JPP Journal of Pharmacy and Pharmacology

JPP 2009, 61: 989–1003

Received January 10, 2009
Accepted March 30, 2009
DOI 10.1211/jpp/61.08.0002
ISSN 0022-3573

Review

Dendrimers as therapeutic agents: a systematic review

Virendra Gajbhiye, Vijayaraj K. Palanirajan, Rakesh K. Tekade and Narendra K. Jain

Pharmaceutics Research Laboratory, Department of Pharmaceutical Sciences, Dr Hari Singh Gour University, Sagar, India

Abstract

Objectives Dendrimers by virtue of their therapeutic value have recently generated enormous interest among biomedical scientists. This review describes the therapeutic prospects of the dendrimer system.

Key findings Their bioactivity suggests them to be promising therapeutic agents, especially in wound healing, bone mineralisation, cartilage formation and tissue repair, and in topical treatments to prevent HIV transmission. Findings also demonstrate their potential as anti-prion, anti-Alzheimer's, anticoagulant, antidote, anti-inflammatory and anticancer agents. One of the dendrimer-based formulations with activity against herpes simplex virus (VivaGel from Starpharma) has successfully completed phase I clinical trials and is expected to be available on the market soon.

Summary All reports cited in this review demonstrate the use of dendrimers as medical therapeutics in different ailments. The review focuses on the current state of therapeutic potential of the dendrimer system.

Keywords anti-Alzheimer; anticancer; anti-HIV; anti-prion; dendrimer

Introduction

The dendritic architecture derives its name from a Greek word 'dendron' because the dendrimer resembles a regular branch of a tree; it was explored for the first time by Vogtle and colleagues (US patents 4289872 and 4410688).[1] This was soon followed by the emergence and exploration of different types of dendritic macromolecules as carriers for drug delivery,[2–4] gene delivery,[5] targeting,[6] solubilisation,[7] diagnosis,[8] chemical catalysis[9] and as multivalent ligands for interesting biological applications.[10,11] They are highly branched monodispersed molecules with low polydispersity and are endowed with micelle-like behaviour and nanoscale container properties.[12,13] Their reasonable cost of manufacture, toxicological profile and biocompatibility distinguish them from other nanosised species used for polyvalent or multivalent drug discovery.

Recently, dendrimers have generated interest in the discovery of drugs by virtue of their activities against prion diseases,[14] Alzheimer's disease,[15] inflammation,[16] HIV,[17] herpes simplex virus (HSV),[18] bacteria[19] and cancer.[20] They are among few therapeutic agents to cure prion diseases. Dendrimers prevent formation of amyloid fibrils and disaggregate previously formed fibrils,[15] and prevent viral adhesion and replication.[17] Polycationic dendrimers react with bacterial membranes and disturb their integrity.[21] Dendrimers have capabilities as heparin analogues[22] and collagen mimetics.[23] They have been shown to be efficacious in tissue repair[24] and in preventing the formation of scar tissue.[25] Dendrimers also have potential for neutralising toxins[26] and removing drug and metal overdose from the body.[27,28] Use of these nanostructures as bioenzymes[29] and biosensors[30] has also been reported. A dendrimer-based product, 'VivaGel' from Starpharma, which is essentially a formulation for intra-vaginal use as an anti-viral agent, has successfully completed phase-I clinical trials and is expected to be available on the market soon.[31]

There is a plethora of review articles on dendrimers but most of these have generally touched upon the drug delivery potential of these nanocarriers. Here we review the therapeutic potential of dendritic polymers for different ailments. These therapeutic applications, if established, could be used to achieve additive/synergistic effects between drugs and dendrimers. The review is organised into subsections, each dealing with therapeutic application of dendrimer in different diseases.

Correspondence: Virendra Gajbhiye, Pharmaceutics Research Laboratory, Department of Pharmaceutical Sciences, Dr Hari Singh Gour University, Sagar-470 003, India. E-mail: cme_virendra@yahoo.co.in

Novel dendritic scaffolds in the treatment of prion disease and Alzheimer's disease

Fatal neurodegenerative disorders such as prion diseases cause conformational changes in the normal cellular form of prion protein (PrP) to an infectious scrapie isoform (PrP^{Sc}).[32] These non-pathological infectious proteins are rich in β-structures, which lead to the formation of amyloid fibril-like structures. Exposure of branched polyamines like G4 polyamidoamine (PAMAM) and polypropylene imine (PPI) dendrimers was reported to eliminate measurable prion infectivity from neuroblastoma ScN2a cells.[33] They have been shown to clean scrapie-infected ScN2a cells of PrP, which depends on the high density of surface primary amino groups and highly branched structure. In PPI-treated and control ScN2a cells incubated in mice for 200 and 61 days, respectively, the titre of infectious prion in ScN2a cells was reduced from a 50% infective dose (ID50) of ~10^6 units per 100 mm plate to less than 10^2 units per plate. PrP 27-30 was purified from RML-prion-infected mouse brain, and an in-vitro assay was used to determine the molecular target of these branched polyamines on purified PrP 27-30. PrP 27-30 was made protease sensitive by dendrimer, with reduced infectivity. During experiments, when 15 μg RML prion rods/ml were incubated with and without 60 μg PPI G4/ml, prion infectivity was reduced from 10^7 to 10^5 ID50 units/ml. Similarly, PAMAM G3 and G4 dendrimers have also been shown to reduce infectivity.[33]

Prion diseases exist as different phenotypic strains with differences in the conformation of PrP^{Sc},[34] and susceptibility of these prions to dendrimer depends on both prion strain and PrP sequence. PPI-induced conformational changes were observed *in vitro* in the Mo(RML), Mo(22a) and Mo(139A) mouse strains but not in Mo(Me7) and Mo (87V), which were found to be resistant. The investigators suggested that these strains are more resistant to denaturation than susceptible strains. As dendrimers assist denaturation, they provide infected PrP protease sensitivity more efficiently at lower pH values. Electron microscopy showed that the RML prion rods were disaggregated after incubation with PPI dendrimers for 2 h at 37°C, accompanied by loss in β-sheet secondary structure. Results indicate that approximately one PPI molecule was required for five PrP 27-30 molecules in purified RML prion preparations to induce disaggregation. As PPI requires acidic condition to provide protease sensitivity, the authors predicted accumulation chiefly in the lysosomes of living cells.[33]

As Alzheimer's disease is also associated with the formation of amyloid fibrils, use of dendrimer has also been suggested in this condition.[15] Klajnert and colleagues explored the effect of pH on the ability of PPI dendrimers to inhibit amyloid activity *in vitro* and characterised the aggregation processes at the molecular level.[35] Alzheimer's peptide Aβ 1-28 and a segment of prion protein PrP 185-208 were selected for this study. The β-sheet structure content in prion peptides seems to be enhanced at lower pH compared with neutral pH,[36] suggesting that prion replication occurs chiefly in acidic cellular compartments such as lysosomes and endosomes. Amyloidogenesis was faster at lower pH value, but was different for PrP 185-208 and Aβ 1-28. Hydroxyl-terminated dendrimers were ineffective even at higher concentrations, indicating that the amino groups at the periphery were necessary for maximum inhibitory activity. The β-sheets are stabilised by salt bridges formed by intramolecular interactions between His-187 and Glu-196 in PrP 185-208. The positively charged dendrimers have highest affinity for protonated Glu residues at pH 5.9, resulting in disruption of interactions between His-187 and Glu-196. For Aβ 1-28, a salt bridge is formed between Asp-7 and His-13. At pH 5.9 Asp-7 is approximately 99% protonated and hence becomes an attractive target for cationic dendrimers.[35]

The Aβ peptides were also reported to have high affinity for sialic acid residues.[37] The toxicity of Aβ in human neuroblastoma cells was shown to be reduced by sialic acid–PAMAM dendrimer complexes at micromolar concentration, three orders of magnitude lower than soluble sialic acid. Activity depended on the generation size of the dendrimers. Patel and colleagues reported G4 to be most effective in protecting cells from Aβ toxicity.[38] Phosphorus-containing dendrimers (PDs) can also associate with hydrophobic proteins like PrP^{14} and can remove PrP^{Sc} from ScN2a cells infected with 22L strain. In-vitro studies in cell lines demonstrated this phenomenon to be dose dependent, with PD-G4 and PD-G5 being more efficient than PD-G3, possibly due to higher surface crowding on the former. They also found that, when the treatment was ceased, PrP^{Sc} had not reappeared after 5 weeks, and no infectivity was detected in a cell-to-cell transmission assay, indicating that PDs completely disposed of prion infectivity. Results also suggested that the preformed aggregates were rapidly disrupted by the PD *in vitro*, and at higher doses they completely blocked PrP^{Sc} accumulation in the spleen of C57BL/6 mice. PD had similar efficacy to MS-8209, which is an analogue of amphotericin B able to inhibit PrP^{Sc} replication by 70–90%.[14]

Klajnert and colleagues also examined the effect of PAMAM dendrimers (G3, G4 and G5) on amyloid aggregation of PrP 185-208 and Aβ 1-28. The fluorescence of thioflavin T (Th T) dye was used to monitor the formation of amyloid fibrils *in vitro*. The results indicated that the G3 PAMAM dendrimer slows the elongation reaction at a concentration of 0.1 μmol/l in the case of Aβ 1-28 fibril formation. At 1 μmol/l, G4 and G5 PAMAM dendrimers completely inhibited the formation of Aβ 1-28 fibrils. For PrP 185-208, 1 μmol/l G3, G4 or G5 PAMAM completely inhibited fibril formation. To evaluate the ability of PAMAM dendrimers to disaggregate amyloid fibrils, increasing concentration of dendrimers were added to previously formed aggregates and changes in Th T fluorescence observed. Dendrimers were found to be capable of disrupting the previously formed aggregates, and higher generation dendrimers were more effective and at lower concentrations. The authors suggested three possible mechanisms for this inhibition of amyloid fibril formation by the dendrimers: binding to the peptide, breaking the fibrils and blocking the free ends of the fibrils (Figure 1).[15]

Dendritic nano-architecture and anti-inflammatory activity

Anti-inflammatory properties of G4 PAMAM dendrimer were revealed during the biodisposition study of a G4

