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REVIEW

Designing dendrimers for biological applications

Cameron C Lee[1], John A MacKay[2], Jean M J Fréchet[1] & Francis C Szoka[2]

Dendrimers are branched, synthetic polymers with layered architectures that show promise in several biomedical applications. By regulating dendrimer synthesis, it is possible to precisely manipulate both their molecular weight and chemical composition, thereby allowing predictable tuning of their biocompatibility and pharmacokinetics. Advances in our understanding of the role of molecular weight and architecture on the *in vivo* behavior of dendrimers, together with recent progress in the design of biodegradable chemistries, has enabled the application of these branched polymers as anti-viral drugs, tissue repair scaffolds, targeted carriers of chemotherapeutics and optical oxygen sensors. Before such products can reach the market, however, the field must not only address the cost of manufacture and quality control of pharmaceutical-grade materials, but also assess the long-term human and environmental health consequences of dendrimer exposure *in vivo*.

As polymer science has evolved over the past two centuries, the number of compositions and architectures of macromolecules synthetically accessible has also grown. The ability to easily tune the size, chemistry, topology and ultimately the properties of polymers through chemical synthesis inevitably has led to their widespread use in a variety of technological applications. The myriad properties and functions that can be designed into polymeric systems are prompting the medical community to use polymers in drug delivery, tissue engineering and biological imaging.

The highly branched and symmetrical molecules known as dendrimers are the most recently recognized members of the polymer family, with the first dendrimer reports published in the late 1970s and early 1980s by the groups of Vögtle[1], Denkewalter[2], Tomalia[3] and Newkome[4]. Since these pioneering studies were done, many hundreds of research groups from diverse scientific disciplines have joined the field, leading to numerous advances in the synthesis, analysis and application of these polymers[5]. Their unique branched topologies confer dendrimers with properties that differ substantially from those of linear polymers, and therefore their behaviors and possible uses have and should continue to be evaluated independently from linear polymers. In this review, we relate how the unique properties associated with the dendrimer structure have been exploited in the past few years for biomedical applications (**Table 1**), with emphasis on how the chemical composition and topology of dendrimers influence their biocompatibility and pharmacokinetic profiles.

Dendrimer chemistry and structure

A dendrimer is a polymeric molecule composed of multiple perfectly branched monomers that emanate radially from a central core, reminiscent of a tree, whence dendrimers derive their name (Greek, *dendra*). When the core of a dendrimer is removed, a number of identical fragments called dendrons remain, the number of dendrons depending on the multiplicity of the central core (2, 3, 4 or more). A dendron can be divided into three different regions: the core, the interior (or branches) and the periphery (or end groups) (**Fig. 1**). The number of branch points encountered upon moving outward from the core of the dendron to its periphery defines its generation (G-1, G-2, G-3); dendrimers of higher generations are larger, more branched and have more end groups at their periphery than dendrimers of lower generations.

Two examples of a polyester dendrimer synthesis are illustrated in **Figure 2**. The synthesis can be either divergent (upper portion of **Fig. 2**), which results in an exponential-like growth[6], or convergent (bottom portion of **Fig. 2**), in which case dendrons are grown separately and attached to the core in the final step. As evident from **Figure 2**, dendrimers are prepared in a stepwise fashion[3,4,7,8], similar to the methods used for solid-phase polypeptide and oligonucleotide syntheses, and therefore the products are theoretically monodisperse in size, as opposed to traditional polymer syntheses where chain growth is statistical and polydisperse products are obtained. A monodisperse product is extremely desirable not only for synthetic reproducibility, but also for reducing experimental and therapeutic variability. In practice, a monodisperse product can be easily obtained for low-generation dendrimers (up to G-3), but sometimes at higher generations the inability to purify perfect dendrimers from dendrimers with minor defects that are structurally very similar results in a deviation from absolute monodispersity, albeit typically a slight one.

A dendrimer may be based on practically any type of chemistry, the nature of which can determine its solubility, degradability and biological activity (**Fig. 3**). Some of the commonly encountered types of dendrimers in biological applications are based on polyamidoamines[6], polyamines[7], polyamides (polypeptides)[9],

[1]Department of Chemistry, University of California, Berkeley, California 94720-1460, USA. [2]Department of Biopharmaceutical Sciences & Pharmaceutical Chemistry, University of California, San Francisco, California 94143-0446, USA. Correspondence should be addressed to F.C.S. (szoka@cgl.ucsf.edu).

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Table 1 Biological applications of dendrimers and polymer/protein-dendrimer hybrids

Application	Dendrimer chemistry	References (*in vivo* applications italicized)
Bioimaging (magnetic resonance imaging, O_2 sensing)	PAMAM	*41*, *42*
	Polypeptide	24, *44*, *45*
Drug carrier (anticancer therapy)	PAMAM	22, *30*, *33*
	Polyether	15
	Polyester	23, *56*
	Polypeptide	87, *88*, *89*
	Self-immolative	69, 77
Drug/vaccine (prion-clearing agents, multivalent binding inhibitors)	Polypeptide	*20*, *49*, *90*
	Hydrocarbon	19
	PAMAM	47
Gene carrier	PAMAM	38, 39, *40*
Scaffold for tissue repair	Polypeptide	50
	Polyester	51

poly(aryl ethers)[8], polyesters[10,11], carbohydrates[12] and DNA[13,14]. By far the most common dendrimer scaffold is that of the polyamidoamine (PAMAM) dendrimers, which are available commercially with a wide variety of generations and peripheral functionalities (SigmaAldrich and Dendritic Nanotechnologies).

