also receiving considerable attention.

One of the most active areas of research about dendrimers concerns their biological properties. Numerous reviews in this field have been published recently,[4] but none of them focused on the interactions of dendrimers with another fascinating molecular architecture: DNA (deoxyribonucleic acid). Chemically, DNA is also a precisely shaped polymer of simple units, with a backbone made of phosphates and sugars to which one of the four natural bases is linked. The

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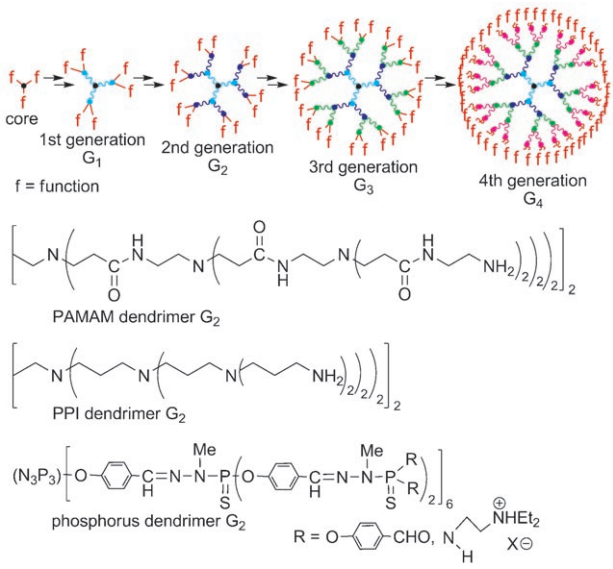

Scheme 1. Schematized step-by-step divergent synthesis of dendrimers (generation after generation) and structure of the dendrimers that are used the most for interacting with DNA.

key point of the well-known double helix structure is the hybridization, due to the supramolecular phenomenon of bases pairing,[5] in which purine bases (adenine and guanine) are hydrogen-bonded to complementary pyrimidine bases (cytosine and thymine), creating A–T pairs (two hydrogen bonds) and G–C pairs (three hydrogen bonds) (Figure 1).

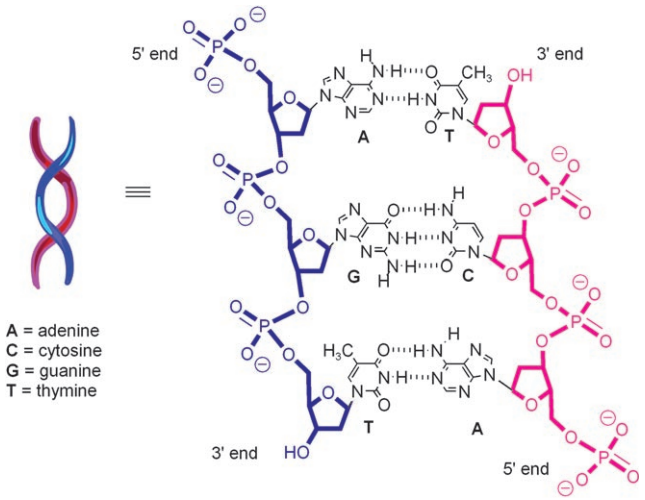

Figure 1. Hybridization occurs through supramolecular recognition of complementary bases (adenine with thymine, and cytosine with guanine) linked to the phosphate deoxyribose backbones, as illustrated by one example.

Infinite types of combinations between the double helix structure of DNA and the globular hyperbranched structure of dendrimers can be foreseen, affording original entities of

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interest not only for chemists but also for physicists and biologists. This paper will display the most significant examples connecting the field of dendrimers to that of DNA, but it is not an exhaustive review (more than 500 papers were already published). All the work relevant to this topic concerns three main aspects: i) the synthesis of dendrimers composed in part or totally of oligonucleotides (short fragments of DNA); ii) the use of dendrimers covalently linked to one or a few oligonucleotides to create microarrays usable as biosensors; and iii) the noncovalent interactions between dendrimers and DNA, useful for creating nanostructures for physicists, but mostly used in view of genetic therapy.

DNA as Structural Constituents of Dendrimers

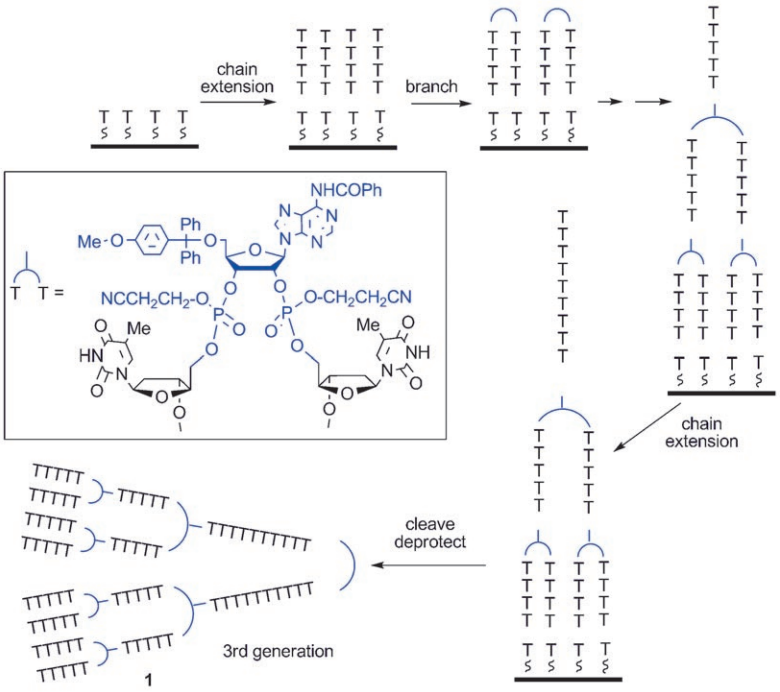
The first use of DNA or nucleic acids to elaborate dendritic structures dates back to 1993. Classical automated solid-phase synthesis of single-stranded oligonucleotides was used, with a phosphoramidite reagent to join neighboring oligomers together, thus forming the branching points. Chain elongation and branching steps were repeated to synthesize the successive generations (up to generation 3) in a convergent manner (Scheme 2). Then, the oligomers were concomitantly cleaved from the support, affording dendrimers of the type **1** shown also in Figure 2. Characterization indicated the presence of some defects in the branching, due to the convergent strategy on support.[6] Later on, a divergent strategy was used, also in the solid phase.[7] No attempt of self-assembly was carried out. Nevertheless, most of the work carried out with oligonucleotide-containing dendrimers takes profit of hybridization (self-assembly of complementary oligonucleotides), to elaborate sophisticated dendritic architectures.