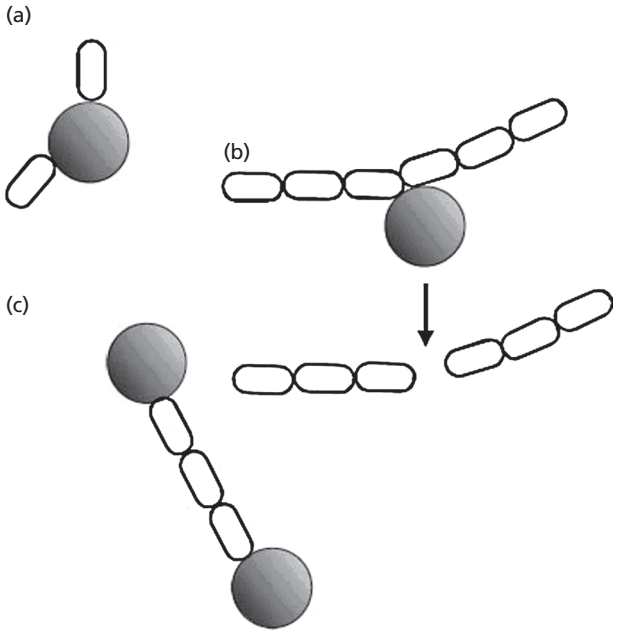

Figure 1 Schematic diagram of possible mechanisms of dendrimer-mediated inhibition of amyloid fibril formation by (a) binding to the peptide, (b) breaking the fibrils and (c) blocking the free ends of the fibrils (reproduced from Klajnert *et al.*[15] with the permission of Elsevier)

dendrimer–indometacin complex in the carrageenan-induced rat paw oedema model by Chauhan and colleagues.[16] Inhibition of oedema formation was higher with the G4–indometacin complex (8 mg/kg G4; 1.6 mg/kg indometacin) than with indometacin alone. In the case of free indometacin, percentage of inhibition was higher than with G4 until 4 h, after which no significant rise in the activity was observed. G4 dendrimer has mild anti-inflammatory activity at 4 and 8 mg/kg, whereas significantly higher anti-inflammatory activity was seen at 16 mg/kg. At a dose of 16 mg/kg hydroxyl-functionalised G4 PAMAM dendrimer has considerable anti-inflammatory activity, analogous to that of G4 dendrimer. However, G4 dendrimer had superior activity to G4-OH PAMAM dendrimers. The G4.5 dendrimer had very little activity, and that too was diminished within 4 h.[13,36]

In mouse models of inflammation induced by subcutaneously implanted cotton pellets, G4 dendrimer formulation and G4–indometacin were found to be more effective than the standard indometacin formulation; no significant difference was observed between the G4 dendrimer and G4–indometacin complex. The superior activity of G4 over indometacin was observed continuously until the 14th day.[16,39]

The effects of dendrimer on various pro-inflammatory mediators such as cyclo-oxygenase (COX)-1, COX-2, nitric oxide and interleukin (IL)1β show that all these dendrimers (G4, G4-OH and G4.5) have significant inhibitory effects on nitric oxide production in rat peritoneal macrophages. G4 and G4-OH showed similar inhibition of nitric oxide, and both were more potent in this respect than G4.5. Both G4 and G4-OH have also shown significantly superior inhibition of COX-2 enzyme compared with G4.5. The authors found that the in-vivo anti-inflammatory activity of these dendrimers was chiefly attributable to selective inhibition of COX-2; inability to inhibit COX-1 further infers that the dendrimers are potent and safe anti-inflammatory agents.[16,39]

Interaction on the endothelial surface between selectin and O-glycosylated protein ligands leads to enhanced leucocyte counts, with inflammatory and cell-mediated immune responses.[40] Owing to multiple surface reactive sites and sulfate groups at the periphery, branched polyethylene oxide (PEO) polymers inhibit selectin binding to O-glycosylated protein ligands *in vivo*.[40] Rele and colleagues, using a trichloroacetimidate glycosylation method using BF_3OEt_2 as Lewis acid activator, glycosylated all arms of 12-OH-terminated PEO branched polymer (G2). They then carried out deacetylation followed by lactose sulfation to form sulfated-oligosaccharide-functionalised PEO glycoclusters 1c, 2c and 3c (Figure 2a), among which 3c was a dendrimer-like glycopolymer. Using thioglycollate-induced acute inflammation in mice, the anti-inflammatory potency was shown to be valency dependent: 1c and 2c had little activity whereas 3c at an intravenous dose of 0.5 mg reduced mobilisation of neutrophils and macrophages to the epithelium by 86 and 60%, respectively. Inhibition of adhesion of U937 cells to immobilised selectin in the presence of 3c also confirmed the ability of 3c to block selectin *in vivo*. The inhibitory activity was found to be dose dependent (IC50 2.4 nM) for L-selectin; 3c did not inhibt P-selectin or E-selectin activity, indicating selective inhibition of L-selectin. 3c also limited leucocyte extravasation and chemokine binding to endothelium, leading to anti-inflammatory effects.[40]

Dendrimeric polyglycerol sulfates and carboxylates as heparin analogues

Heparin is widely used as an antithrombotic drug but presents numerous limitations. Turk and colleagues[41] have developed new synthetic highly branched heparin analogues: dendritic polyglycerol sulfate, synthesised by sulfation of polyglycerol,[42] and dendritic polyglycerol carboxylate, synthesised by carboxylation of polyglycerol (Figure 2b).[43] Citrated human platelet pooled plasma was used in a classic coagulation assay to evaluate the anticoagulant activity of the polyglycerol derivatives. The polyglycerol sulfates showed a concentration-dependent prolongation of activated partial thromboplastin time of 5.7–8.1% and prolongation of thrombin time of 15.7–33.6% compared with standard unfractionated heparin (UFH).[41]

Turk and colleagues[41] also performed complement-induced haemolysis assays using the microtitre plate technique described by Alban and colleagues[42] to evaluate the influence of polylycerol derivatives on classic complement activation (CCA) and alternative complement activation (ACA). The unfunctionalised polyglycerol and polyglycerol carbonates were found to be inactive in the coagulation assay and the complement-induced haemolysis assays. On the other hand, the polyglycerol sulfates showed concentration-dependent inhibition of haemolysis induced by CCA as well as ACA. The authors suggested that the sulfate groups are essential for the effect and cannot be replaced by other

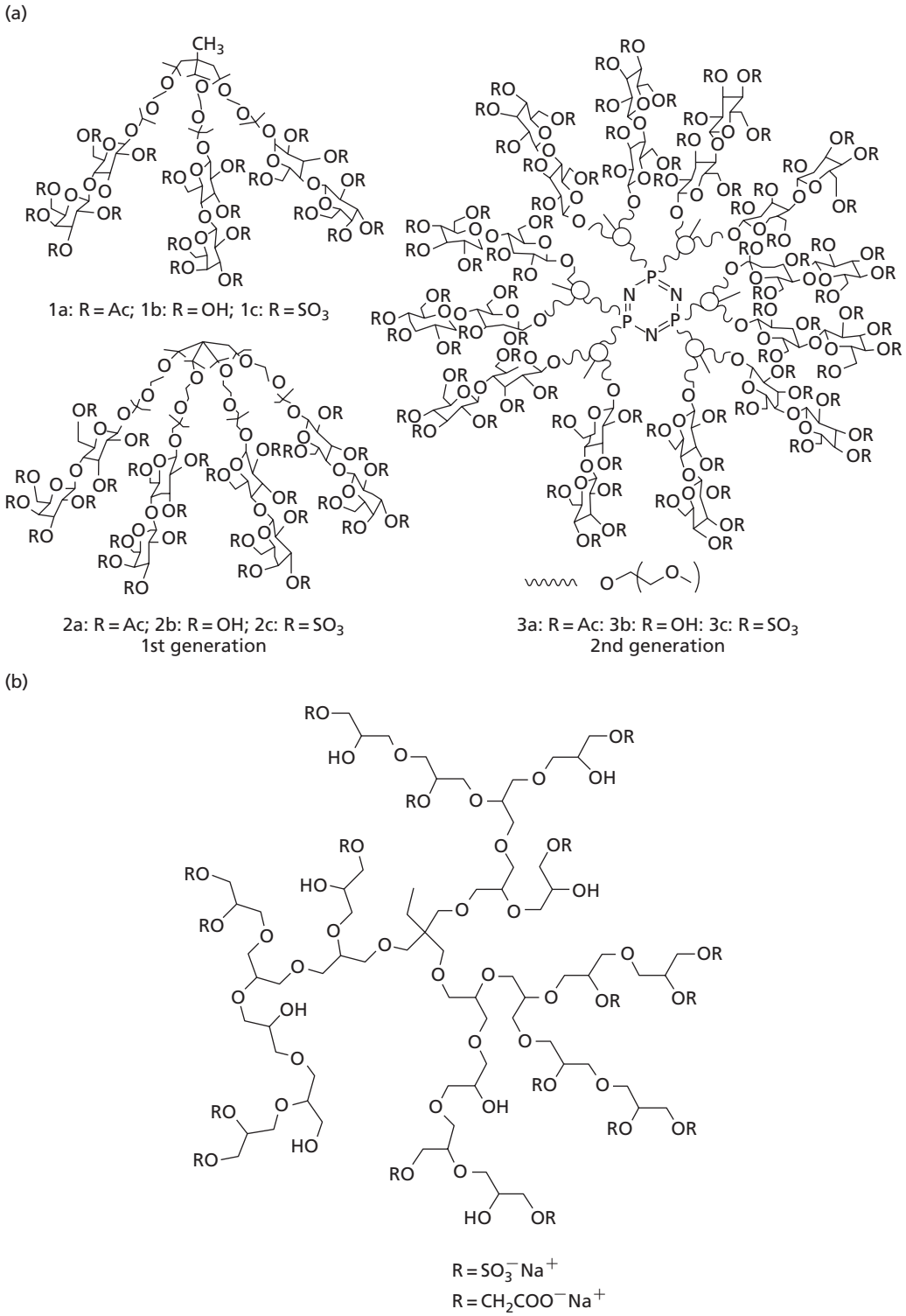

Figure 2 Schematic diagram of (a) saccharide-functionalised polyethylene oxide star and 'dendrimer-like' polymers as selectin ligands with anti-inflammatory activity (adapted from Rele[40]) and (b) polyglycerol sulfate and carboxylate as heparin analogues (reproduced from Turk *et al.*[41] with permission; Copyright American Chemical Society)

negatively charged residues such as carboxylates. According to the investigators, the polyglycerol sulfates exhibit lower anticoagulant activity than heparin but have higher anti-complement activities. They also suggested that potential bleeding risk associated with the use of UFH due to weak anti-complement activity can be overcome by polyglycerol sulfates because of their improved efficacy : risk ratio, which is 300 for polyglycerol sulfates and 1 for UFH.[41]

Functionalised dendrimeric structures as antimicrobial agents

Pathogens invade host cells in particular by affixing to carbohydrates on the cell surface. Pioneer explorations for prevention of host–pathogen interaction are being developed as more is learnt about the interaction pathway. The potential of dendrimers to interact with specific sites on pathogens makes them excellent antimicrobials.

The antibacterial activity of cationic dendrimers is due to electrostatic interaction between negatively charged bacteria and positively charged dendrimer.[21] Dendrimer biocides are bacteriostatic at low concentration by causing a slight change in membrane permeability. At higher concentration they denature the membrane protein and start to penetrate the lipid bilayer, causing leakage of potassium ions. This leads to complete disintegration of the bacterial membrane and a corresponding bactericidal effect.[21] Queiroz and colleagues conjugated dendritic polyglycerol (PGLD) with O-carboxymethylated chitosan to obtain PGLD–chitosan dendrimer (PGLD–Ch), which was then boronated to form (PGLD–Ch) B dendrimer. (PGLD–Ch)B was found to suppress bacterial proliferation when tested against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*[44] Tam and colleagues[45] developed dendrimeric peptides by tethering a tetrapeptide (R4) and an octapeptide (R8) on a lysine core. The R4 contains a putative microbial surface recognition BHHB (B = basic, H = hydrophilic amino acid) pattern found in protegrins and tachyplesins, and is a potent antimicrobial. R8 contains one R4 and a degenerated R4 repeat. The tetra- and octavalent R4 and R8 dendrimers were found to be active against 10 organisms in high- and low-salt condition antimicrobial assays, whereas their R4 and R8 monomers and divalent dendrimers showed little or no activity.[45]

Recently Cheng and colleagues reported that the microbiological activity of loaded antimicrobial drugs is selectively enhanced in the presence of PAMAM dendrimers, which could be exploited for delivery benefits.[46]

Antiviral activity of dendrimers

Use of dendrimers as antiviral agents has been one of the most successful avenues in dendrimer-based drug discovery. Dendrimers either prevent binding of viruses to the target cell surface or prevent replication of the viral genome.

