Expert Opinion

Dendritic systems in drug delivery applications

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The design of well-defined particulate carrier systems with controlled size, shapes and physicochemical characteristics is becoming a focal point in the field of biomedicine and drug delivery. Dendrimers are one of the emerging technologies of recent times and have served as a unique platform to achieve the development as novel drug delivery scaffolds. Dendrimers may be engineered to meet the specific needs of biologically active agents, which can either be encapsulated within dendrimers or chemically attached to these units. The large number of active functional groups on the surface of dendrimers allows them to be meticulously tailored and to act as nano-scaffolds or nano-containers of various categories of drugs. The architecture of modified dendrimers has posed a challenge to drug delivery, in particular with respect to their *in vivo* metabolic fate. The drug delivery applications of dendrimers presented in this article provide an insight of their potential and substantiate the major roles for the future of these nanoconstructs.

Keywords: biocompatibility, biopharmaceutical issues, covalent interaction, dendrimers, drug delivery, encapsulation, nanoconstructs, routes of administration

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1. Introduction

The classical concepts in drug delivery have undergone significant changes in the last half a century. Drug targeting is a clear example of where there has been an improvement in the understanding of biopharmaceutics, the optimisation of drug delivery (maximum therapeutic benefit and no or minimal toxicity), patient compliance and in the development stringent regulatory issues.

1.1 Novel systems in drug delivery

Novel approaches to delivering a drug to a target site can be achieved through the development of satisfactory methods to deploy medications intact to specifically targeted parts of the body in a manner that can control the therapy by means of either a physiological or chemical trigger. Molecular constructs used as drug carriers should be preferably in the nanometeric size range. In addition, uniformity in their size and other characteristics, such as hydrophilicity and macromolecularity, can overcome the risk of faster clearance from liver, spleen or glomerular filtration. Precise control of the molecular structure by synthesising nano-scaffolds and nano-macromolecular grafts can improve access to the target site. The majority of particles that fulfill these requirements are amphiphilic molecules with a tendency to assemble rapidly in aqueous phase to form micelles above a specific concentration (critical micelle concentration). The interior hydrophobic core formed in such arrangements of monomer units has been exploited as a carrier of hydrophobic drugs. These highly dynamic micelles can rearrange themselves, with minor modifications in solution, on exposure to external energy such as agitation, sonication, or extrusion through a filter membrane, to form lipid bilayers.

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The design and development of vesicular carriers, such as liposomes, which encapsulate an aqueous chamber between dual lipid layers, are capable of carrying hydrophilic biologically active agents. Unfortunately, highly dynamic micelles depend not only on their monomer's characteristics, but also on solution conditions, such as concentration, ionic strength, pH and temperature. This may not be favourable to maintain the intactness of the carrier in the diverse environment of a biological system. Liposomes meet the majority of the requirements of an ideal carrier, but are essentially considered energetically metastable, and are capable of rearranging to form planar bilayers [1,2]. Various new approaches have been adopted to overcome this important biostability issue, and consist of systems such as water-in-oil-in-water double emulsions, monodisperse systems such as nanospheres and microspheres, and nanocapsules.

1.2 Drug delivery nanoartifacts

Nanomedicine has emerged as a promising concept for monitoring, repairing, constructing and controlling human biological systems at the cellular level. The recent developments in nanotechnological tools in drug delivery have potentiated the strength of nanomedicine. At present, nanotechnology research has focused on two major types of complementary systems: nanoscale architectures or nanoparticles, and nanoporous systems. Engineering novel nanoscale drug carriers could be a realistic approach, as the biological processes in a living biological system are precisely dependent on molecular machines and clearly defined structures.

The particle size and surface characteristics (charge or hydrophobicity) determine the distribution and fate of polymeric nanoparticles [3]. Particles smaller than 200 nm are not easily cleared by the reticuloendothelial system, but large particles (> 200 nm) can be removed in this way [4]. This sequestration acts as a barrier to targeting cells or tissues in the body and limits the application of many particulate carriers [5]. The most recent work in nanomedicine or nanopharmceuticals comprises nanoscale systems in the size range of 5 – 500 nm [6]. The submicron size range of nanosystems offers excellent opportunities to cross physiological barriers and access different tissues, followed by efficient cellular uptake. Moreover, these nanosystems have the potential to effectively carry and target drugs by virtue of their colloidal size range and ability to conjugate site-specific ligands. One such example is a marketed preparation, Abraxane® (Abraxis BioScience), which incorporates the anticancer drug paclitaxel within albumin nanoparticles. Various nanosystems, such as polymeric nanoparticles, liposomes, carbon nanotubes, quantum dots and dendrimers, have been successfully functionalised for efficient biomedical use [7]. A few other examples include nanoemulsions, nanocapsules and nanocrystals [6]. Nanocages, nanogels, nanofibres, nanoshells, nanorods, for example, are some of the more recent nanosystems. The precise and accurate evaluation of processes, such as cellular internalisation, presents a major hurdle for these systems, which remain hidden to the human eye. Only a few years ago, physicists believed that it was impossible to resolve details closer than 200 nm – the limit imposed by Abbe's Law, whereby the resolution of a light microscope cannot be more accurate than half of the wavelength of light entering the microscope. However, this was achieved recently with a resolution of up to 15 nm using a Stimulated Emission Depletion (STED) microscope [8]. Numerous reports on nanoscale biodegradable polymeric constructs for protein and drug conjugates, including dendrimer and dendronisation technology, are being published on novel concepts that have emerged as a platform for next-generation therapy [9]. In addition to being nano-sized, having a multifunctional nature, being conjugated to a targeting ligand and a having a macromolecular character are among the critical features in the majority of these carriers.