A few examples of dendrimers possessing a single oligonucleotide linked to the core are known. The first examples of type **2** dendrimers, also called dendrons (Figure 2) possess a 15 mer single-stranded oligonucleotide connected to the core of polyglycol dendrimers.[8] These compounds were used as probes to oligonucleotide arrays possessing the complementary oligonucleotide, and also as primer in the PCR (Polymerase Chain Reaction, a technique that allows to exponentially amplify a piece of DNA by in vitro enzymatic replication[9]). The oligonucleotide at the core of compounds of type **2** can be chosen in order to allow the hybridization with another dendron possessing the complementary oligonucleotide, affording compounds of type **3** (Figure 2). This supramolecular approach offers a versatile strategy for designing new dendritic nanostructures, by varying the size, shape, and surface substituents of both dendritic entities involved.[10]

A similar approach consists of randomly conjugating one or a few oligonucleotides to the surface of dendrimers. Hybridization of two dendrimers possessing two complementary oligonucleotides on their surface affords compounds of type **4** (Figure 2). Such compounds were first used to study their physical properties and shape (AFM, dynamic light scattering).[11] Later on, the random approach was extended to fluorescent labels on one dendrimer (fluorescein) and folic acid on the other dendrimer, for cancer cell specific targeting. Hybridization of both dendrimers offers the potential for developing combinatorial therapeutics.[12]

In the case of the full substitution of all the terminal groups of dendrimers by oligonucleotides such as compounds of type **5** (Figure 2), various types of hybridizations can be performed. The simplest case consists of reacting linear complementary oligonucleotides to afford compounds of type **6**,[13,14] either in solution, or linked to a solid surface. In case of two dendrimers possessing several single-stranded complementary oligonucleotides, stable nanostructures were obtained in some cases by self-assembly, such as compounds of type **7** (Figure 2).[13,15]

Dendrimers exclusively composed of oligonucleotides associated by hybridization were first described in 1997.[16] A heterodimer composed of two single-stranded nucleic acid



Scheme 2. Schematized convergent synthesis of single-stranded oligonucleotide dendrimers on solid support.

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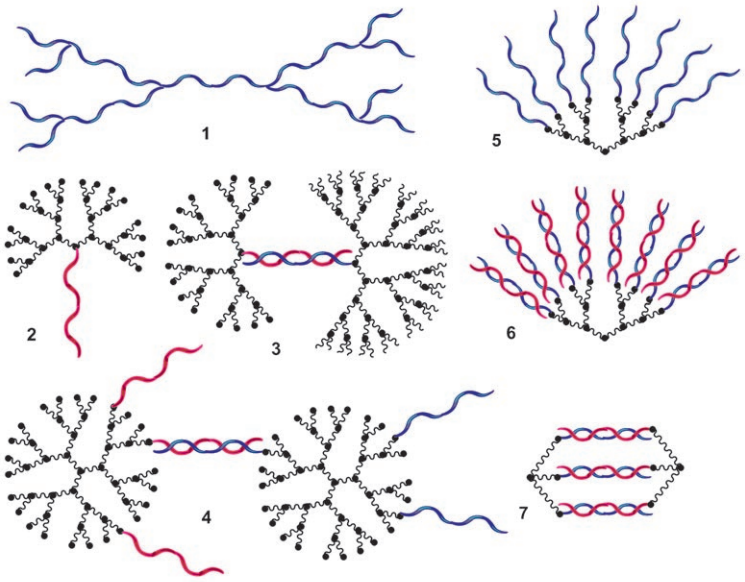
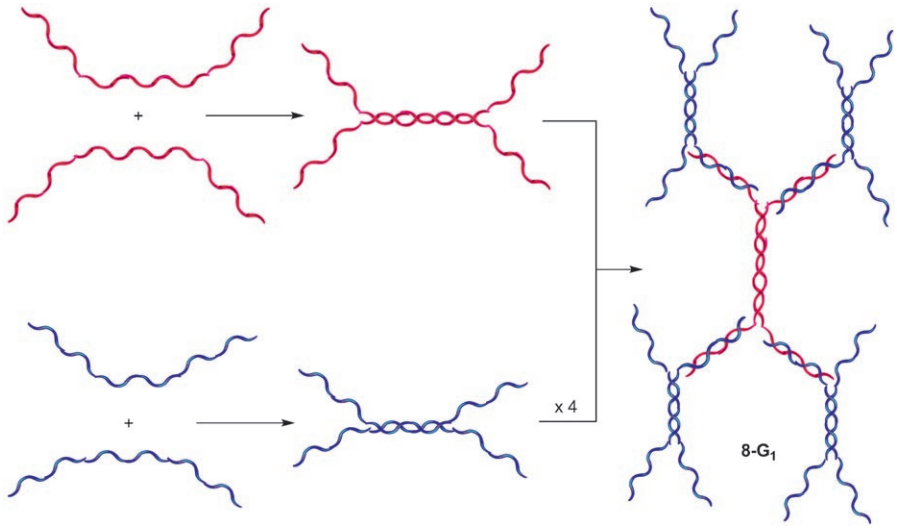