Prevention of virus binding to the target cell surface

Multiple carbohydrate binding proteins present on viruses play a critical role in invasion of the host cell. Carbohydrates present on the target cell surface can be linked to various dendrimers, which can act as receptors for viruses. Thus, glycodendrimers with carbohydrates at the periphery were tested for their ability to prevent binding of the influenza virus to host cell surface carbohydrates.[47,48] Sialic-acid-functionalised dendrimers investigated by Reuter and colleagues against influenza have 5×10^4-fold superior haemagglutination inhibiting activity than monomeric sialic acid.[49] PAMAM dendrimers functionalised with sialic acid (Figure 3a) completely protected mice against infection with murine influenza pneumonitis.[50]

Sialylated branched oligosaccharides containing 11 sugars per sequence were linked to polyacrylamide and given as an aerosol to mice before intranasal influenza inoculation, resulting in increased survival of the host.[51] Carbosilane dendrimers functionalised with sialyllactose (a trisaccharide) were also found to be effective inhibitors of influenza hemagglutinin-induced agglutination of erythrocytes.[52] The non-cytotoxic polysulfated galactose-derivatised poly(propyleneimine) dendrimers inhibit the infection of laboratory-isolated HIV-1 as efficiently as dextran sulfate.[53]

A number of pathogens, including Ebola, simian immunodeficiency virus (SIV), HIV, dengue, hepatitis C and cytomegalovirus use dendritic-cell-specific ICAM-3-grabbing non-integrin (DC-SIGN) receptors to infect host cells.[54–63] Mannosylated Boltorn (BoltornH30sucMan) dendrimers (Figure 3b) have been shown to inhibit DC-SIGN-mediated cell entry in an Ebola-pseudotyped viral model at nanomolar concentrations (IC50 337 nM).[54]

Prevention of viral genome replication

Formation of complexes between polyanionic compounds (modified PAMAM dendrimers) and HIV, HSV and many other viruses has been reported in the literature.[64] Polyanionic dendrimers have been shown to target the viral life cycle in addition to preventing its binding to the host cell. Carboxylated fullerene-based dendrimers are capable of inhibiting both viral protease and reverse transcriptase (purified and intracellular) in acutely HIV-infected primary human lymphocytes.[65] Sulfonated dendrimer BRI2923 (Figure 3c) has been shown to inhibit viral enzymes – reverse transcriptase and integrase – as well as cell entry of HSV *in vitro*.[17]

Sulfonated polylysine dendrimer with a benhydrylamine core has been shown to inhibit viral absorption, infection and DNA synthesis by the infected cell when tested against HSV.[66] Bernstein and colleagues have also investigated the potential of sulfonated polylysine dendrimer with a benhydrylamine core *in vivo* in mice and guinea-pigs and found that they protected against intravaginal challenge with HSV.[67] Sulfonated dendrimer also protected female pigtail macaques against intravaginal infection with SIV/HIV chimera virus.[31] Similar results were obtained by Bourne and colleagues upon administration of sulfonated (BRI-2999) and carboxylated (BRI-6741) dendrimers (Figure 3d) to mice prior to exposure to HSV.[68] Recently, Dixon and colleagues[69] have reported inhibitory action of sulfonated naphthyl polyphyrins against HIV infection of cells *in vitro*. Besides polyanionic dendrimers, unmodified polycationic PAMAM dendrimers have also been shown to inhibit viral replication by disrupting the interaction between HIV Tat protein and trans-acting response element (TAR) RNA, which is crucial in the life cycle of HIV-1. Unmodified polycationic PAMAM dendrimer showed greater affinity towards TAR-RNA than viral Tat protein.[70]

Bernstein and colleagues[67] also reported that the outer sulfonic acid surface of lysine-based dendrimers attaches through thiourea (SPL2999) or amide (SPL7013, SPL7015 and SPL7032) linkages (Figures 4a and b). They evaluated these compounds *in vivo* in mouse and guinea-pig models of HSV-2-induced genital herpes infection. All the compounds

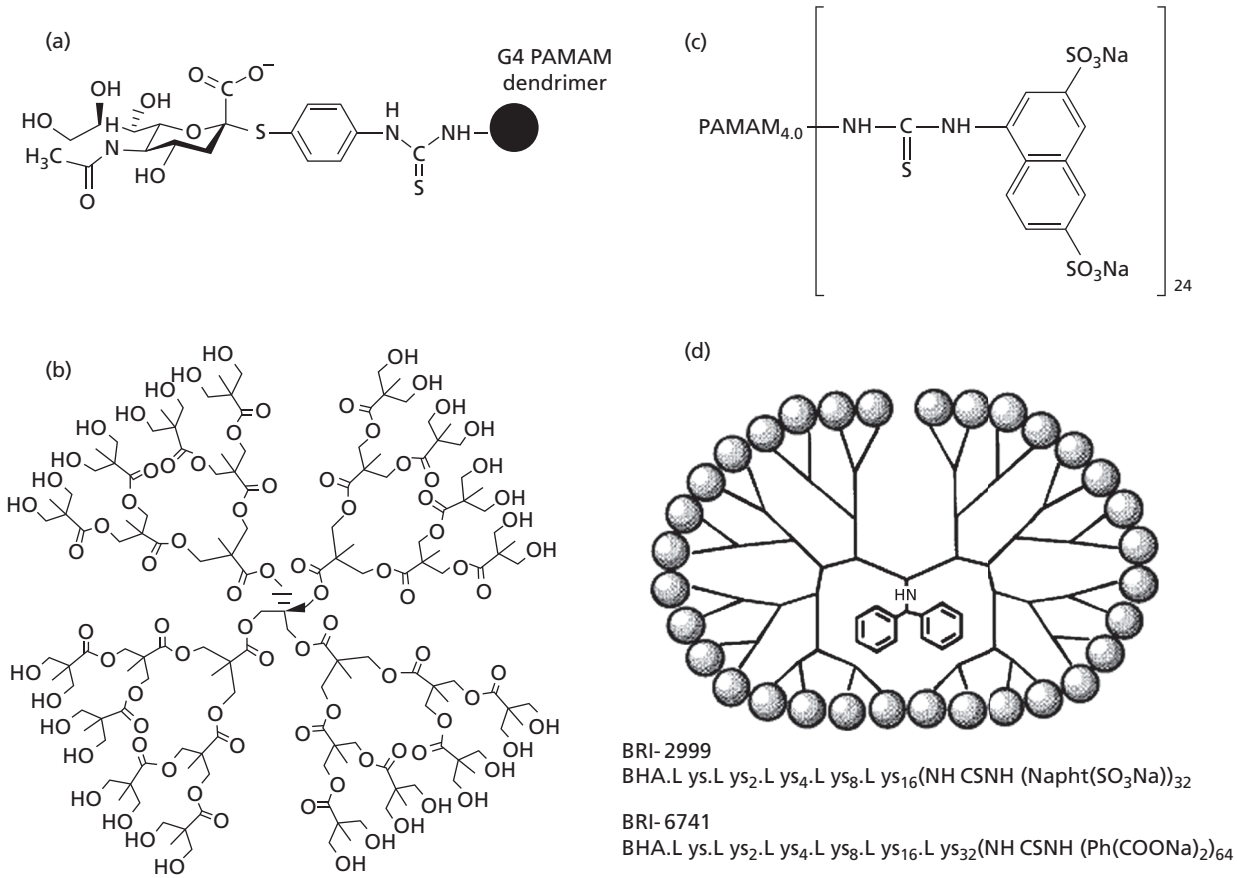

Figure 3 (a) Sialic acid–PAMAM dendrimer, which inhibits hemagglutination activity against influenza virus (reproduced from Landers *et al.*[50], with permission from Chicago Journals). (b) Third-generation Boltron dendritic polymer used in inhibition of DC-SIGN-mediated cell entry of Ebola-pseudotyped virus (adapted from Lasala *et al.*[54] with the permission of the American Society for Microbiology). (c) Sulfonated BRI2923 dendrimer inhibitors of viral replication (reproduced from Witvrouw *et al.*[17]) with the permission of the American Society for Pharmacology & Experimental Therapeutics). (d) Sulfonated (BRI-2999) and carboxylated (BRI-6741) dendrimers showing activity against herpes simplex virus infection (reproduced from Bourne *et al.*[68] with the permission of the American Society for Microbiology).

showed similar activities against HSV-2 *in vitro*. When these compounds were tested as 10% solutions in animal models, significant protection against the disease as well as the infection was observed. When SPL7013 compound was evaluated at different concentrations, it provided significant protection even at a low concentration of 1 mg/ml. At a concentration of 10 mg/ml the compound provided protection from disease for at least 1 h following administration. The SPL7013 compound was then used for further development of three products that differ in the concentration of glycol and glycerin (1V, 2V and 3V), all three of which provided significant protection against the infection. The 2V formulation was further evaluated and showed protection at a concentration of 1% for 30 min in two experiments and at least 1 h after application in one experiment when evaluated in mice. Dose-dependent protection was observed for the 2V formulation in the guinea-pig model of genital herpes at 3% and 5% concentrations when applied 5 min before virus challenge. The authors concluded that despite the increased size, vaginal vault area and higher dose of virus used in the guinea-pig model, the high activity of SPL7013 was maintained.[67] The 3% SPL7013 formulation exhibited the same safety profile as the 1% formulation and demonstrated no cervicovaginal irritation when tested in macaques.[31] Further evaluation of the 3% formulation for rectal safety and efficacy against *Chlamydia trachomatis* was carried out. In a phase I clinical trial of VivaGel, which contains 0.5–3% (wt/wt) SPL7013, similar results were obtained for both placebo and active gel test groups, indicating that the formulation did not affect the microbiology of either ecosystem.[31]

The anti-HIV activity of dendrimers and glycodendrimers is being extensively studied worldwide. A recent study by our group indicates that PPI and manosylated PPI dendrimer alone possess some anti-HIV activity. These nanocarrier systems are being explored further for controlled and targeted delivery of anti-HIV drugs.[71]

Dendrimer-based inhibitors of bacterial toxins

Bacteria and bacterial toxin mimic viruses in infecting host cells by adhering to cell surface carbohydrates. Toxins produced by various strains of bacteria such as shiga toxin, shiga-like toxin (verotoxin), tetanus toxoid, cholera toxin and