Perhaps the most exploited property of dendrimers is their multivalency. Unlike in linear polymers, as dendrimer molecular weight and generation increases, the terminal units become more closely packed, a feature exploited by many investigators as a means to achieve concentrated payloads of drugs or spectroscopic labels for therapeutic and imaging applications. The many end groups can also greatly modulate a dendrimer's solubility: hydrophilic end groups can make water soluble a dendrimer with a hydrophobic core (J.M.J.F. and colleagues[15]), whereas hydrophobic peripheral moieties can make a dendrimer with a hydrophilic interior soluble in oil[16]. Dendrimer multivalency is particularly useful when multiple copies of ligands are affixed to the periphery of the molecule. The resulting interaction between a dendritic array of ligands and a cell or other target bearing multiple receptors leads to a greatly increased avidity between the dendrimer and the cell compared with the binding of the monovalent ligand to the cell[17,18]. Thus having multiple weak binders on a dendrimer can turn it into a high-affinity reagent. Dendrimer multivalency has lent itself to applications ranging from the prevention of tumor cell adhesion and metastasis by carbohydrate-modified dendrimers (*in vitro*)[19] to the inhibition of HIV infection by sulfate-modified dendrimers in primate studies[20].

The highly congested branching that makes up the bulk of the dendrimer interior can have interesting effects on its conformation. For example, at low generations, a dendrimer typically has a floppy, disc-like structure, but at higher generations (usually >G-4), the polymer adopts a more globular or even spherical conformation. Typical dendrimers can be prepared to about G-10 with maximum diameters of ~10 nm; at higher generations the exponentially increasing mass of the dendrimer cannot fit within its linearly expanding spherical diameter. The nanometer sizes and globular shapes of high-generation dendrimers are reminiscent of some proteins, and have prompted many to suggest that they may possess distinctly different nanoenvironments at their cores and their peripheries (see Hecht and J.M.J.F.[21]). This 'core-shell' architecture has been exploited for the encapsulation of chemically sensitive functionality and molecules that are incompatible with the environment external to the dendrimer, such as catalysts (e.g., metallophthalocyanines)[21], drug molecules (e.g., indomethacin, doxorubicin, methotrexate and 10-hydroxycamptothecin)[15,22,23] or chromophores (e.g., metalloporphyrins)[24].

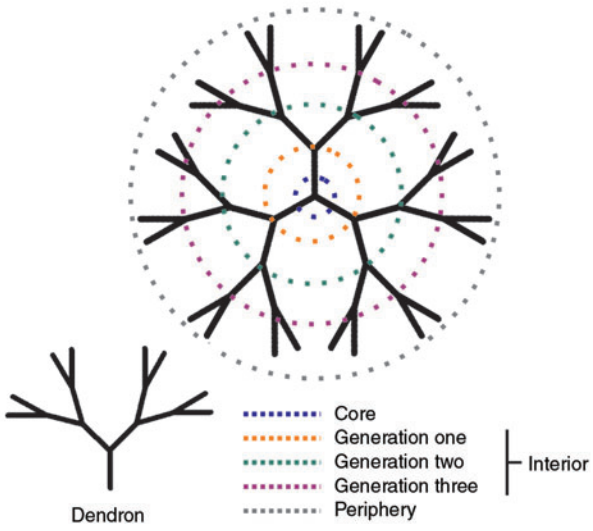

Figure 1 Anatomy of a dendrimer. A dendrimer and dendron are represented with solid lines. The colored, broken lines identify the various key regions of the dendrimer.

Biological applications

The early use of dendrimers in biology and medicine has been reviewed (chemistry, characterization, use in cell culture, use as transfection reagents and use as carriers of contrast material)[25–28]; however, new *in vivo* applications and new dendrimer architectures have appeared in the past few years.

Drug and gene delivery. By attaching a drug to a suitable carrier it is possible to enhance its aqueous solubility, increase its circulation half-life, target the drug to certain tissues, improve drug transit across biological barriers and slow drug metabolism. Optimization of these features to maximize drug bioavailability to diseased tissues while minimizing drug exposure to healthy tissues, results in improved therapeutic efficacy. A variety of carriers, including small-molecule substrates for cellular receptors and transporters, proteins, soluble polymers, micro/nanoparticulate polymers and liposomes, have been used for this purpose[29].

Numerous reports on the *in vitro* efficacy of purely dendrimer-based drug carriers have been published, but only a few *in vivo* therapeutic studies exist. One of the earliest examples of anti-tumor drug delivery with dendrimers was achieved by complexing cisplatin (20–25% by weight) to the surface groups of a G-4 carboxylate-terminated PAMAM dendrimer[30]. Conjugation of cisplatin to the dendrimer led to a tenfold increase in cisplatin solubility, but the drug also caused cross-linking between dendrimers, resulting in aggregates with diameters of 30–40 nm. When administered intravenously to mice, the aggregates targeted subcutaneous tumors via

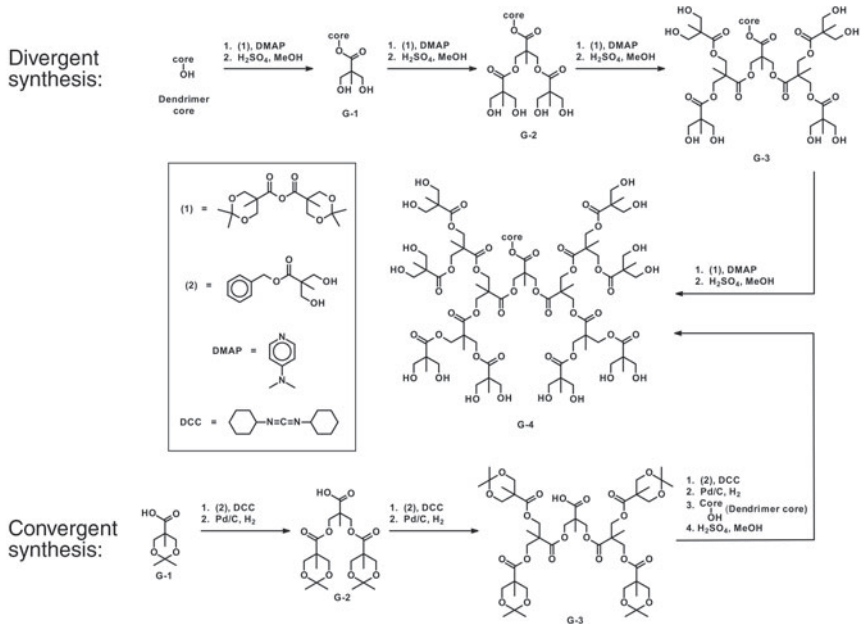

Figure 2 Synthesis of a polyester dendron. An example of a typical dendrimer synthesis via divergent (top) and convergent (bottom) approaches[35] through G-4. Note that in the convergent approach, dendrons are grown separately and attached to the dendrimer core in the final steps; in the divergent approach, dendrons are grown outwards starting from the dendrimer core. Dendrimer synthesis is stepwise and results in a product with a defined structure, unlike typical polymerization reactions.