Figure 2. Different types of dendritic structures incorporating oligonucleotides (nucleic acids) in their structure.

oligomers possessing a central double-stranded waist and four single-stranded arms for binding was used as monomer. The assembly of the dendrimer proceeds in layers as shown in Scheme 3 for the first-generation **8-G$_1$**, possessing 12 single-stranded arms on the periphery. This method was first applied up to generation 6 (2916 single-stranded arms).[16]

These types of dendritic nucleic acids possess numerous interesting properties. In a first example, they were used as very sensitive probes for DNA biosensors (see below).[17] Another example of strong signal amplification using these nucleic acid dendrimers was observed when attaching as terminal groups special oligonucleotides suitable to undergo an extension due to polymerase activity and an excision due to exonuclease activity. DNA polymerase catalyses the generation of pyrophosphate, which is enzymatically converted to ATP and subsequently detected by bioluminescence with luciferase. Measurement of the fluorescence issued from the reaction of a monomeric oligonucleotide and of the generations 4, 6, and 8 of the nucleic-acid dendrimers bearing this monomer as terminal functions shows that the magnitude of the signal parallels the increase of the number of terminal functions. Thus, this method was able to detect a few attomoles (10^{-18}) of generation 4 and a few zeptomoles (10^{-21}) of the generation 8, that is a few hundreds of molecules of dendrimers (Scheme 4).[18] The peripheral groups of the nucleic acid dendrimers shown in Scheme 3 can also be used to graft randomly both specific oligonucleotides and fluorophores. These compounds were used to identify some specific cells in vivo in chicken embryo.[19] They are commercially available under the name of 3DNA, and are particularly suitable as labels for improved detection in microarray experiments and flow cytometry.[20]

Another series of nucleic-acid dendrimers was obtained later in a relatively analogous way, using also single-stranded oligonucleotides having partial complementary sequences. In this case, Y-shaped DNA was obtained (named D_3 **9-G$_0$** in Scheme 5) as core. Using the same principle, an AB_2 monomer (also Y-shaped DNA) was elaborated. Assembly by ligating three equivalents of AB_2 with one equivalent of the core D_3 affords the first generation dendrimer B_6 **9-G$_1$**. Reacting six equivalents of a CD_2 monomer elaborated in the same way, with B_6 affords the second-generation **9-G$_2$** (Scheme 5). The alternate use of AB_2 and CD_2 monomers was carried out up to the fifth generation.[21] A, B, C, and D represent different sequences of oligonucleotides, with A and D being complementary, as well as C and B; on the other hand, A and C, as well as D and B, are mis-



Scheme 3. Synthesis of dendritic nucleic acid structures by hybridization of linear oligonucleotides. Only the first generation is shown.

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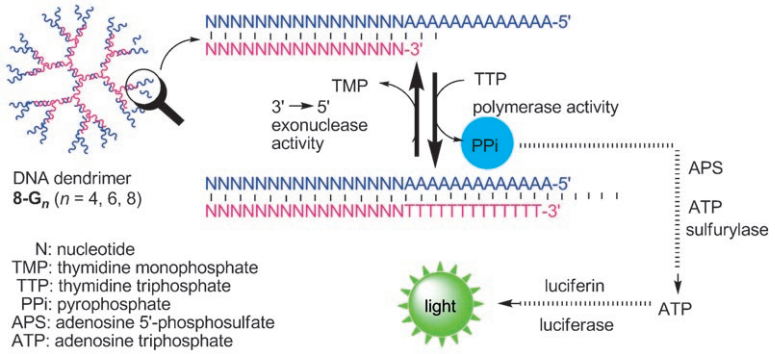
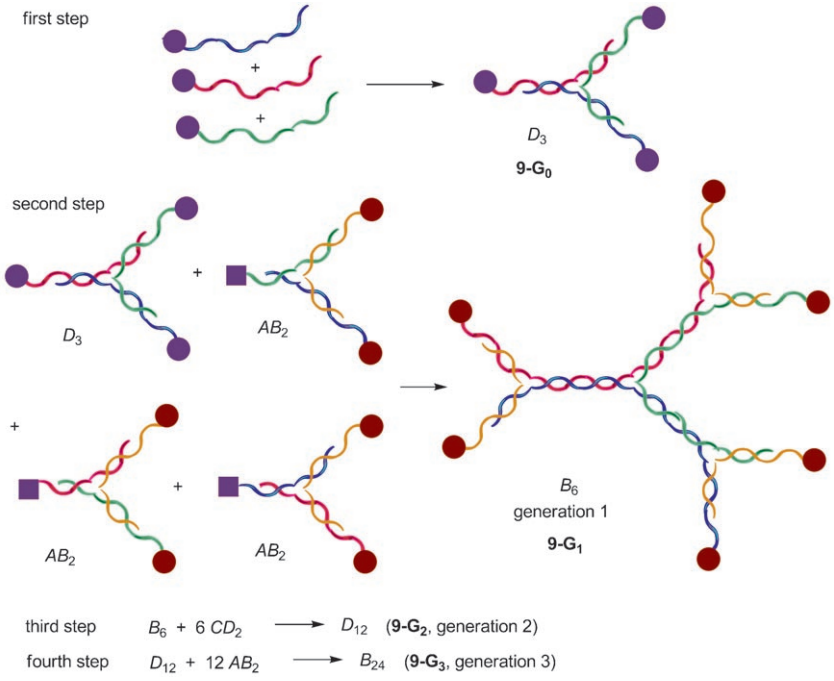