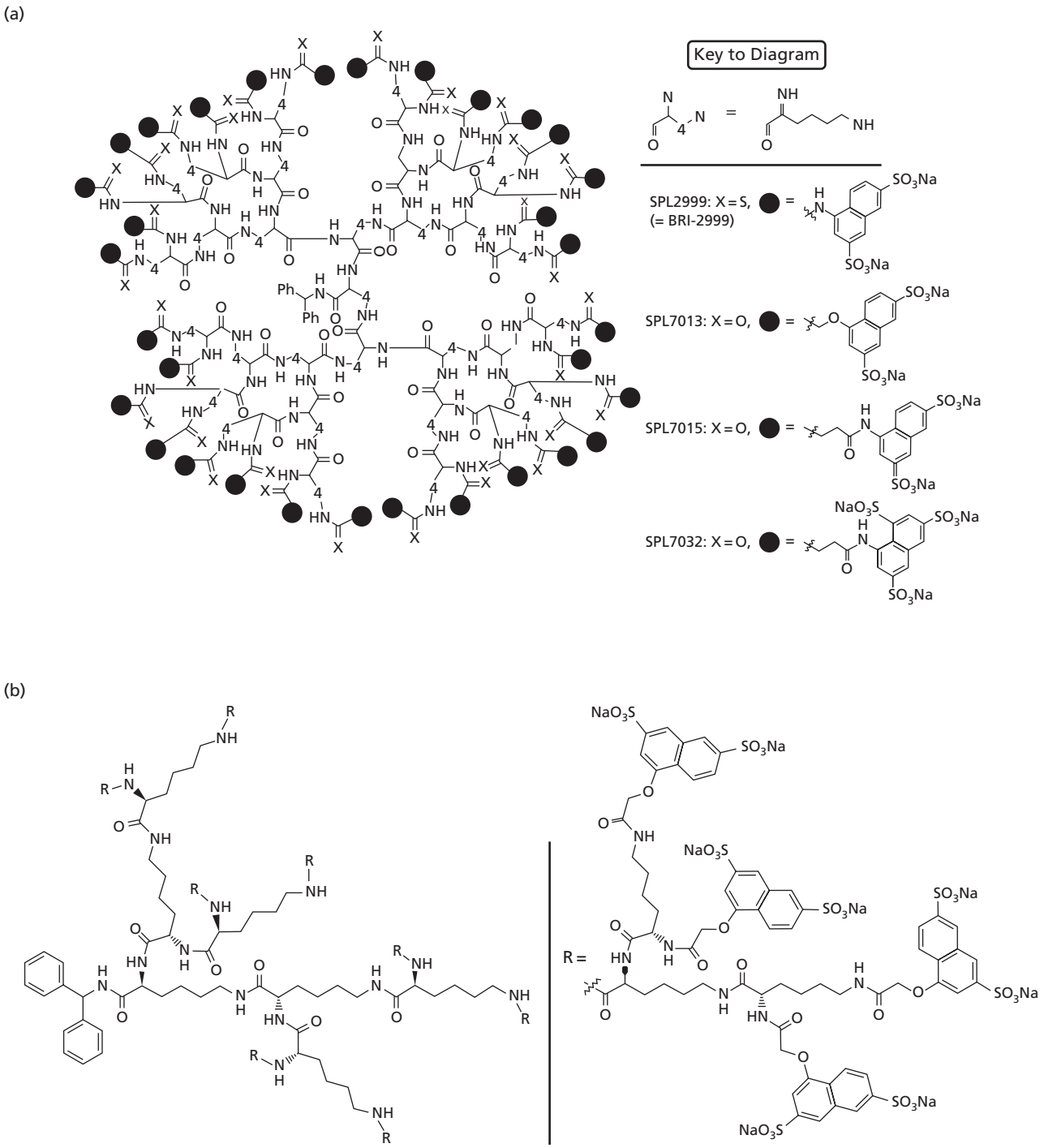

Figure 4 Schematic diagram of (a) sulfonic-acid-functionalised lysine dendrimers (reproduced from Bernstein *et al.*[67] with the permission of the American Society for Microbiology) and (b) SPL7013 (Vivagel) dendrimer used against herpes simpex virus (HSV-2) infection (reproduced from McCarthy *et al.*[71] with permission; Copyright American Chemical Society).

botulinum toxin cause life-threatening illness. These toxins chiefly consist of two subunits A and B; the A subunit is responsible for enzymatic activity while the B subunit is responsible for host cell binding. Multivalent carbohydrate inhibitors of their binding had been developed against shiga and shiga-like toxins.[72] An oligosaccharide-derivatised dendrimer has been developed in which 4–8 primary amino groups containing poly(propyleneimine) and PAMAM dendrimer were used as the core for covalent attachment of phenylisothiocyanate (PITC) derivatised galbeta1-3galNAc-beta1-4[sialic acid alpha2-3]-galbeta1-4glc (oligo-GM1) residues.[73,74] These oligo-GM1–PITC dendrimers inhibited

binding of ^{125}I-labelled cholera toxin B subunit and heat-labile enterotoxin of *Escherichia coli* to GM1-coated wells at a molar concentration 5–15-fold lower than GM1 and more than 1000-fold lower than that of free oligosaccharide.[70] Galactose-functionalised dendrimers have also been found to reduce the binding efficiency of cholera toxin and are superior to corresponding monomers.[75–77]

Yamada and colleagues[78] also synthesised carbosilane dendrimers bearing three, four and six galabiose (Ga1α1-4Gal) units and found them to be effective against shiga toxins produced by *E. coli* O157:H7. They concluded that an intra-sugar distance between branches of approximately 29 Å was required for increased binding activity against shiga toxin. Dendritic compounds with a glucose core linked with divalent sugar moieties by each hydroxyl group also neutralised shiga toxins in mice.[79,80] Mannosylated dendrimers have also been found to inhibit *E. coli* (K12) strain due to presence of multiple mannosyl residues and an alpha-oriented aglycon.[81]

Reduction of drug toxicity

Anticancer drugs such as methotrexate and 6-mercaptopurine are hepatotoxic. Melamine dendrimers have been shown to reduce the organ toxicity of these drugs in C3H mice by increasing their solubility. Animals were given subchronic doses of methotrexate and 6-mercaptopurine with and without solubilising dendrimer, and the level of alanine transaminase (ALT) was used to probe liver damage. ALT was reduced to 27% and 36% with encapsulated methotrexate and 6-mercaptopurine, respectively, compared with non-encapsulated drugs.[27]

Treatment of haemochromatosis

Iron overload caused by haemosiderosis in β-thalassaemia major can be reduced with the help of orally active iron chelators. Hexadentate-terminated dendrimers such as hydropyridinone-based dendrimer reduce the absorption of iron (III) from the intestine and can be used to treat iron overload in haemochromatosis and thalassaemia intermedia.[28]

Anti-tumour effects of dendritic nanostructures

The knowledge of carbohydrate receptors and their ligands has proved useful in targeting and treatment of tumours, including breast and renal melanomas. Changes in the precise collection of surface carbohydrates on cells showed the way for the malignant transformation process. This stratagem has been explored for investigating immune reactions against tumours.[82,83] Glycodendrimers have emerged as convincing targets for carbohydrate-binding proteins on tumour cells. Mice injected with B16 melanoma showed enhanced mean survival time when treated with PAMAM-based glycodendrimers (Figure 5a), from about 27 days in the control group and groups receiving various other treatments, to 42 days in the glycodendrimer-treated group.[84] Both survival and inhibition of tumour growth were dose dependent following intraperitoneal injection. Ex-vivo cytotoxicity assays showed enhanced natural killer (NK) cell activity, which clearly established that multivalent glycodendrimers were able to elicit an anti-tumour immune response in tumours.[84,85]

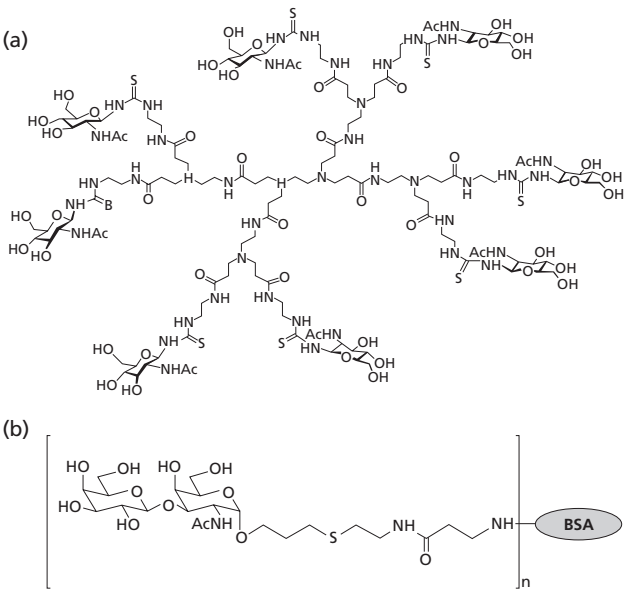

Figure 5 (a) Schematic diagram of octavalent N-acetyl-glucosamine PAMAM dendrimer (PAMAM-GlcNAc8) used against B16 melanoma in mice (reproduced from Vannucci *et al.*[84] with the permission of Spandidos Publications). (b) T-antigen bearing neoglycoconjugates have improved inhibitory activity compared with monovalent antigen for cancer marking (reproduced from Roy *et al.*[87] with permission; Copyright American Chemical Society).

A single dose of glycodendrimers might be an alternative to multiple injections of glycolipid-coated liposomes, which elicited cellular immunity by NKR-P1 cells. The CD4 lymphocyte subset was subsequently elevated, leading to a permanent immune response against the tumour.[20,86]

Roy and colleagues conjugated the breast-cancer-associated T-antigen carbohydrate marker to novel glycodendrimers based on N, N′-bis (acrylamido) acetic acid core by thiolated T-antigen.[87] An acid derivative of T-antigen was also conjugated to polyamine dendrimer by amide bond. These multivalent conjugates (Figure 5b) showed improved inhibition compared with the monovalent antigen. The glycodendrimers synthesised with 2,2′-bipyridine-4,4′-dicarboxylic acid chloride and the aminated sugar derivatives show inhibitory potency against monomeric allyl α-GalNAc (breast-cancer-associated T-antigen carbohydrate marker). The di- and tetravalent bipyridyl clusters exhibited 87-fold amplification of inhibitory properties compared with the monomeric complements.[88] PAMAM glucosamine 6-sulfate has anti-angiogenic activity as a result of its ability to block endothelial cell proliferation mediated by fibroblast growth factor-2 and neo-angiogenesis in human Matrigel and placental angiogenesis assays.[25]

Collagen-mimetic and tissue repair dendrimers

Collagen is a fundamental constituent of extracellular matrixes on which tissues are built in vertebrates, and comprises about one-third of proteins in animals.[89] It is a vital component of connective tissues such as bone, cartilage, skin and tendon, and its high tensile strength is due to its triple helical structure.