a passive targeting mechanism known as the enhanced permeation-and-retention effect[31,32], and tumor levels of platinum (from cisplatin) were fivefold greater for the dendrimer-drug aggregates than for the free drug at equivalent doses. In the B16 murine subcutaneous tumor model, a single intravenous administration of the dendrimer-cisplatin aggregates given at 15 mg/kg cisplatin equivalents/body weight slowed the rate of tumor growth significantly relative to saline-treated mice, whereas unconjugated cisplatin administered at the maximum tolerated dose of 5 mg/kg did not.

PAMAM dendrimers have also been used as antitumor targeted carriers of methotrexate[33]. The peripheral amines of G-5 PAMAM dendrimers were first partially modified with acetyl groups to reduce dendrimer surface charge. The acetylated PAMAM was subsequently functionalized with folate as a targeting ligand, a fluorophore (fluorescein) and ~9% by weight of methotrexate, all in the same molecule. After intravenous administration in mice with subcutaneous tumors, radiolabeled or fluorescently labeled folate dendrimers accumulated and were taken up intracellularly by human KB tumors overexpressing the folic acid receptor; the concentration of targeted dendrimer in the tumor was five to ten times higher than that of a control dendrimer lacking the folate ligand. Treatment of mice bearing subcutaneous KB tumors with 15 biweekly intravenous injections of the methotrexate-folate-fluorescein–modified dendrimer significantly reduced the rate of tumor growth relative to saline-treated mice. The small diameter of the dendrimers (<5 nm) resulted in their rapid clearance from the blood through the kidney, and although such rapid elimination means that the modified carrier does not have to be biodegradable to prevent bioaccumulation, it also means a significant amount of drug was lost via renal elimination.

Similar to *in vivo* drug delivery studies with lipid vesicles, these data clearly demonstrate dendrimers can be modified with multiple groups in a manner that allows various labels, targeting ligands and drugs to be statistically incorporated into one delivery package. However, it is important to note that advances in dendrimer synthesis have also enabled the precise placement of two or more components in distinct ratios on a dendrimer scaffold[34–37].

Gene delivery has been accomplished using a variety of positively charged dendrimers, including PAMAMs, to form DNA complexes

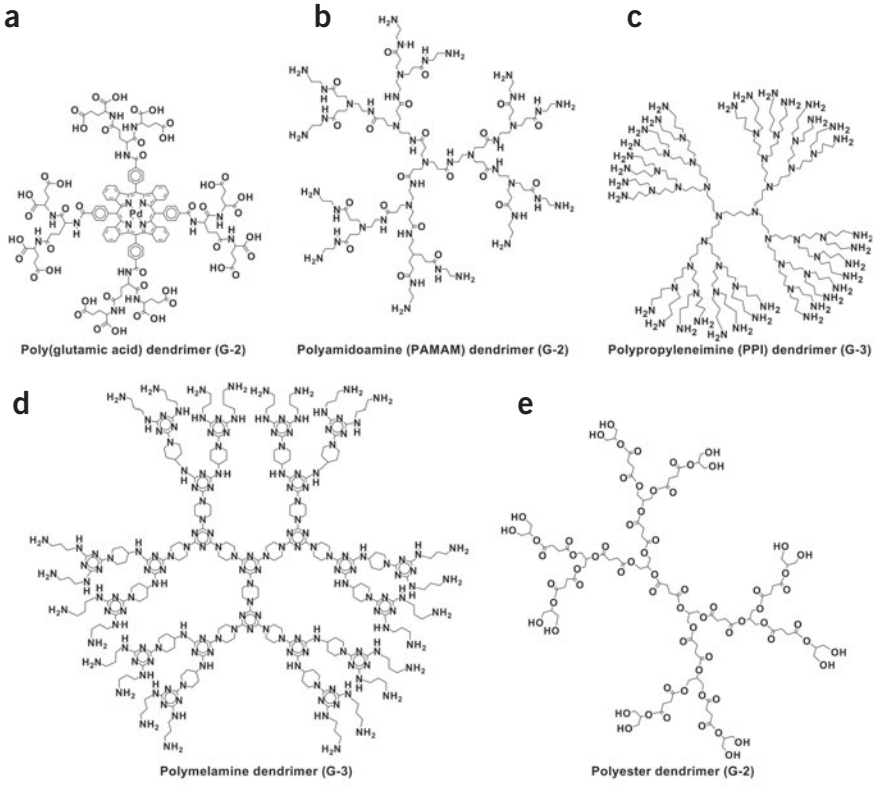

Figure 3 The variety of dendrimers used in biology. A few examples of the types of dendrimer chemistries used in biological applications. (**a**) G-2 poly(glutamic acid) dendrimer[45]. (**b**) G-2 polyamidoamine (PAMAM) dendrimer[6]. (**c**) G-3 polypropyleneimine (PPI) dendrimer[7]. (**d**) G-3 polymelamine dendrimer[59]. (**e**) G-2 polyester dendrimer[11].