Scheme 4. Schematic representation of the principle of detection of DNA dendrimers terminated by special single-stranded oligonucleotides. The polymerase activity extends the recessive 3′-end. For each TTP molecule incorporated, a PPi molecule is liberated. Once extension is completed, the template is regenerated by the 3′→5′ exonuclease activity for another round of polymerase activity. This process affords a high concentration of PPi, which is enzymatically converted to ATP. The amount of ATP generated is detected by firefly luciferase and luciferin.



Scheme 5. Synthesis of nucleic acid dendrimers using branched monomers elaborated from three oligonucleotides. The colours pink, blue, green and orange are only used to differentiate each strand (all the AB_2 monomers are identical).

matched. This principle (use of AB_2 and CD_2 monomers with reaction of A only with D, and of C only with D) was already reported for "classical" dendrimers (not with DNA).[22]

Due to the specificity of the hybridization, each terminal group of the oligonucleotide dendrimers shown in Scheme 5 can be precisely functionalized, in particular to graft different fluorescent dyes, in different ratios, as well as a probe for molecular recognition. This method provides "nanobarcodes", usable for the detection of the DNA of several pathogens by fluorescence microscopy, by blotting assays, and by flow cytometry,[23] but also for studying diffusion properties.[24] An analogous procedure was applied starting from agarose beads as solid support.[25] Complex dendritic architectures were recently obtained by similar self-assembly pathways,[26] as well as assemblies of discrete gold nanoparticles.[27] The covalent grafting of dendrons to DNA was also used for the direct metallization of specific DNA strands, in order to increase the conductivity, affording molecular wires.[28]

Dendrimers for DNA Microarrays and Biosensors

The growing demand for molecular medicine, forensic applications, preservation of food and environment safety, has stimulated the search for quick and reliable analytical methods. Biosensors, such as DNA microarrays, are more and more playing a major role in this field.[29] Typical devices consist of an oligonucleotide (the probe) immobilized at discrete positions on surface activated slides; generally a robot is used for spotting the oligonucleotides. The sample to be analyzed is constituted of complex mixtures of fluorescently labeled nucleic acids containing the target. The hybridization probe–target is quantified by fluorescence (see upper part of Figure 3). In some cases, a dehybridization (stripping) process can be carried out after the experiment to regenerate the probe, and reuse it in subsequent experiments. The degree of sophistication of these devices increases continuously to progress towards ultra-sensitive methods. Several examples have reported the role played by dendritic structures for improving the sensitivity and reliability of such devices, by ensuring the moving of the probe away the solid surface for improved hybridizations. A recent review emphasized the role of dendrimers,[30] thus, only leading and up-dated examples will be reported here.

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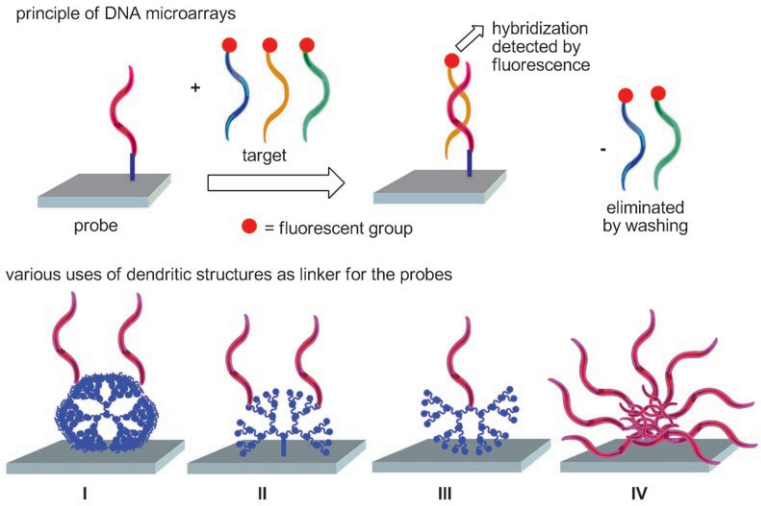

Figure 3. Upper part: principle of detection by DNA sensors. Lower part: various types of dendritic structures used as spacer between the solid surface and the oligonucleotide probe; from left to right: I) dendrimer deposited on the surface and then functionalized by the probe; II) dendrimer created step-by-step on the surface, then functionalized by the probe; III) dendron deposited on the surface, then the core is functionalized by the probe; IV) all the terminal groups of the dendrimer are probes.