Natural collagen has potential uses in surgical sutures, haemostatic agents and tissue replacements for blood vessels, valves and cartilage,[90–94] although its utilisation is limited by disruption of its structural configuration during purification and sterilisation. Collagen obtained from human cadavers and bovine sources induces disease transmission and allergic responses.[94] There is thus a need for synthetic and artificial material analogous to collagen. Kinberger and colleagues[95] explored the synthesis and conformational properties of 162-residue collagen-mimetic dendrimers (TMA[TRIS{(Gly-Pro-Nleu)$_6$-OMe}$_3$]$_3$) as shown in Figure 6, which exhibited improved stability in triple helical structure compared with equivalent scaffold terminated structures. Gly-Pro-Nleu and Gly-Nleu-Pro sequences were used to synthesise collagen-mimetic dendrimers. With the aim of synthesising the dendrimeric structure, they removed Z-protecting groups from *N*-(benzyloxycarbonyl)-tris(carboxyethoxymethyl) aminomethane (Z-TRIS(OH)$_3$) and reacted it with trimesoyl chloride. They have also reported the synthesis of an analogous dendrimer using a Gly-Nleu-Pro sequence containing a β-alanine spacer. Thermal denaturation monitored by optical rotation and circular dichroism measurement showed that dendrimers exhibited a triple-helical structure. Their results also indicated that collagen-mimetics synthesised from the Gly-Nleu-Pro sequence were thermally more stable triple-helical structures than the equivalent structures formed from the Gly-Pro-Nleu sequence.[95]

Recently, the same investigators have reported the synthesis of a new collagen mimetic dendrimer composed of the Gly-Pro-Nleu sequence fabricated on a G1 PAMAM core.[23] By donation of amine and amide electrons, PAMAM dendrimers complexed with transition metals (e.g. Cu^{2+} and Ni^{2+}).[95,96] Copper(II) acts as an enzyme cofactor for cross-linking of fibril collagens, which is vital for connective tissues.[97] Bone mineralisation and wound healing involving collagen are also assisted by metal cofactors.[98,99] Hence, it was suggested that synthesis of PAMAM collagen-mimetic conjugates and their resultant complexation with metal cofactors may have extraordinary biological properties. They converted G0.5 PAMAM dendrimer to free acid and then allowed it to react with excess H-Gly-Pro-Nleu-OMe in the presence of O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate to synthesise PAMAM G0.5 [Gly-Pro-Nleu-OMe]$_8$, which after purification was again allowed to react with H-(Gly-Pro-Nleu)$_5$-OMe to form the triple helical structure of the PAMAM collagen-mimetic conjugate. They observed that at 22°C the PAMAM and trimesic acid collagen-mimetic dendrimers retained their triple helical structure, whereas the tris-assembled structure was almost entirely denatured.[23]

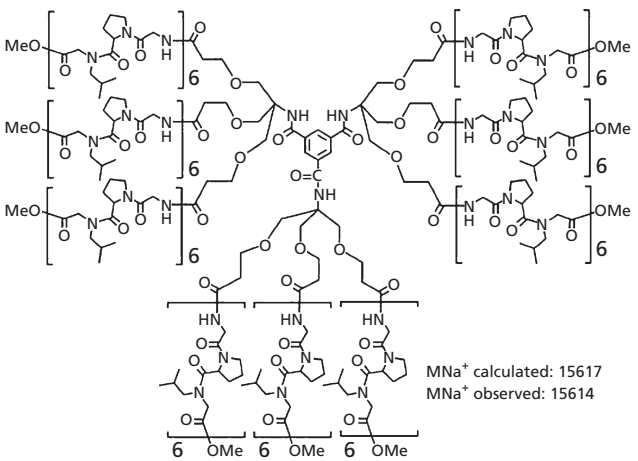

Figure 6 Schematic diagram of the collagen-mimetic dendrimer (TMA[TRIS{(Gly-Pro-Nleu)$_6$-OMe}$_3$]$_3$) (reproduced from Kinberger *et al.*[95]) with permission; Copyright American Chemical Society).

Cartilage degeneration is distressing to millions of individuals worldwide in the form of osteoarthritis and trauma. Treatment involves anti-inflammatory drugs, total joint replacement, discectomy and chondrocyte transplantation.[100–103] Tissue engineering strategies may significantly improve patient care because clinical treatments stated above are either scarce or long term. Hydrogel derived from dendrimer-based polymers provides a multivalent and modular base for the design and optimisation of novel macromers for tissue engineering and also offer an alternative to those based on a linear structure.[24] Compared with linear polymers, multivalent branched dendrimer structures provide higher cross-linking densities, with the potential to accomplish the apparently conflicting necessities of high mechanical strength and water content that are obligatory for cartilage repair. In addition, dendritic branched constitutions can accommodate considerable degradation before the cross-linked network breaks down, upholding mechanical integrity during degradation. Sontjens and colleagues characterised the swelling, mechanical and degradation properties of biodendrimer scaffolds consisting of a poly(ethylene glycol) core and methacrylated poly(glycerol succinic acid) dendrimer terminal blocks, as well as the ability of the dendrimer-based hydrogel to support articular chondrocytes and extracellular matrix synthesis *in vitro*.[24] The biodendrimer showed very small changes in volume during cross-linking, demonstrating that it did not detach from the wound site and did not exacerbate the trauma. The biodendrimer hydrogel was also shown to support cartilaginous extracellular matrix production, and encapsulated chondrocytes also retain rounded morphology with no sign of differentiation, and produced extracellular matrix similar to native articular cartilage, including type II collagen and proteoglycans.

The biocompatible and biodegradable photo-cross-linkable derivative of poly(ethylene glycol), glycerol and succinic acid copolymer (Figure 7) successfully sealed a 4.1 mm corneal laceration by physical entrapment, where an interpenetrating network had been formed between the cross-linked copolymer and the tissue.[104] This dendritic copolymer was found to seal the wound better than conventional sutures and can withstand greater pressures and stresses placed on or around the wound site. The procedure was about 5-fold faster than suturing the wound, potentially diminishing surgical time and interventions. In addition to this, the cross-linked gel of hybrid linear-dendritic copolymer is transparent, elastic and has a beneficial adhesive property for an ophthalmic sealant.[104]

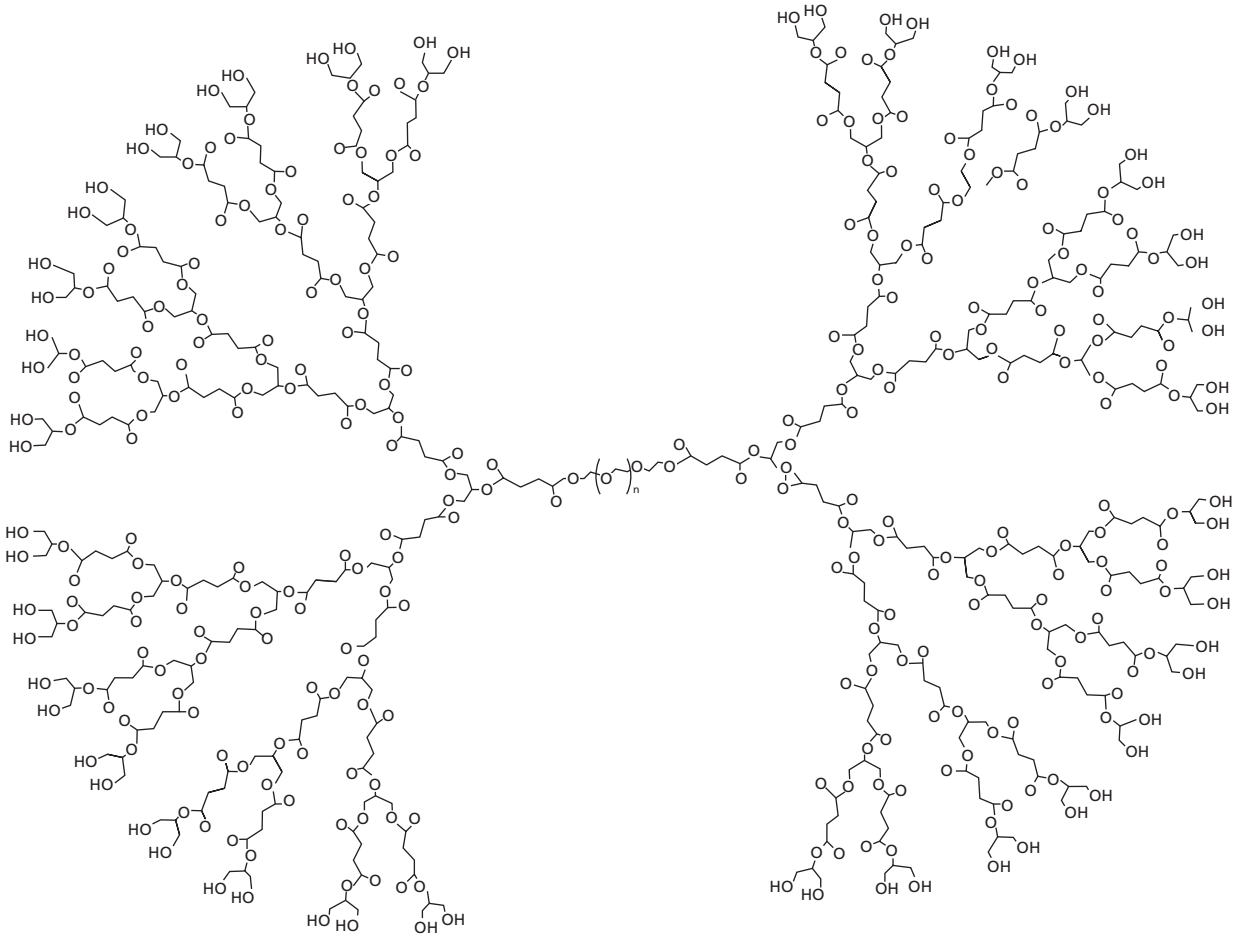

Figure 7 Schematic diagram of photo-cross-linkable derivative of poly(ethylene glycol), glycerol and succinic acid copolymer, which seals corneal lacerations (reproduced from Carnahan *et al.*[105] with permission; Copyright American Chemical Society).

Prevention of scar tissue formation after glaucoma surgery

Glaucoma, which is a key cause of permanent blindness, can be treated surgically by creating a new channel to drain aqueous humour from the eye, which diminishes the intraocular pressure and prevents damage to the optic nerve. However, surgery can lead to scarring because of a persistent inflammatory and angiogenic response that supports fibroblast proliferation. Anti-cancer drugs such as 5-fluorouracil and mytomycin C prevent scar tissue formation[105] but cause sight-threatening complications, including severe infection and thin, leaky tissue. The success of surgical treatment in rabbits was increased from 30% to 80% by combination treatment with dendrimer-glucosamine (Figure 8) and dendrimer-glucosamine 6-sulfate at doses of 60.3 and 30.15 mg, respectively.[25] No systemic infection by bacteria, viruses or fungi was observed in the 30 days after treatment, and there was no haematological, clinical or biochemical (including blood glucose) toxicity. No substantiation of persistent inflammation or neoangiogenic response was found, and formation of scar tissue was minimal with dendrimer-glucosamine and dendrimer-glucosamine 6-sulfate compared with placebo-treated rabbits.