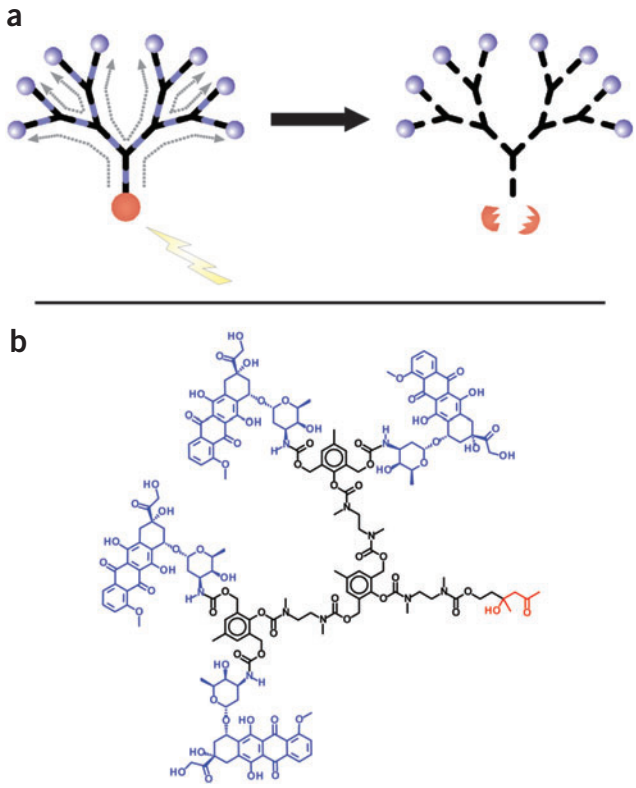

Figure 4 Self-immolative dendrimers. (**a**) Upon chemical reaction at the core of the dendron (e.g., an enzymatic or photochemical reaction), the entire dendrimer is broken down into identical low molecular weight fragments, ultimately resulting in the release of all peripheral groups. (**b**) Chemical structure of a hypothetical self-immolative dendrimer. Dendrimer degradation is initiated upon reaction of the β-hydroxy ketone (red) at the core with a catalytic antibody[69], ultimately resulting in the release of four molecules of doxorubicin (blue).

and transfect cultured cells with lower toxicities and higher efficiencies than conventional polyamine transfection agents (Haensler and F.C.S.[38]). Interestingly, work from one of our groups (F.C.S.[39]) shows dendrimers with imperfect or 'fractured' structures are the most effective, a finding possibly related to their greater structural flexibility. Kits using this dendrimer-based technology are commercially available (SuperFect, Qiagen, Hilden, Germany) and studies successfully using this strategy for the treatment of subcutaneous tumors in a murine model have been reported[40]. As with other cationic carriers, issues related to the toxicity associated with the positive charge of the PAMAMs must be solved if such systems are to be successful in the clinic.

Imaging. *In vivo* imaging is an increasingly useful tool in biomedicine, as it is noninvasive and provides a wealth of information regarding the native states of a variety of tissue types. The earliest *in vivo* uses of dendrimers were as carriers for magnetic resonance imaging contrast reagents[41,42], an application that has been reviewed elsewhere[43].

Another noninvasive imaging application of dendrimers involves photonic oxygen sensing. Because the concentration of oxygen in certain tumors can indicate whether the tumor will respond to treatment, methods to accurately determine this parameter are desirable[44]. By encapsulating hydrophobic metalloporphyrins in the cores of variously sized poly(glutamic acid) (**Fig. 3**), poly(aryl ether), or poly(ether amide) dendrimers, Vinogradov and coworkers[24,45] have prepared water-soluble oxygen sensors whose phosphorescence is quenched upon collision with dissolved oxygen. Once present in the tissue of interest, the dendrimer sensor can be induced to phosphoresce by irradiation with visible light or multiple photons of near-infrared light[45,46]. The phosphorescence lifetime of the dendrimers is inversely related to the oxygen concentration (via the Stern-Volmer equation) and can be measured both *in vitro* and *in vivo*[44]. With current systems, light absorption and scattering by tissues limits the depth of penetration for such applications; however, as photophysical technology improves, the solubilizing and steric-stabilizing core-shell architecture provided by dendrimers will be essential for the success of accurate, noninvasive optical imaging.

Intrinsic drug properties. Whereas the majority of dendrimer designs have been used as carriers for drugs and nucleic acids, some dendrimers act as drugs themselves. Supattapone and coworkers[47] discovered that branched polyamines, including PAMAM dendrimers and hyperbranched polymers, stimulate the removal of prion proteins present in infected cells. The branched architecture appears essential to this application because linear polyamines and small-molecule amines are ineffective.

Multivalent display of ligands on the surface of a dendrimer has also proven to be a viable method of inhibiting multivalent binding between cells, viruses, bacteria, proteins and combinations thereof[17,18,48]. For example, a G-4 poly(L-lysine) dendrimer bearing sulfate groups at its periphery is being evaluated as an anti-viral topical ointment[20,49] (Vivagel; Starpharma, Melbourne, Australia). By binding electrostatically in a multivalent fashion to viral envelope proteins (complementary ligands for CD4 receptors on a cell surface), the dendrimer is able to block adsorption and subsequent entrance of the virus into cells. When applied topically as a gel in the vagina, the dendrimers prevent the infection of female macaques with vaginally administered simian HIV[20]. Although similar results have been achieved previously with linear polyanions, dendrimer polysulfates should be easier to move from the laboratory to the clinic because of their monodispersity, which translates into a more consistent product.