The simplest method consists in covalently grafting the dendrimers on a glass (or silica or quartz) slide beforehand activated by reacting in particular aminotriethoxysilanes. If the dendrimer has not the suitable functional groups to react directly with the NH_2 surface groups (in particular PAMAM dendrimers), another intermediate linker such as a diisothiocyanate is needed. This type of homobifunctional reagent also induces the cross-linking of dendrimers to improve the stability of the array, and allows the grafting of oligonucleotides on the surface of the dendrimer (slides I, Figure 3). The stability was confirmed by the constancy of the intensity of the fluorescence after 100 simulated regeneration cycles.[31] However, the cross-linking diminishes the number of sites suitable for the grafting of the oligonucleotides, thus the intermediate linkers were modified in subsequent attempts to avoid cross-linking. A 10-fold increase of the fluorescence intensity was observed using these slides modified by PAMAM dendrimers, compared to slides modified by a linear linker. Furthermore, single nucleotide polymorphism can be detected (only one base different from the perfect target on a 212 mer single-stranded DNA induces a decrease of the signal intensity of 60–92% depending on the position of the mismatch).[32] Other types of dendrimers having also NH_2 terminal groups were used, in particular PPI dendrimers, but in this case no real improvement was observed when compared to nondendritic arrays.[33]

If the dendrimers possess aldehyde terminal groups, such as phosphorus containing dendrimers, they are directly linked to the slide functionalized by NH_2.[34] Furthermore, the remaining aldehyde groups are also suitable for the direct grafting of amino-modified oligonucleotides. Several generations of these dendrimers were tested (from 1 to 7), and the best signal-to-noise ratio was obtained with generations 4 to 7, emphasizing the importance of the size of the dendrimer. A 1000-fold increase of the fluorescence intensity was observed when compared with simple aldehyde functionalized slides.[35] Comparison with twelve commercially available activated slides shows that only the presence of the dendrimers allows the detection of picomoles of targets (10^{-12}).[36] This high sensitivity is particularly interesting for studies involving very low amounts of biological materials. Furthermore, these slides can be reused several times using dehybridization/hybridization sequences, and allows the detection of mismatching. The same type of slides functionalized by phosphorus dendrimers also allowed the elaboration of microstructured liposome arrays, by grafting on the liposomes oligonucleotides complementary to the oligonucleotides bound to the array.[37]

Besides "true" dendrimers, dendrons were also used to create DNA microarrays of type II (Figure 3). In the first example, the dendrons were created step-by-step on the solid surface from nucleating points. However, a mixture of branched products was obtained, and not a precise dendron.[38] On the other hand, true dendrons can be considered as cone-shaped compounds that are able to generate mesospacing when linked to a solid.[39] Coupling one oligonucleotide to the core provides DNA probes with ample space for hybridization (type III, Figure 3), resulting in high hybridization yields (80–100%). These DNA microarrays have high sensitivity and selectivity and are usable in a wide range of temperatures (37–50°C).[40] These arrays were also used for measuring the force of DNA hybridization with an AFM probe covered by the complementary oligonucleotide.[41]

Besides the spotting of the probe oligonucleotides by a robot which was used for most of the above-mentioned examples, dendrimers were also used in the patterning of sensors for biomolecules. A silicon wafer covered by PEG (polyethyleneglycol) was patterned by e-beam to generate silanol groups, then surrounded by an aldehyde terminated SAM (self-assembled monolayer). PAMAM dendrimers having NH_2 terminal groups were then covalently linked on the precise places where the aldehyde groups were placed. Then, the probe oligonucleotide was linked to the dendrimer as for classical DNA arrays, and the efficiency of the detection of the target was checked classically by fluorescence.[42] Another method for patterning consists in using stamps for microprinting. The particular advantage of this technique is that it allows the replication of microarrays. A

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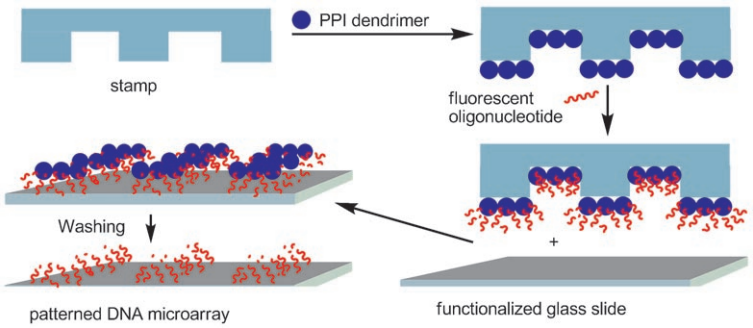
stamp was inked with PPI dendrimers, and subsequently incubated with fluorescein-labeled DNA. After transfer printing onto the functionalized slide, the substrate was rinsed to wash out the dendrimers, affording the patterned DNA microarray, analyzed by fluorescence (Scheme 6).[43]



Scheme 6. Microcontact printing of DNA with a stamp functionalized by dendrimers.