Dendrimer-glucosamine inhibited lipopolysaccharide–Toll-like receptor 4-mediated pro-inflammatory mediator synthesis from immature human dendritic cells and macrophages but allowed activation and maturation of dendritic cells. On the other hand, dendrimer-glucosamine 6-sulfate inhibited the proliferation of human umbilical vein endothelial cells mediated by fibroblast growth factor-2, rendered into a considerable anti-angiogenic effect in two human model systems of new blood vessel formation. This study was the first to report the simultaneous targeting of pro-inflammatory mediators and neoangiogenesis by dendrimers, with the capability of preventing scar tissue formation with higher levels of safety following surgery.[22]

These therapeutic effects of dendrimers, coupled with their nanoscopic size, low polydispersity, ease of synthesis and stability,[106–110] demand their further scientific exploration.

Dendrimers in photodynamic therapy

Since the approval of Photofrin as a photosensitiser for photodynamic therapy (PDT), the use of PDT for the treatment of cancer as well as non-neoplastic lesions has increased dramatically.[111,112] This approach involves tumour

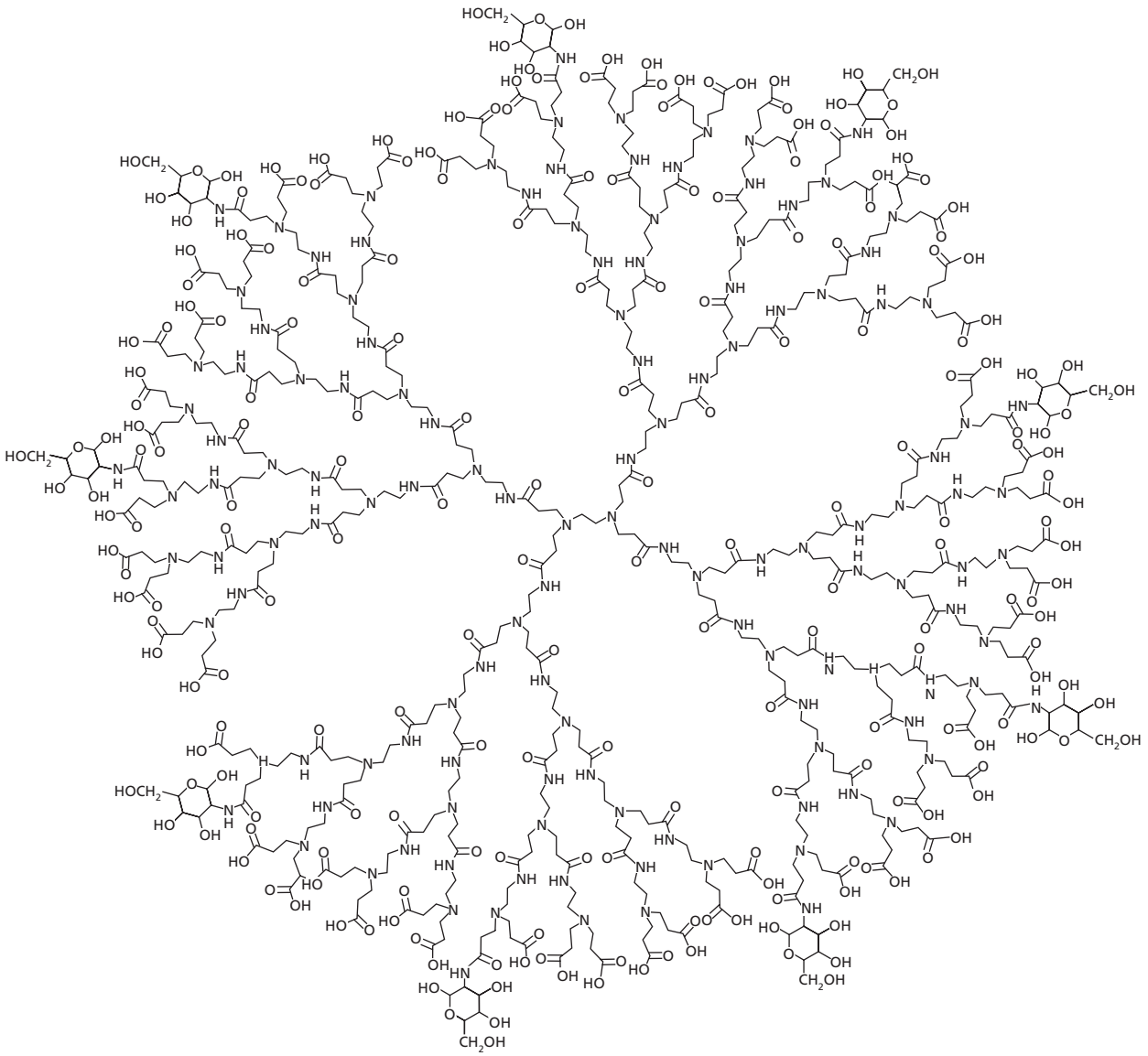

Figure 8 Schematic diagram of glucosamine-conjugated PAMAM dendrimer, which inhibits scar tissue formation (reproduced from Shaunak *et al.*[25]) with the permission of the Nature Publishing Group).

localisation of dendrimeric porphyrin conjugates, employing a variety of receptor-mediated events. This is then followed by the irradiation of dendrimeric porphyrin architecture. At the targeted site, localised porphyrin core dendrimers bring about the conversion of light energy to chemical energy, generating singlet oxygen, which is an electronically excited species.

To explore the avenues of PDT, Nishiyama and colleagues synthesised the 3.0G-polyaryl ether dendrimer porphyrins and evaluated these as a novel supramolecular class of photosensitisers for PDT.[113] Such dendrimeric scaffold remained localised in lysosomes and other intracellular compartments. On the other hand, protoporphyrin IX (PpIX), which is a hydrophobic and relatively low molecular weight photosensitiser, diffused through the cytoplasm but not to the nucleus. The dendrimer porphyrins showed lower toxicity than PpIX in terms of disruption of membranes and intracellular organelles, in agreement with other reports on PDT.[114–116] Furthermore, dendrimer porphyrins showed about 140-fold lower dark toxicity than PpIX, signifying their selective photosensitising effect.[113]

In order to further explore dendritic prodrugs in PDT, 3.0G aryl ether dendrimer porphyrin with 32 primary amine groups on the periphery, and pH-sensitive polyion complex (PIC) micelles composed of the porphyrin dendrimer and pegylated poly (aspartic acid) were evaluated as new photosensitisers for PDT in the Lewis lung carcinoma cell line. Compared with the dendrimer porphyrin, cellular uptake of the dendrimer porphyrin incorporated into the PIC micelle was relatively low; however, the latter exhibited enhanced photodynamic effects. Moreover, the use of PIC micelles as a delivery system reduced the dark toxicity of the cationic dendrimer porphyrin, probably because of the biocompatible PEG shell.[117] Dichtel

and colleagues have explored the porphyrin core dendrimers, conjugating numerous two-photon-absorbing chromophores, which upon irradiation at longer wavelength tend to efficiently generate singlet oxygen species.[118]

Recently, Yamamoto and colleagues reported that metal-assembling dendritic phenylazomethines could also significantly improve the electron transfer reaction as a protein-like catalyst.[119] Later the same group showed that the lanthanide-ion-assembling dendritic phenylazomethines with a cobalt porphyrin core may also act as an efficient catalyst for the CO_2 electrochemical reduction with a very low potential.[120] Cobalt tetraphenylporphyrin, which is a model of the core unit, is known to catalyse the reduction.[121,122] It was suggested that the electron exchange kinetics between the core and metal complexes should be very fast in order to accelerate the electron transfer process. In the case of dendritic phenylazomethine complexes, the time scale of the electron transfer was much faster than nanoseconds, and was confirmed by a fluorescence quenching experiment.[123]

Conclusions

Dendrimers have emerged as important tools for drug discovery because of their ease of surface modification as well as their ability to interact with charged functional groups. The examples discussed in this review suggest that dendrimers may have applications in prion infections and Alzheimer's disease. They are also very efficient anti-virals, acting by both inhibiting cell entry and preventing replication of pathogens. They have shown immense potential against HIV, which is a major problem worldwide. The collagen-mimetic dendrimers complexed with metal cofactors may have potential in wound healing and bone mineralisation. Cartilage formation, tissue repair and prevention of scar tissue formation, where they appear promising alternatives to conventional sutures, are among the most novel properties of therapeutic dendrimers. A few but sound examples illustrate the potential applications of dendrimers as therapeutics against cancer, and warrant further exploration. Pro-inflammatory mediators and selectin inhibition show that the dendrimer can be a very selective anti-inflammatory agent. Heparin analogues may be potential alternatives to heparin and other anticoagulants. Dendrimeric antidotes and chelators may prove useful for removing various toxic substances from the body. The use of dendrimeric nano-architectures as vaccines and as blood substitutes also has an interesting future. These horizons of pharmaceutical and therapeutic relevance of dendrimers are likely to widen in the near future.

The field of dendrimer therapeutics is still in its infancy, but the explosion of curiosity in this field makes it appropriate to review current knowledge regarding dendrimer chemistry. Because of their multivalency, dendrimers are extensively cited as a means to generate improved vaccines, and in this line peptide-,[124,125] glyco- and glycolipid-containing dendrimers have been described.[126] However, unwanted immunogenicity (antigenicity) of dendrimers designed for other therapeutic uses could limit their clinical development, although this is difficult to define at present. Few have systematically studied the cellular and humoural effects of dendrimers. A vast array of biocompatible hybrid dendritic architectures are currently being investigated.[127–130] Further research is needed to assure the safety of long-term administration. For in-vivo applications, there is a requirement for cautiously designed toxicology and toxicokinetic studies for each dendrimer type, the protocols being customised to address the likely clinical use. For this, assistance can be taken from past clinical experience with other macromolecular and polymer therapeutics. Several decades of clinical knowledge with these systems provides insight into likely adverse reactions and an understanding of fundamental mechanisms. In conclusion, it can be envisaged that safe and effective dendrimer-based therapeutics could become a reality in the near future with the help of a logical blueprint developed by understanding of the biological processes.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This review received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

1. Wehner BW, Vogtle F. "Cascade and nonskid-chain like" synthesis of molecule cavity topologies. Vogtle Synthesis. 1978, 155; US patents 4289872 (1981) and 4410688 (1985).
2. Patri AK *et al*. Dendritic polymer macromolecular carriers for drug delivery. *Curr Opin Chem Biol* 2002; 6: 466–471.
3. Jansen JFGA. Encapsulation of guest molecules into a dendritic box. *Science* 1994; 266: 1226–1229.
4. Gajbhiye V *et al*. Dendrimeric nanoarchitectures mediated transdermal and oral delivery of bioactives. *Indian J Pharm Sci* 2008; 70: 431–439.
5. Schatzlein AG *et al*. Preferential liver gene expression with polypropylenimine dendrimers. *J Control Release* 2005; 101: 247–258.
6. Patri AK *et al*. Targeted drug delivery with dendrimers: comparison of the release kinetics of covalently conjugated drug and non-covalent drug inclusion complex. *Adv Drug Deliv Rev* 2005; 57: 2203–2214.
7. Gupta U *et al*. Dendrimers: novel polymeric nanoarchitecture for solubility enhancement. *Biomacromolecules* 2006; 7: 649–658.
8. Wiener EC *et al*. Dendrimer-based metal chelates: a new class of magnetic resonance imaging contrast agents. *Magn Reson Med* 1994; 31: 1–8.
9. Wu L *et al*. Phosphine dendrimer-stabilized palladium nanoparticles, a highly active and recyclable catalyst for the Suzuki-Miyaura reaction and hydrogenation. *Org Lett* 2006; 8: 3605–3608.
10. Gajbhiye V *et al*. Pharmaceutical and biomedical potential of PEGylated dendrimers. *Curr Pharm Design* 2007; 13: 415–429.
11. Heldt JM *et al*. Preparation and characterization of poly (amidoamine) dendrimers functionalized with a rhenium carbonyl complex and PEG as new IR probes for carbonyl metallo immunoassay. *J Organometallic Chem* 2004; 689: 4775–4782.