Scaffolds for tissue repair. Although most of the applications discussed so far describe dendrimers as soluble, homogeneous compounds, they may also be used as insoluble supports for the delivery of therapeutic molecules. For example, Grinstaff and coworkers[24,45] have shown that dendrimers' high functional-group densities and low solution-viscosities make them useful as injectable sealants for corneal wounds. In this work, the peripheries of biodegradable polyester dendrimers are functionalized with reactive groups that can cross-link and form an insoluble hydrogel matrix upon activation[11,50,51]. For example, when the dendrons are functionalized with polymerizable acrylate groups, cross-linking can be induced by photoinitiation of polymerization with ultraviolet light. The ability of the sealants to maintain their integrity at and above typical intraocular pressures was confirmed by *ex vivo* experiments on lacerated eyes (human and nonhuman). Maximum intraocular pressures before rupture in eyes sealed with the dendrimers were comparable with those attained by the more common and labor-intensive suturing method. Because the strength and solubility of the hydrogels formed can be readily tuned by varying the generation or chemical composition of the dendrimers, these types of materials should be useful in a variety of sealing applications in other organs[11]. One can envision a multifunctional dendrimer serving both as an adhesive and also as

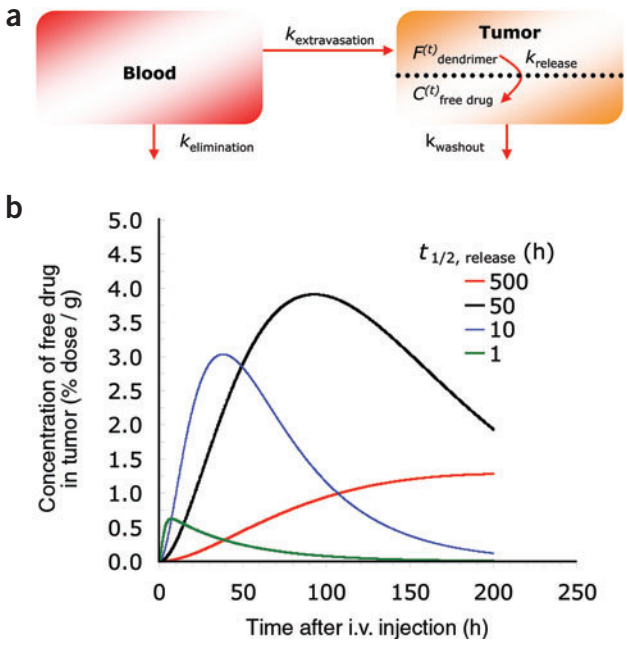

Figure 5 A simplified mathematical model predicting drug concentration in a tumor. (**a**) Diagram of the blood, tumor and first-order rate constants considered. (**b**) Calculated free drug concentration in the tumor as a function of time after injection for a 0.3 mg subcutaneous mouse tumor assuming the injected polymer had $k_{elimination}$ = 0.016 h^{-1}, $k_{extravasation}$ = 0.0015 h^{-1}, and carried doxorubicin with $k_{washout}$ = 0.023 h^{-1}. The four curves represent drug concentration profiles in the tumor for hypothetical polymer-drug linkages with first-order release half-lives of 1, 10, 50 and 500 h.

a signaling device to promote wound healing by displaying growth factors on its surface.

Biocompatibility

The success of dendrimers as carriers or biomaterials will depend in large part on their biocompatibility—whether dendrimers elicit an undesirable response from their biological host. Long-term accumulation of low molecular weight compounds is not often a problem because they are excreted in the urine or in the feces after metabolism. However, injected polymers are not eliminated as easily, especially if they are not readily degraded into smaller units[52] or are too large to be filtered via the kidneys. Thus for dendrimers, which can be classified as low molecular weight or polymeric depending on their generation, acceptable biocompatibility must be accompanied by a reasonably fast renal elimination rate or biodegradation rate.

***In vitro* toxicity.** In most cases, the nature of a dendrimer's numerous end groups dictate whether or not it displays significant toxicity. For example, cationic dendrimers with terminal primary amino groups, such as PAMAM and polypropyleneimine (PPI) dendrimers (**Fig. 3**), generally display concentration-dependent toxicity and hemolysis[53–55], whereas dendrimers containing only neutral or anionic components have been shown to be much less toxic and less hemolytic[23,54,56–58]. Cytotoxicity of amino-terminated dendrimers can be lessened by partial or complete modification of the dendrimer periphery with negatively charged or neutral groups[54,55,59]. The toxicity of cationic PAMAM dendrimers increases with each generation,

Box 1 Impact of drug release rate on intratumoral drug concentration

Dendrimers and high molecular weight polymers can target tumors by the enhanced permeation-and-retention (EPR) effect[31,32]. A long blood circulation half-life is a major requirement for EPR targeting; however, the release rate of the drug within the target site is also a critical variable under control of the polymer chemist. Without an appropriately rapid release rate, the drug may not achieve a high enough concentration at the site to be effective, but dendrimers with extremely rapid release may lose too much drug before entering the tumor. To gain insight into these interdependent parameters associated with EPR drug delivery, a quantitative model, similar to others proposed[79], can be constructed (**Fig. 5a**).

Of the four kinetic parameters in the model, the adjustment of the elimination rate has received the most attention. The rate constant of elimination, $k_{elimination}$, is predominantly a function of renal, liver and splenic clearance. Large dendrimers achieve a long elimination half-life by exceeding the renal filtration cutoff (J.M.J.F, F.C.S and colleagues[56,57]). In contrast, the rate constant of extravasation, $k_{extravasation}$, is difficult to manipulate by polymer chemistry because it depends upon bulk properties, such as the tumor size, the convective flow to the tumor and the vascular permeability. In a recent study, a dendronized linear polymer showed a mouse blood half-life of 44 h. At 48 h post-infusion, ~5% of polymer was in the tumor, enabling an estimate for the half-life of extravasation at about 450 h[84].

Although elimination and extravasation constants predict the fraction of dendrimer in the tumor over time, $F^{(t)}_{dendrimer}$, the concentration of free drug in the tumor must be estimated in order to predict the therapeutic effect. From $F^{(t)}_{dendrimer}$ the rate of drug generation can be modeled by a first order release parameter, $k_{release}$. The release parameter is a function both of the chemistry of drug attachment and the local environment of the polymer. Most importantly, the release rate can be modulated by the chemistry used to attach the drug. The last parameter in the model, $k_{washout}$, specifies the rate of free drug elimination from the tumor, and this parameter is primarily a function of the drug itself. The drug washout rate can be inferred from the terminal half-life of drug elimination; for example, the low molecular weight drug doxorubicin has a terminal half-life of about 30 h. Short of selecting a different drug, the washout rate is not under the control of the polymer chemist. Lastly, the mass balance between the generation and washout of free drug in the tumor enables estimation of the concentration over time, $C^{(t)}_{free\ drug}$.