A very recent example of a biosensor enabling an enhanced detection of DNA hybridization used nanotubes of dendrimers elaborated inside an ordered porous alumina membrane. The nanotubes were obtained by layer-by-layer deposition of negatively and positively charged phosphorus dendrimers on the pore walls.[44] After deposition of three bilayers of anionic and cationic dendrimers, a first layer of negatively charged quantum dots (QD) was deposited. The alternate deposition of polycationic dendrimers with several other layers of luminescent quantum dots (three different quantum dots of type ZnCdSe alloys with different emission wavelengths) offered a graded-bandgap structure. Then probe oligonucleotides were immobilized, and hybridized with target fluorescent labeled oligonucleotides (Figure 4). An efficient excitation energy transfer occurs by FRET (Förster resonance energy transfer) from the outer to the inner surface of the dendritic nanotubes, and allowed the detection of hybridization with a high sensitivity.[45]

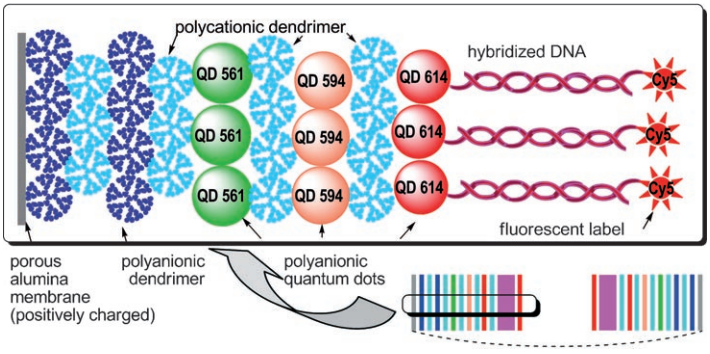


Figure 4. Dendrimer nanotubes as graded-bandgap structures elaborated with charged phosphorus dendrimers and ZnCdSe quantum dots inside a porous alumina membrane, then surrounded by DNA linked to a fluorescent label. The enlargement of one wall is schematized.

Besides fluorescence, a few other methods have been used to detect hybridization occurring with dendritic DNA sensors. The first example concerns the nucleic acid dendrimers shown in Scheme 3. The generation 4, especially designed to possess single-stranded terminal arms specific to a waterborne pathogen, was entrapped in polyphenol for immobilization onto a quartz-crystal microbalance (slide IV in Figure 3). Hybridization with the DNA target issued from this pathogen produces a weight increase, which induces a large modification of the resonance frequency. An important increase of the sensitivity of detection is observed, but it is lower than expected, indicating that not all the oligonucleotide arms are hybridized with the target.[17] The same detection technique was applied to phosphorus dendrimers linked on one side to a resonating piezoelectric membrane and to an oligonucleotide on the other side. The complementary oligonucleotide (the target) is biotin-labeled, and is then used to trap streptavidin–gold colloid conjugates. The deposition of the gold colloids induces a modification of the resonance frequency of the membrane; the estimated mass sensitivity was better by a factor of several hundreds than the state of the art values for such devices.[46] Surface plasmon resonance[47] and electrochemistry[48] were also used to detect the efficiency of dendritic (PAMAM dendrimers) DNA sensors.

Noncovalent Interactions Dendrimers/DNA

In most cases using dendrimers as sensitive biosensors, the dendrimer and the oligonucleotide are covalently linked. However, there exist also numerous works concerning noncovalent interactions between positively charged dendrimers and DNA (generally double-stranded). As expected, the major binding force for DNA–dendrimers complexes are electrostatic interactions.[49] Most of this work was carried out with the aim of performing transfection experiments. However, we will first give some insights on the studies carried out to understand how the interaction occurs (some examples relevant to this topic were previously reviewed);[50] indications about the potential of these complexes for physical technologies will be also given.

An important part of this work was carried out with PAMAM dendrimers, which possess easily cationized amino terminal and internal groups. Nitroxide-labeled PAMAM dendrimers studied by EPR provided information about the interaction DNA/dendrimers (Figure 5): i) DNA can "wrap" around generation 6 better than around generation 2; ii) higher protonation decreases the interaction with DNA for

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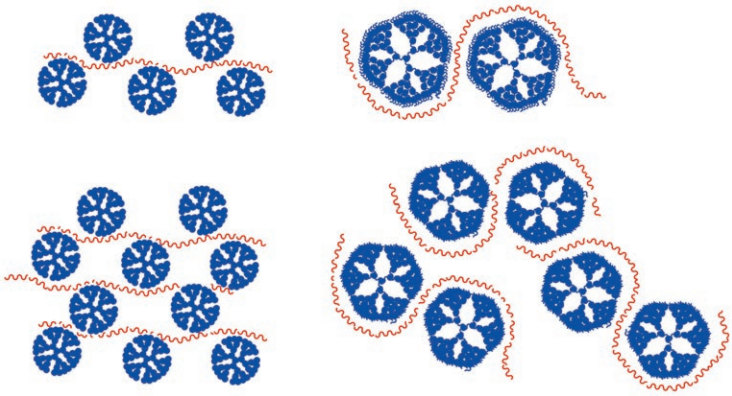

Figure 5. Possible types of interactions between dendrimers and DNA, depending on the size of dendrimers, the length of DNA, and the ratio of charges.