12. Bosman AW *et al*. About dendrimers: structure, physical properties, and applications. *Chem Rev* 1999; 99: 1665–1688.
13. Klajnert B, Bryszewska M. Dendrimers: properties and applications. *Acta Biochimica Polonica* 2001; 48: 199–208.
14. Solassol J *et al*. Cationic phosphorus containing dendrimers reduce prion replication both in cell culture and in mice infected with scrapie. *J Gen Virol* 2004; 85: 1791–1799.
15. Klajnert B *et al*. Influence of dendrimer's structure on its activity against amyloid fibril formation. *Biochem Biophy Res Commun* 2006; 345: 21–28.
16. Chauhan A *et al.* Composition and complexes containing a macromolecular polymer as potential anti-inflammatory agents. US Patent 20030180250 A1, 2003.
17. Witvrouw M *et al*. Polyanionic (i.e., polysulfonate) dendrimers can inhibit the replication of human immunodeficiency virus by interfering with both virus adsorption and later steps (reverse transcriptase/integrase) in the virus replicative cycle. *Mol Pharmacol* 2000; 58: 1100–1108.
18. Gong Y *et al*. Evidence of dual sites of action of dendrimers: SPL-2999 inhibits both virus entry and late stages of herpes simplex virus replication. *Antiviral Res* 2002; 55: 319–329.
19. Chen CZ *et al*. Quaternary ammonium functionalized poly (propylene imine) dendrimers as effective antimicrobials: structure-activity studies. *Biomacromolecules* 2000; 1: 473–480.
20. Pospisil M *et al*. Glycodendritic ligand of c-type lectin receptors as therapeutic agents in experimental cancer. *Adv Exp Med Biol* 2001; 495: 343–347.
21. Chen CZ, Cooper SL. Interaction between dendrimer biocides and bacterial membranes. *Biomaterials* 2002; 23: 3359–3368.
22. Turk H *et al*. Dendritic sulfates as new heparin analogues and potent inhibitors of the complement system. *Bioconjug Chem* 2004; 15: 162–167.
23. Kinberger GA *et al*. The design, synthesis, and characterization of a PAMAM-based triple helical collagen mimetic dendrimer. *Tetrahedron* 2006; 62: 5280–5286.
24. Sontjens SHM *et al*. Biodendrimer-based hydrogel scaffolds for cartilage tissue repair. *Biomacromolecules* 2006; 7: 310–316.
25. Shaunak S *et al*. Polyvalent dendrimer glucosamine conjugates prevent scar tissue formation. *Nat Biotechnol* 2004; 22: 977–984.
26. Yamada A *et al*. Synthesis and vero toxin-binding activities of carbosilane dendrimers periphery-functionalized with galabiose. *Tetrahedron* 2006; 62: 5074–5083.
27. Neerman MF *et al*. Reduction of drug toxicity using dendrimers based on melamine. *Mol Pharmacol* 2004; 1: 390–393.
28. Zhou T *et al*. Iron binding dendrimers: a novel approach for the treatment of haemochromatosis. *J Med Chem* 2006; 49: 4171–4182.
29. Kofoed J *et al*. Artificial aldolases from peptide dendrimer combinatorial libraries. *Org Biomol Chem* 2006; 4: 3268–3281.
30. Losada J *et al*. Bioenzyme sensors based on novel polymethylferrocenyl dendrimers. *Anal Bioanal Chem* 2006; 385: 1209–1217.
31. Jiang YH *et al*. SPL7013 gel as a topical microbicide for prevention of vaginal transmission of SHIV89.6P in macaques. *AIDS Res Hum Retroviruses* 2005; 21: 207–213.
32. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science* 1982; 216: 136–144.
33. Supattapone S *et al*. Branched polyamines cure prion-infected neuroblastoma cells. *J Virol* 2001; 75: 3453–3461.
34. Safar J *et al*. Eight prion strains have PrP^{Sc} molecules with different conformations. *Nat Med* 1998; 4: 1157–1165.
35. Klajnert B *et al*. Molecular interactions of dendrimers with amyloid peptides: pH dependence. *Biomacromolecules* 2006; 7: 2186–2191.
36. Selvaggini C *et al*. Molecular characteristics of a protease-resistant, amyloidogenic and neurotoxic peptide homologous to residues 106–126 of the prion protein. *Biochem Biophys Res Commun* 1993; 194: 1380–1386.
37. Heegaard PMH *et al*. Amyloid aggregates of the prion peptide PrP 106–126 are destabilised by oxidation and by action of dendrimers. *FEBS Lett* 2004; 577: 127–133.
38. Patel D *et al*. Attenuation of β-amyloid induced toxicity by sialic acid-conjugated dendritic polymers. *Biochim Biophys Acta* 2006; 1760: 1802–1809.
39. Chauhan AS. Macromolecular compound as potential anti-inflammatory agents. WO/2003/080121, 2003.
40. Rele SM *et al*. Dendrimer-like PEO glucopolymers exhibit anti-inflammatory properties. *J Am Chem Soc* 2006; 127: 10132–10133.
41. Turk H *et al*. Dendritic polyglycerol sulfates as new heparin analogues and potent inhibitors of the competent system. *Bioconjug Chem* 2004; 15: 162–167.
42. Alban S *et al*. Synthesis of laminarin sulfates with anticoagulant activity. *Arzneim Forsch Drug Res* 1992; 42: 1005–1008.
43. Gait MJ *et al*. Synthetic analogues of polynucleotides. Part XII. Synthesis of thymidine derivatives containing an oxyacetamido- or an oxyformamido- linkage instead of a phosphodiester group. *J Chem Soc Perkin Trans* 1974; 1: 1684–1686.
44. Queiroz de AAA *et al*. Physicochemical and antimicrobial properties of boron-complexed polyglycerol-chitosan dendrimers. *J Biomater Sci Polym Ed* 2006; 17: 689–707.
45. Tam JP *et al*. Antimicrobial dendrimeric peptides. *Eur J Biochem* 2002; 269: 923–932.
46. Cheng Y *et al*. Polyamidoamine (PAMAM) dendrimers as biocompatible carriers of quinolone antimicrobials: an in vitro study. *Eur J Med Chem* 2007; 42: 1032–1038.
47. Tsvetkov DE *et al*. Neoglycoconjugates based on dendrimeric poly(aminoamides). *Bioorg Khim* 2002; 28: 518–534.
48. Roy R. Synthesis and some applications of chemically defined multivalent glycoconjugates. *Curr Opin Struct Biol* 1996; 6: 692–702.
49. Reuter JD *et al*. Inhibition of viral adhesion and infection by sialic-acid-conjugated dendritic polymers. *Bioconjug Chem* 1999; 10: 271–278.
50. Landers JJ *et al*. Prevention of influenza pneumonitis by sialic acid-conjugated dendritic polymers. *J Infect Dis* 2002; 186: 1222–1230.
51. Gambaryan AS *et al*. Polymeric inhibitor of influenza virus attachment protects mice from experimental influenza infection. *Antiviral Res* 2002; 55: 201–205.
52. Matsuoka K *et al*. Sugar chain-containing carbosilane dendrimer compounds, process for producing the same verotoxin neutralizers and antiviral agents. US Patent 2004/0040554, 2004.
53. Kensinger RD *et al*. Novel polysulfated galactose-derivatized dendrimers as binding antagonists of human immunodeficiency virus type 1 infection. *Antimicrob Agents Chemother* 2004; 48: 1614–1623.
54. Lasala F *et al*. Mannosyl glycodendritic structure inhibits DC-SIGN-mediated Ebola virus infection in cis and in trans. *Antimicrob Agents Chemother* 2003; 47: 3970–3972.
55. Geijtenbeek TB, van Kooyk Y. Pathogens target DC-SIGN to influence their fate DC-SIGN functions as a pathogen receptor with broad specificity. *APMIS* 2003; 111: 698–714.
56. van Kooyk Y *et al*. A fatal attraction: Mycobacterium tuberculosis and HIV-1 target DC-SIGN to escape immune surveillance. *Trends Mol Med* 2003; 9: 153–159.
57. Takada A *et al*. Human macrophage C-type lectin specific for galactose and N-acetylgalactosamine promotes filovirus entry. *J Virol* 2004; 78: 2943–2947.