A representative plot is presented here (**Fig. 5b**), which was constructed using pharmacokinetic data for an intravenously administered dendronized linear polymer studied in our laboratories[84]. The plot illustrates that for a short release half-life (1 h) much of the drug would be released before entering the tumor. The concentration profiles peak near the 10- and 50-h release half-lives, but decrease dramatically for the 500-h release half-life. This plot illustrates the importance of engineering the rate of drug release from the polymer by selecting the most appropriate chemistry of attachment. Derivation of the model can be found in the **Supplementary Discussion** online.

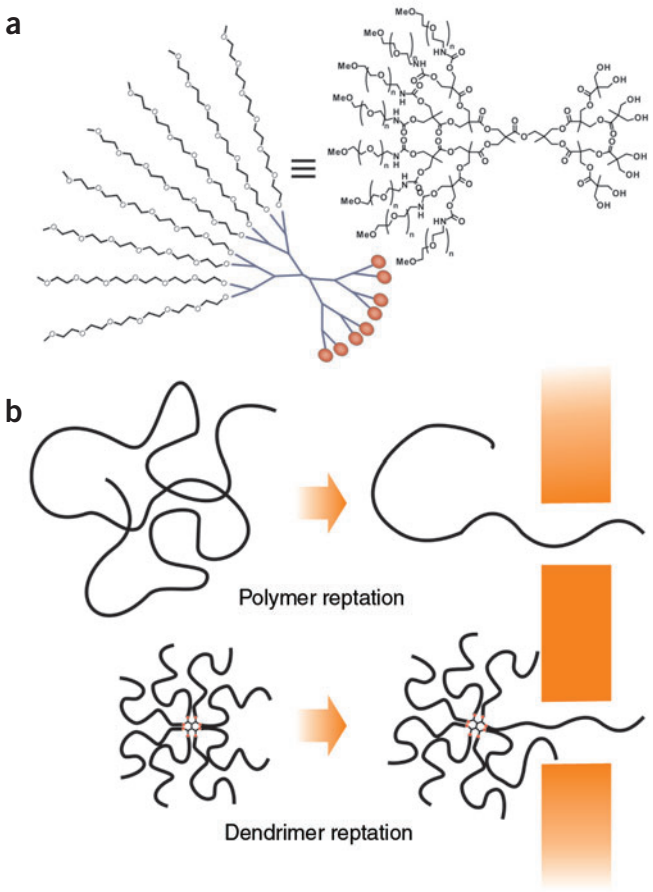

Figure 6 The effect of polymer architecture on glomerular filtration. (**a**) A cartoon and structural representation of a G-3 polyester dendrimer-poly(ethylene oxide) hybrid. The dendrimer generation determines the compactness of the resulting hybrid (higher generation are more compact and less deformable). (**b**) Polymers with sizes larger than the pores in the renal filtration membrane can potentially pass through by end-on reptation of the chain ends. Depicted here is the hypothetical reptation of two polymers with identical molecular weights through a pore. Although the loosely coiled linear polymer has a larger diameter than the more compact dendrimer-polymer hybrid, the linear polymer is more deformable and is eliminated through the pores at a greater rate.

but, surprisingly, cationic PPI dendrimers do not follow this trend[53,54]. The mechanism of cell death for cationic dendrimers is proposed to be attributable to necrosis and/or apoptosis, although it has not been precisely determined for all dendrimer types and can differ among cell lines[60,61].

***In vivo* toxicity.** *In vivo* toxicity correlates reasonably well with *in vitro* toxicity. Mice tolerate low intraperitoneal doses of positively charged PAMAM dendrimers (~10 mg/kg)[53]. Acute and subchronic toxicity studies in mice with melamine dendrimers (**Fig. 3**) bearing cationic surface charges revealed that intraperitoneally administered doses above 10 mg/kg produced liver toxicity, as demonstrated by increased levels of alanine transaminase in serum and liver necrosis upon histopathological analysis; administration of a 160-mg/kg dose of dendrimer by the same route resulted in 100% mortality within 12 h[62]. When ~50% of the cationic groups of a structurally similar dendrimer were replaced with neutral polyethylene oxide chains, no acute or subchronic toxicity was observed after intraperitoneal or intravenous injection of doses greater than 1 g/kg[59]. Similarly, a family of noncharged polyester dendrimers showed very low toxicity[56].

Degradation. Biodegradability of dendrimers is a valuable attribute that can prevent bioaccumulation and the possible toxic effects associated with its occurrence. The most widely studied dendrimers, PAMAMs, are hydrolytically degradable only under harsh conditions because of their amide backbones[39], and hydrolysis proceeds slowly at physiological temperatures.

More promising in terms of hydrolytic degradability are dendrimers based on polyester backbones (**Figs. 2** and **3**)[11,63,64]. In one example, polyester dendrimers have been carefully designed such that the ester hydrolysis products are nontoxic, natural metabolites[11], whereas in another instance high molecular weight polyester dendrimers and dendronized polymers have been shown to degrade to putative excretable and nontoxic lower molecular weight species[57,65].

Dendrimers and dendrons containing thiol-reactive disulfides within their branches have been prepared that should possess the ability to cleave under the reducing conditions encountered inside of cells[66,67]. In addition, dendrimers composed of bonds that are enzyme substrates have been prepared and represent another avenue by which dendrimers can be biodegraded[63,68,69]. However, it has generally been observed that the assembly of enzymatically labile polypeptides[70] or oligonucleotides[71] into a dendritic array often increases their resistance to enzymatic degradation.

Photolytically labile dendrimers may allow external initiation and spatially addressable dendrimer degradation. Dendrimers in which the dendrons are released from the core[72], in which the dendrimer peripheral groups are cleaved[73] or in which the entire dendrimer degrades into identical small-molecule fragments[74] upon ultraviolet irradiation have been prepared. Although the limited tissue permeability of ultraviolet light could hamper the applicability of these specific systems *in vivo*, it might be possible to use lower frequency irradiation[75] or more tissue-permeable radiation (that is, X-rays) to access alternative bond-cleavage mechanisms.