large dendrimers but increases for small dendrimers; iii) formation of supramolecular structures induces a protection against specific nucleases; iv) extensive precipitation of PAMAM/DNA adducts takes place for a large number of ratios x (x = positive charges of dendrimers/base pairs of DNA), due to charge neutralization (only aggregates with $0<x<1$ and $200<x$ are soluble).[51] In some cases, the aggregates form ordered structures, characterized by a different degree of DNA ordering: a condensed nematic phase was observed for $2=x<4$, whereas a long-range ordered square lattice was formed at $x=4$, using PAMAM G_4 dendrimers.[52] With PAMAM G_2 on mica, 2D densely packed DNA nanostructures were created.[53] AFM images with G_6 showed two different phases, a condensed phase and an extended phase (a PAMAM/DNA fiber), and indicated that the dendrimers are bent around DNA more than the DNA bends around the dendrimers.[54] In the case of single-stranded DNA, a theoretical approach indicates that the complexation is surprisingly sequence dependant, with the binding constant of PAMAM G_4 for homologous oligonucleotides (a single type of base) being T < A < C < G.[55] Between PPI dendrimers and double stranded DNA, water soluble complexes were obtained at $1 < x$ with G_4 and G_5, but not with G_1 and G_2. It was shown that native DNA complexed with an excess of dendrimer was compacted.[56] A deeper insight in the structure using synchrotron X-ray studies showed that the complexes are columnar mesophases consisting of arrays of DNA rods intercalated with dendrimers.[57]

Several types of dendritic structures were specially engineered for studying their interaction with DNA. The self-assembly between a barbell-like triblock copolymer comprising two polylysine dendrons linked by their core to a linear PEG and plasmid DNA was studied by electrophoretic shift assay and AFM. A very important difference was observed between the generations 3 and 4 of the dendrons: only G_4 was able to form spherical particles with DNA and to protect it against DNase attack.[58] Cylindrically shaped dendronized polymers having G_2 or G_4 dendrons attached to aminopolystyrene were complexed with DNA. Scanning force microscopy data indicated that DNA wraps around the dendronized polymers.[59] Low molecular weight dendrons having spermine as terminal groups were shown to bind strongly to plasmid DNA; G_1 affords spherical aggregates of approximately 100 nm in diameter, and 400 nm for G_2, showing that both G_1 and G_2 efficiently condense DNA.[60] Attachment of a protein at the core of the same dendrons does not modify the high affinity to DNA, allowing to convey DNA affinity to proteins that do not have natural DNA-binding ability.[61] On the other hand, introducing UV-responsive linkers between the spermine and the dendrons affords photocleavable entities, able to bind reversibly to DNA.[62]

Final examples of interactions between double-stranded DNA and dendrimers were not carried out in view of transfection experiments. PAMAM dendrimers were used as stationary phases to deposit porphyrin-intercalated DNA onto ITO (indium titanium oxide) electrodes. The resulting electrodes generated photocurrent waves in response to irradiation with visible light; the intensity increased with the generation (G_1 to G_4).[63] On the other hand, positively charged phosphorus dendrimers and long DNA (3 μm length) were used for the elaboration of microcapsules, starting form microparticles ($\varnothing = 4$ μm) covered by the layer-by-layer deposition technique (4× each). By dissolving the microparticle templates in HCl, microcapsules composed of DNA and dendrimers layers in alternation were obtained, illustrating the high stability of the complexes. Most of the capsules are parts of small aggregates, due to the length of DNA, which can be linked to two capsules.[64]

Dendrimers as Transfecting Agents

The delivery of therapeutic nucleic acids is one of the major challenges of genetic therapies. This challenge has been addressed using a variety of viral and non-viral delivery systems, but all have some severe disadvantages: lack of safety for viral vectors and poor efficiency for synthetic vectors. We have seen in the previous part of this paper that polycationic dendrimers strongly interact with DNA, thus their use as synthetic vectors has been recognized very early,[65] and has now produced a huge quantity of publications. A review has gathered most of the early work in this topic,[66] thus we will focus on the most representative, and also the most recent examples.

Most of this work has been carried out for the delivery of plasmid DNA, to express the gene of interest, which will *increase* the activity of the target (in particular by production of a therapeutic protein). A small part of this work concerns antisense oligonucleotides or siRNA (silent), which generally will *reduce* the target activity. A mechanism can be proposed for the use of dendrimers as transfecting agents, even if all the steps are not fully understood (Figure 6). The first step is the association of dendrimers and DNA, affording what has been called a dendriplexe.[67] The binding of the

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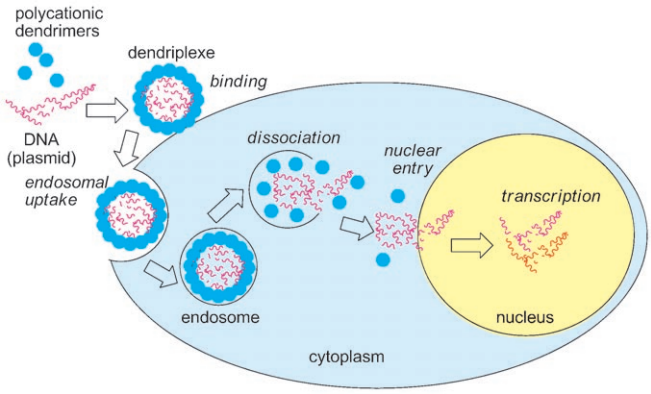

Figure 6. Possible way for transfecting cells using dendrimers.

dendriplexe (positively charged) to the cell membrane (negatively charged) is generally based on electrostatic attraction. Endosomal uptake of this dendriplexe allows the internalization inside the cytoplasm. Dissociation occurs presumably under the influence of cationic lipids. The transfer from the cytoplasm to the nucleus is a critical step, but nuclear entry is not fully understood (with or without dendrimers). The behavior of the dendrimer after the transfection is also unclear.