58. Marzi A *et al*. DC-SIGN and DC-SIGNR interact with the glycoprotein of Marburg virus and the S protein of severe acute respiratory syndrome coronavirus. *J Virol* 2004; 78: 12090–12095.
59. Borges RA, Schengrund CL. Dendrimers and antivirals: a review. *Curr Drug Targ* 2005; 5: 247–254.
60. Lozach PY *et al*. C-type lectins L-SIGN and DC-SIGN capture and transmit infectious hepatitis C virus pseudotype particles. *J Biol Chem* 2004; 279: 32035–32045.
61. Wang QC *et al*. DC-SIGN: binding receptors for hepatitis C virus. *Chin Med J (Engl)* 2004; 117: 1395–1400.
62. Rojo J, Delgado R. Glycodendritic structures: promising new antiviral drugs. *J Antimicrob Chemother* 2004; 54: 579–581.
63. van Kooyk Y, Geijtenbeek TB. DC-SIGN: escape mechanism for pathogens. *Nat Rev Immunol* 2003; 3: 697–709.
64. Ross MB, George H. Antiviral dendrimers. European patent, WO9534595, 1995.
65. Schinazi RF, Brettreich M, Hirsch A. Water-soluble dendrimeric fullerene as anti-HIV therapeutic. US patent, 2003/0036562 A1, 2003.
66. Gong Y *et al*. Evidence of dual sites of action of dendrimers: SPL-2999 inhibits both virus entry and late stages of herpes simplex virus replication. *Antiviral Res* 2002; 55: 319–329.
67. Bernstein DI *et al*. Evaluations of unformulated and formulated dendrimer-based microbicide candidates in mouse and guinea pig models of genital herpes. *Antimicrob Agents Chemother* 2003; 47: 3784–3788.
68. Bourne N *et al*. Dendrimers, a new class of candidate topical microbicides with activity against herpes simplex virus infection. *Antimicrob Agents Chemother* 2000; 44: 2471–2474.
69. Dixon DW *et al*. Sulfonated naphthyl porphyrins as agents against HIV-1. *J Inorg Biochem* 2005; 99: 813–821.
70. Zhao H *et al*. Polyamidoamine dendrimers inhibit binding of Tat peptide to TAR RNA. *FEBS Lett* 2004; 563: 241–245.
71. McCarthy TD *et al*. Dendrimer as drug: discovery and preclinical and clinical development of dendrimer-based microbicides for HIV and STI prevention. *Mol Pharm* 2005; 2: 313–318.
72. Dutta T, Jain NK. Targeting potential and anti-HIV activity of lamivudine loaded mannosylated poly (propyleneimine) dendrimer. *Biochim Biophys Acta* 2007; 1770: 681–686.
73. Kitova EN *et al*. The observation of multivalent complexes of Shiga-like toxin with globotriaoside and the determination of their stoichiometry by nanoelectrospray Fourier-transform ion cyclotron resonance mass spectrometry. *Glycobiology* 2001; 11: 606–611.
74. Thompson JP, Schengrund CL. Oligosaccharide-derivatized dendrimers: defined multivalent inhibitors of the adherence of the cholera toxin B subunit and the heat labile enterotoxin of E. coli to GM1. *Glycoconj J* 1997; 14: 837–845.
75. Thompson JP, Schengrund CL. Inhibition of the adherence of cholera toxin and the heat-labile enterotoxin of Escherichia coli to cell-surface GM1 by oligosaccharide-derivatized dendrimers. *Biochem Pharmacol* 1998; 56: 591–597.
76. Merritt EA *et al*. Characterization and crystal structure of a high-affinity pentavalent receptor-binding inhibitor for cholera toxin and E. coli heat-labile enterotoxin. *J Am Chem Soc* 2002; 124: 8818–8824.
77. Fan E *et al*. Structural biology and structure-based inhibitor design of cholera toxin and heat-labile enterotoxin. *Int J Med Microbiol* 2004; 294: 217–223.
78. Yamada A *et al*. Synthesis and vero toxin-binding activities of carbosilane dendrimers periphery-functionalized with galabiose. *Tetrahedron* 2006; 62: 5074–5083.
79. Mulvey GL *et al*. Assessment in mice of the therapeutic potential of tailored, multivalent Shiga toxin carbohydrate ligands. *J Infect Dis* 2003; 187: 640–649.
80. Nagahori N *et al*. Inhibition of adhesion of type 1 fimbriated Escherichia coli to highly mannosylated ligands. *Chembiochemistry* 2002; **3**: 836–844.
81. Nagahori N *et al*. Inhibition of adhesion of type 1 fimbriated Escherichia coli to highly mannosylated ligands. *Chembiochemistry* 2002; 3: 836–844.
82. Cohen J. Cancer vaccines get a shot in the arm. *Science* 1993; 262: 841–843.
83. McCoy JPJr, Chambers WH. Carbohydrates in the function of natural killer cells. *Glycobiology* 1991; 1: 321–328.
84. Vannucci L *et al*. Effects of N-acetyl-glucosamine-coated glycodendrimers as biological modulators in the B16F10 melanoma model *in vivo*. *Int J Oncol* 2003; 23: 285–296.
85. Vannucci L *et al*. A new experimental model for colorectal carcinogenesis in the rat. *J Environ Pathol Toxicol Oncol* 1994; 13: 59–61.
86. Pospisil M *et al*. Cancer immunomodulation by carbohydrate ligands for the lymphocyte receptor NKR-P1. *Int J Oncol* 2000; 16: 267–276.
87. Roy R *et al*. Synthesis of N, N′-bis (acrylamido) acetic acid-based T-antigen glycodendrimers and their mouse monoclonal IgG antibody binding properties. *J Am Chem Soc* 2001; 123: 1809–1816.
88. Roy R, Kim JM. Cu(II)-self-assembling bipyridyl-glycoclusters and dendrimers bearing the Tn-antigen cancer marker: syntheses and lectin binding properties. *Tetrahedron* 2003; 59: 3881–3891.
89. Jenkins CL, Raines RT. Insights of the conformational stability of collagen. *Nat Prod Rep* 2002; 19: 49–59.
90. Stenzel KH *et al*. Collagen as a biomaterial. *Annu Rev Biophys Bioeng* 1974; 3: 231–254.
91. Miller M *et al*. Clinical observation on use of an extruded collagen sutures. *Arch Surg* 1964; 88: 167–174.
92. Browder IW, Litwin MS. Use of absorbable collagen for hemostatis in general surgical patients. *Am Surg* 1986; 52: 492–494.
93. Huynh T *et al*. Remolding of an acellular collagen graft into a physiologically responsive neovessel. *Nat Biotechnol* 1999; 17: 1083–1086.
94. Ellingsworth LR *et al*. The human immune response to reconstituted bovine collagen. *J Immunol* 1986; 136: 877–882.
95. Kinberger GA *et al*. Collagen mimetic dendrimers. *J Am Chem Soc* 2002; 124: 15162–15163.
96. Ottaviani MF *et al*. Internal structure of silver-poly(amidoamine) dendrimer complexes and nanocomposites. *Macromolecules* 2002; 35: 5105–5115.
97. Zhao M *et al*. Preparation of nanoclusters within dendrimer templates. *J Am Chem Soc* 1998; 120: 4877–4878.
98. Ouzzine M *et al*. Expression of active, human lysyl oxidase in *Escherichia coli*. *FEBS Lett* 1996; 399: 215–219.
99. Filmon R *et al*. Poly(2-hydroxy ethyl methacrylated)-alkaline phosphatase: a composite biomaterial allowing in vitro studies of bisphosphonates on the mineralization process. *J Biomater Sci Polym Ed* 2000; 11: 849–868.
100. Arul V *et al*. Biotinylated GHK peptide incorporated collagenous matrix: a novel biomaterial for dermal wound healing in rats. *J Biomed Mater Res B Appl Biomater* 2005; 73: 383–391.
101. Karakurum G *et al*. Comparative effect of intra-articular administration of hyaluronan and/or cortisone with evaluation of malondialdehyde on degenerative osteoarthritis of the rabbit's knee. *Tohoku J Exp Med* 2003; 199: 127–134.
102. Tropiano P *et al*. Lumbar total disc replacement. *J Bone Joint Surg Am* 2006; 88: 50–64.
103. Tayrose GA *et al*. Costal cartilage autografts to simulated degenerative intervertebral discs in the rat. *Spine* 2006; 3: E863–E866.

104. Zhang Y *et al*. Transduced bovine articular chondrocytes affect the metabolism of cocultured nucleus pulposus cells in vitro: implications for chondrocyte transplantation into the intervertebral disc. *Spine* 2005; 30: 2601–2607.
105. Carnahan MA *et al*. Hybrid dendritic-linear polyester-ethers for in situ photopolymerization. *J Am Chem Soc* 2002; 124: 5291–5293.
106. Wells AP *et al*. Cystic bleb formation and related complications in limbus versus fornix based conjunctival flaps in paediatric and young adult trabeculectomy with mitomycin-C. *Ophthalmology* 2003; 110: 2192–2197.
107. Tekade RK *et al*. Exploring dendrimer towards dual drug delivery: pH responsive simultaneous drug-release kinetics. *J Microencapsul* 2008, 15 Sept (Epub ahead of print). (DOI 10.1080/02652040802312572).
108. Gajbhiye V *et al*. PEGylated PPI dendritic architectures for sustained delivery of H_2 receptor antagonist. *Eur J Med Chem* 2009; 44: 1155–1166.
109. Tekade RK *et al*. Surface engineered dendrimers for dual drug delivery: A receptor up-regulation and enhanced cancer targeting strategy. *J Drug Targ* 2008; 16: 758–772.
110. Gajbhiye V *et al*. Novel PEGylated PPI dendritic nanostructures for sustained delivery of anti-inflammatory agent. *Curr Nano* 2008; 4: 267–277.
111. Ackroyd R *et al*. The history of photodetection and photodynamic therapy. *Photochem Photobiol* 2001; 74: 656–669.
112. Qiang Y *et al*. Medical progress. Photodynamic therapy for malignant and non-malignant diseases: clinical investigation and application. *Chin Med J* 2006; 119: 845–857.
113. Nishiyama N *et al*. Light-harvesting ionic dendrimer porphyrins as new photosensitizers for photodynamic therapy. *Bioconjug Chem* 2003; 14: 58–66.
114. Jevprasesphant R *et al*. Engineering of dendrimer surfaces to enhance transepithelial transport and reduce cytotoxicity. *Pharm Res* 2003; 20: 1543–1550.
115. Malik N *et al*. Dendrimers: relationship between structure and biocompatibility *in vitro*, and preliminary studies on the biodistribution of ^{125}I-labelled polyamidoamine dendrimers *in vivo*. *J Control Release* 2000; 65: 133–148.
116. Roberts JC *et al*. Preliminary biological evaluation of polyamidoamine (PAMAM) Starburst dendrimers. *J Biomed Mater Res* 1996; 30: 53–65.
117. Zhang GD *et al*. Polyion complex micelles entrapping cationic dendrimer porphyrin: effective photosensitizer for photodynamic therapy of cancer. *J Control Release* 2003; 93: 141–150.
118. Dichtel WR *et al*. Singlet oxygen generation via two-photon excited FRET. *J Am Chem Soc* 2004; 126: 5380–5381.
119. Yamamoto K, Imaoka T. Dendrimer complexes based on fine-controlled metal assembling. *Bull Chem Soc Jpn* 2006; 79: 511–526.
120. Imaoka T *et al*. Metal assembly in novel dendrimers with porphyrin cores. *J Am Chem Soc* 2003; 125: 340–341.
121. Fujita E *et al*. Carbon dioxide activation by cobalt (I) macrocycles: factors affecting carbon dioxide and carbon monoxide binding. *J Am Chem Soc* 1991; 113: 343–353.
122. Behar D *et al*. Cobalt porphyrin catalyzed reduction of CO_2 radiation, chemical, photochemical, and electrochemical studies. *J Phys Chem* 1998; 102: 2870–2877.
123. Imaoka T *et al*. Probing stepwise complexation in phenylazomethine dendrimers by a metallo-porphyrin core. *J Am Chem Soc* 2005; 127: 13896–13905.
124. Tam JP. Synthetic peptide vaccine design, synthesis and properties of a high density multiple antigenic peptide system. *Proc Natl Acad Sci USA* 1988; 85: 5409–5413.
125. Ota S *et al*. Cellular processing of a multibranched lysine core with tumor antigen peptides and presentation of peptide epitopes recognized by cytotoxic T lymphocytes on antigen-presenting cells. *Cancer Res* 2002; 62: 1471–1476.
126. Bay S *et al*. Preparation of a multiple antigen glycopeptide (MAG) carrying the Tn antigen: a possible approach to a synthetic carbohydrate vaccine. *J Pept Res* 1997; 49: 620–625.
127. Monika W, Rainer H. A convergent approach to biocompatible polyglycerol click dendrons for the synthesis of modular core-shell architectures and their transport behavior. *Chemistry* 2008; 14: 9202–9214.
128. Hua C *et al*. Synthesis and characterization of linear-dendron-like poly(ε-caprolactone)-*b*-poly(ethylene oxide) copolymers via the combination of ring-opening polymerization and click chemistry. *Macromolecules* 2008; 41: 6686–6695.
129. Tomalia DA, Majoros IJ. Biocompatible dendrimers. US Patent 2006/7078461, 2006.
130. Haba Y *et al*. Synthesis of biocompatible dendrimers with a peripheral network formed by linking of polymerizable groups. *Polymer* 2005; 46: 1813–1820.