Ingenious examples of degradable dendrimers, variously referred to as self-immolative, cascade-release or geometrically disassembling dendrimers, have been reported recently (**Fig. 4**). In these dendrimers, a single chemical reaction at their core[74,76,77] or periphery[78] initiates their complete depolymerization into small, structurally similar units. In reports published to date, mechanisms of depolymerization involve *ortho* and/or *para* quinone methide rearrangement chemistries, but the chemical reactions used to trigger the depolymerization vary. The most biologically relevant triggering mechanisms have employed reactions induced by ultraviolet irradiation or catalytic antibodies[69,74]. Importantly, this disassembly strategy not only results in complete and rapid dendrimer degradation, but also provides a means for release of multiple biologically active species or spectroscopic labels from dendrimer end groups from a single, chemoselective cleavage event. Although the aromatic decomposition products of some of the dendrimers are nontoxic[77], it will be interesting to learn if less hydrophobic aliphatic molecules can be used to increase dendrimer solubility and ensure their biocompatibility.

Pharmacokinetics

An understanding of dendrimer pharmacokinetics is essential for their application in medicine because the bioavailability, toxicity and ultimately efficacy of dendrimer-based drugs and imaging agents will depend on their tissue accumulation profiles, drug release rates (from the polymer) and elimination rates[79].

For example, in anticancer drug delivery, it is known that macromolecules with prolonged circulation times show enhanced accumulation in tumor tissues due to the enhanced permeation-and-retention effect[31,32]. Therefore, knowledge of the blood circulation half-life of a dendrimer chemotherapeutic is a prerequisite for efficient passive tumor targeting. A second important aspect of polymeric drug delivery is the rate of drug release from the dendrimer (**Fig. 5**, **Box 1** and **Supplementary Discussion** online). Any of the variety of chemical linkages employed in the prodrug field can be used to attach drugs to dendrimers with widely variable rates of drug release. Their specific properties are beyond the scope of this review. What is important to point out is that drugs that are loaded into dendrimers using noncovalent hydrophobic or hydrogen-bonding interactions are rapidly released when the dendrimer-drug combination is placed into the biophase[28], and thus drug targeting is not optimal because the drug leaves the carrier before the carrier arrives at its intended target.

Systemic administration. The polymer therapeutics literature indicates that if a medical application requires a long-circulation time, dendrimers must have uncharged or negatively charged surfaces (to limit nonspecific interaction with hepatic tissues) and high molecular weights (to prevent rapid filtration through the glomerular membrane)[30,33,52,79]. In accordance with these predictions, the pharmacokinetic profiles of dendrimers are for the most part, determined by surface charge and dendrimer molecular weight. Polycationic PAMAM dendrimers exhibit fast clearance from the bloodstream upon intravenous or intraperitoneal administration and accumulate either in the liver, kidney, spleen or pancreas[42,53,54]. Modification of the PAMAM surface with hydrophilic polyethylene oxide chains or by acetylation decreases the liver uptake, presumably by steric stabilization of the dendrimer surface and/or by reduction of the positive charge[30,33,42]. PAMAM dendrimers modified to have a negatively charged periphery display substantially longer blood circulation times, with liver accumulation still occurring to a significant extent[30,54].

We have found that neutral, G-4, polyester dendrimers do not show any preferential organ accumulation when administered to mice intravenously and are rapidly excreted in the urine because of their low molecular weights (<12 kDa) and compact dendritic architectures[56]. To make use of this promising biodegradable dendrimer scaffold for antitumor drug delivery using the enhanced permeation and retention effect, we attached polyethylene oxide chains of various lengths to different dendrimer generations to create a small library of dendrimer-polymer hybrids (**Fig. 6a**) spanning a wide range of molecular weights[35]. Importantly, many of these dendrimers were of sizes greater than the reported size limit of 30–40 kDa for renal filtration of polyethylene oxide[80]. Pharmacokinetic studies confirmed that the dendrimers with molecular weights >~40 kDa remained in the blood much longer than the polymers with lower molecular weights[57].

Interestingly, dendrimers of similar absolute molecular weights but different degrees of branching exhibited significantly different elimination rates (when the molecular weights were >40 kDa). As an example, consider the case of two dendrimers with molecular weights of ~40 kDa, one composed of four 10-kDa polyethylene oxide chains attached to a G-2 dendrimer and one composed of eight 5-kDa polyethylene oxide chains attached to a G-3 dendrimer. The more branched, G-3 macromolecule had a significantly greater area under the blood plasma concentration-time curve than the less compact G-2 polymer, and nine-times less polymer was excreted into the urine for the more highly branched dendrimer. This means that more dendrimer-drug would stay in circulation and would have a greater chance of reaching its target if attached to the G-3 rather than the G-2 polymer. This trend held for larger dendrimers pairs with similar molecular weights as well[57].

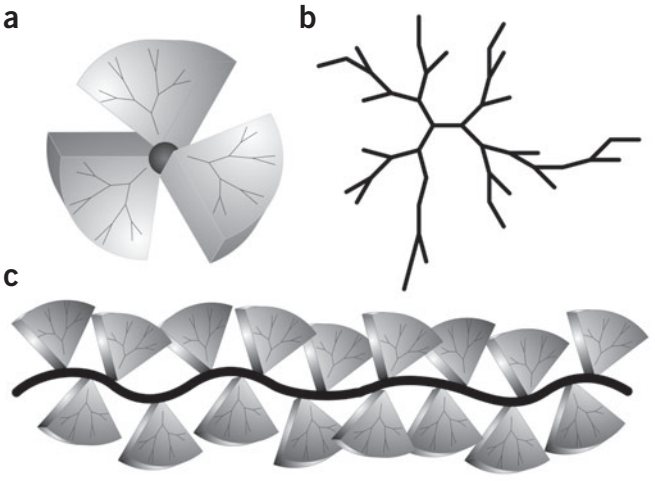

Figure 7 Dendritic polymer architectures. (**a**–**c**) Globular dendrimers (**a**) are the most used members of the dendritic polymer family, but other dendritic polymers exist, including structurally similar hyperbranched polymers (**b**) and rod-like dendronized polymers (**c**).