A very important amount of work dedicated to transfection using dendritic agents concerns PAMAM dendrimers, which have become a standard tool for many cell and molecular biologists. Reviews have been published on this particular topic.[68] The importance of the generation of the dendrimer on the efficiency of transfection was early recognized, the best efficiency for transfecting mammalian cells with the plasmid encoding firefly luciferase being obtained with generation 6 PAMAM,[65] but it appeared later that the specific dendrimer most efficient in achieving transfection varied between different types of cells.[69] Some experiments were carried out in vivo for topical delivery to hairless mouse skin.[70] The delivery of antisense oligonucleotides[71] and RNA[72] using PAMAM dendrimers was also efficient, as shown by the decrease of the expression of the target.

Besides "native" PAMAM dendrimers (NH_2 terminal groups), several types of modifications were attempted, in order to try to increase the efficiency of the transfection. One of the most powerful modifications consists in a thermal degradation of high generation PAMAM dendrimers; it has been shown to induce dramatic enhancement of activity ($>$ 50-fold).[73] It has been shown that these fractured dendrimers (commercialized under the name Superfect) operates via a cholesterol dependant pathway,[74] and is able to deliver gene in vivo to cure established tumors of mice.[75] Another type of modification of PAMAM dendrimers consists in changing their end groups, in particular by poly(ethylene glycol) (PEG). A 20-fold increase in transfection efficiency compared with partially degraded PAMAM was observed when PEG were grafted to the surface of G_5, and the cytotoxicity was very low.[76] A further step in the modification of the terminal groups consisted in using a bifunctional PEG, able to react with the dendrimer on one side and with a brain-targeting ligand (Transferrin or lactoferrin) on the other side. PAMAM-PEG-Tf (or Lf) complexed with DNA was able to cross the blood-brain barrier of mice after intravenous injection to efficiently deliver gene targeted to the brain.[77] Other types of structural modifications used dendrons. Grafting of one PEG at the core of PAMAM dendrons afforded a vector with low toxicity and increased efficiency for gene delivery to mammalian cells.[78] PAMAM dendrons were also used to be grafted by their core to magnetic nanoparticles. These vectors mixed with an antisense oligonucleotide entered into tumor cells and inhibited cell growth; furthermore, the presence of the magnetic particle could be used for medical imaging or hyperthermia.[79] Other interactions between "native" PAMAM and silica nanoparticles were also found efficient for transfection.[80]

Several other types of dendritic structures were tested for transfection experiments, such as those derived from PPI dendrimers. For instance conjugation of arginine to the terminal groups afforded an efficient and low toxic gene delivery system.[81] A branched polymeric derivative easier to synthesize, was used for transfection, but was found less efficient than Superfect.[82] Generation 5 and 6 of poly(lysine) dendrimers (eventually modified by arginine terminal groups) are as efficient or more than Superfect, without significant toxicity or cell specificity.[83] Phosphorus dendrimers of generations 3, 4 and 5, with protonated tertiary amine terminal groups were found efficient transfecting agents of the luciferase gene within 3T3 cells,[84] but also within human cells.[85] The biocompatibility of carbosilane dendrimers having various types of ammonium terminal groups was tested towards blood mononuclear cells, then their interaction with oligodeoxynucleotides was studied in view of transfection experiments in these cells.[86] Besides fully symmetrical dendrimers, several types of Janus dendrimers were synthesized for transfection experiments. Long alkyl chains on one side and ammoniums on the other side afford small amphiphilic dendrimers, sensitive to pH, and more efficient for transfection than Superfect.[87] Analogous compounds of type cationic lipids with a dendritic head group form various lamellar and nonlamellar phases with DNA, and have also efficient transfection properties.[88] Finally, besides classical transfection experiments, more original conditions were used, such as electroporation (an externally applied electric field to increase the permeability of the cell membrane) to transfer DNA/PAMAM complexes in cardiac transplants,[89] or light-induced delivery using polyaryl ether dendrimers having a phthalocyanine as core.[90]

Conclusion

Combination of two of the most particular molecular architectures that nature (DNA) and the chemists (dendrimers) have built has already demonstrated that their synergetic influence one on each other produces particularly fruitful results. Indeed, we have shown that very specific dendritic ar-

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chitectures can be built using DNA, that microarrays usable as biosensors possess a sensitivity increased by several orders of magnitude when they are engineered using dendrimers linked to DNA, and that electrostatic interactions between dendrimers and DNA are able, on one side to produce original condensed phases potentially usable for microelectronics, and on the other side to allow the penetration inside cells of genetic materials for the regulation of the activity of cells (increased production of therapeutic proteins, or inhibition of the growth of cancerous cells).

From the short picture that we have paint in this paper, we can say that the rapid progress made by the technologies implying dendrimers and DNA in the past few years is really impressive; but now, what promises for the future? The elaboration of more sensitive, more specific, and easier to handle biosensors for uses in the preservation of the quality of food and of environment, for medical diagnosis and forensic applications is certainly a development that can be foreseen. Vectors with increased efficiency and stealth characteristics, as well as a decreased toxicity should be synthesized, even if their safe use for human genetic therapy is still a dream. Anyway, it is obvious that breakthrough progresses in the field connecting dendrimers and DNA will need interdisciplinary researches, where physicists, engineers, chemists, biologists, and medical doctors should work together.

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