Box 2 Other dendritic architectures

Hyperbranched polymers are another class of dendritic polymers that are receiving increased attention because they possess dendrimer-like properties and can be prepared in a single synthetic step (**Fig. 7**)[91]. Hyperbranched polymers are typically imperfectly branched and very polydisperse, although methods to make their syntheses more controlled are constantly being refined[92]. If an application is tolerant of these qualities, a variety of hyperbranched materials with different compositions are available commercially in large quantities and at a low cost relative to the more structurally perfect dendrimers (e.g., Hybrane, DSM, Herleen, The Netherlands; Boltorn polyols from Perstorp, Perstorp, Sweden; Lupasol from BASF, Mt. Olive, NJ, USA).

Whereas the majority of dendritic polymer research has focused on globular dendrimers with point cores, recent work has involved the preparation and study of dendritic molecules with polymeric cores. The resulting polymers, called dendronized polymers, bear pendant dendrons at every single repeat unit and at high generations adopt extended, rod-like conformations (**Fig. 7**)[93]. These polymers possess many of the features of dendrimers (that is, multivalency and a core-shell architecture), but their cylindrical shapes are expected to engender them with different physical and biological properties[84,94].

We contend that the differences in blood circulation, time and renal elimination, can be accounted for by the less branched dendrimers' ability to more easily deform and reptate[28] through the pores of the renal filtration membrane and be eliminated into the urine, even though the less branched polymers probably have larger hydrodynamic sizes (**Fig. 6b**). Indeed, for uncharged natural polymers of similar hydrodynamic sizes, compact, cross-linked polymers like Ficoll are cleared less rapidly via glomerular filtration than loosely coiled polymers, such as dextran[81,82]. We think this intuitively pleasing hypothesis is in agreement with theoretical calculations pertaining to the transport of flexible star polymers through pores with diameters smaller than the polymers' radius of gyration, which indicates that the minimum energy required for passage should increase as the square of the arm number[83]. If the reptation hypothesis is correct, the highly branched architecture provided by dendrimers could be a very useful means to modulate their pharmacokinetics, although molecular charge and surface hydrophobicity will still strongly influence biodistribution, and the dendrimer radius must at least approach that of the renal pore (~5 nm[52]) before such behavior is exhibited.

Whether or not other types of dendritic polymers (**Fig. 7**; **Box 2**) also possess systemic pharmacokinetic properties different from those of their linear counterparts remains to be determined, but initial studies in our laboratories (J.M.J.F. and F.C.S.)[84] are currently underway.

Conclusions—why trees?

The majority of the applications of dendrimers discussed in this review use dendrimers as carriers or scaffolds in some capacity (drugs, imaging agents, ligands). Numerous carriers for drug delivery and imaging applications already exist, however, which begs the question: why use dendrimers over other carriers? Of the parenterally administered carriers in use today[29,85], liposomes have found the most commercial success (e.g., Doxil; Ortho Biotech Products, Bridgewater, NJ, USA) because they have high drug loading capacities (10–15,000 drugs/liposome), can be prepared in a variety of sizes (50–10,000 nm), are biodegradable and can be easily modified to display targeting ligands on their surfaces. However, liposomes are multicomponent, noncovalently associated systems that are challenging to formulate and stabilize[86] when compared with macromolecules like dendrimers with covalently associated drugs.

Polymers represent smaller sized carriers (<50 nm) and have a lower payload per particle than do liposomes. Natural polymers such as antibodies are inherently biodegradable and can be designed to target specific tissue types, but their use can be hindered by immunogenicity, high cost and the limited scales on which some of these materials can be obtained. Polymers manufactured via chemical syntheses are perhaps more easily produced on a large scale, but none are currently approved for use in parenteral drug products[85] because of their nonbiodegradability and high polydispersity.

Dendritic polymers can differ significantly from linear polymers in their properties. They have a number of beneficial attributes for biomedical applications, including the following:

- Biodistribution and pharmacokinetic properties that can be tuned by controlling dendrimer size and conformation. This can be achieved with precision by varying dendrimer generation number or by creating dendrimer-polymer hybrids.

- High structural and chemical homogeneity. Dendrimer biological properties can be attributable to a single molecular entity and not a statistical distribution of polymeric or self-assembled materials, facilitating the reproducibility of pharmacokinetic data within and between different synthetic lots.

- Ability to be functionalized with multiple copies of drugs, chromophores or ligands either at their peripheries and/or their interiors. Dendrons also can be used to precisely increase the drug-loading capacity of carriers, such as antibodies[87], and biocompatible polymers like poly(ethylene glycol)[64,88,89].

- High ligand density. Unlike in linear polymers, as a dendrimer's generation increases, the multivalent ligand density at the surface increases, which can strengthen ligand-receptor binding and improve the targeting of attached components.

- Controlled degradation. This can be achieved by judicious choice of dendrimer chemistry, with unique modes of decomposition accessible through use of self-immolative dendrimers.

Despite these advantages, dendrimers face the same challenges that linear polymers encountered moving from the laboratory to the clinic. To be widely adopted, they will also face the extra obstacles of multistep syntheses and associated higher costs of dendrimer preparation. In addition, improved quality control assays will need to be devised to ensure that multicomponent dendritic polymers contain the correct components in the correct ratios.

Nonetheless, the many beneficial attributes of dendrimers described in this review are a strong impetus for considering these tree-like macromolecules as the preferred polymeric carrier for drugs or for imaging agents. Indeed, recent advances in this field promise a veritable forest of biomedical applications arising from these beautiful molecules.

Note: Supplementary information is available on the Nature Biotechnology website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Biotechnology* website for details).

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