The present article critically examines various issues and approaches concerning the potential of dendrimers for drug delivery applications.

2. Why dendrimers?

In last two decades, a polymeric macromolecular system (dendrimers) has gained popularity due to its stability and other desirable features, such as monodispersity, size, well-defined molecular structure, multifunctionality.

Dendrimers were first described in the early 1980s [10] as macromolecular compounds with branches around an inner core [11]. The attractiveness of dendrimers in the field of drug delivery is their nanometre size range, hollow interior channels, and their ability to display multiple copies of surface groups for biological reorganisation processes [12]. Dendrimer molecules are constructs around a small molecule, or core, using connectors and branching units. These are made up of AB_n-type monomers, and the number of peripheral functional groups doubles or triples with each layer or generation of branching units. The final architecture of dendrimers is globular, with hollow internal cavities and a number of terminal groups. This makes the action of these dendritic units more versatility, allowing for drugs to be encapsulated or attached to the surface or peripheral groups [12-14]. The high toxicity of anticancer drug 5-flurouracil (5-FU) has been shown to be reduced after its acylation, to form a polyamidoamine (PAMAM) dendrimer–FU conjugate that, upon hydrolysis, releases free FU for a prolonged duration of action [15]. The terminal groups of dendrimers are primarily responsible for interacting with the molecular environment. The modification of these terminal functionalities adds to the versatility of the dendritic unit as a drug carrier.

Due to their versatile make, with active chemical moieties and a high degree of branching, these ubiquitous dendritic nano-constructs offer unique functional and interfacial opportunities. Some of the most preferred classes among dendrimers that have been proven as better drug delivery

options are PAMAM dendrimers, poly-L-lysine dendrimers, melamine-based dendrimers and poly(propylenimine) (PPI) dendrimers.

2.1 The biocompatibility issue

The biocompatibility of any system is an essential issue when considering it as a drug carrier. As seen for many other cationic systems, including liposomes and micelles, dendrimers with surface groups bearing a cationic charge can readily damage cell membranes, leading to cell lysis. Hence, the issue of non-toxicity is a prerequisite for dendrimers, as for any other prospective drug delivery system, before considering their *in vivo* or advanced use. Dendrimers with free amine groups at their periphery are found to be inherently cationic in nature [16]. In a study by Duncan and co-workers, the effect of dendrimer generation (size), charge and concentration on tissue uptake and serosal transport was studied to establish the potential of such systems in oral delivery. Anionic PAMAM dendrimers, G2.5 and G3.5, displayed rapid serosal transfer rates and lower tissue deposition in contrast to amine-terminated dendrimers. The rates of uptake and transfer were constant with time, without any saturation over the concentration range used. However, an effect of size or conformation was observed with higher-generation (G5.5) dendrimers, displaying greater tissue accumulation [17]. In another study, the *in vitro* and *in vivo* toxicity, along with immunogenicity and biodistribution, of third-, fifth-, and seventh-generation dendrimers indicated 'biological complications' with the seventh generation [18]. This toxicity is directly proportional to the concentration, nature of the functional group (anionic or cationic) and generation number [19,20]. The biocompatibility of PAMAM dendrimers with sodium carboxylate on its surface in one study showed a profound effect of anionic surface functionality [16]. The system has also been evaluated as a carrier of anticancer platinates after intravenous administration [21]. The IC_{50} values for amine-terminated PAMAM dendrimers exhibited cytotoxicity towards human intestinal adenocarcinoma Caco-2 cells [19,22]. The observed difference in the toxicity profile of PAMAM dendrimer compared with relatively flexible linear polymers is possibly due to more rigid and globular conformations of PAMAM dendrimer [20]. The similarity in cytotoxicity and haemolytic effects of PPI and PAMAM dendrimers supports this hypothesis, as both systems posses a rigid and globular conformation that changes with generation number [16,23].

In this regard, it has been observed that the degree of substitution and the type of functionality can make a profound difference in developing a more biocompatible system [20]. Attempts at reducing toxicity have paved the way to methods of masking terminal amine groups or substituting them with other groups, such as hydroxyl [14]. Studies of the toxicity profile of carboxyl-terminated surface functional groups are an example of investigations in this direction [19]. Surface derivatisation is another method of reducing toxicity, and using the same larger hydrophilic moieties, such as PEGs or fatty acids, have contributed to reducing the toxic effects of dendrimers. Studies indicate that an overall reduction of cationic charge of these partially derivatised dendrimers can significantly enhance the biocompatibility of these systems [22,24]. A few areas that still require attention are the metabolic fate of dendrimers in biological systems, and their stability under the varying pH conditions of the gastrointestinal tract, along with actions of different enzymes.

2.2 The biopharmaceutical issue: pharmacokinetics and biodistribution

As the dendrimer is a versatile chemical entity and has a polymeric nanoscale size, it is essential to consider its biopharmaceutical characteristics to confirm its proficiency as a drug carrier. The issues concerning this particular aspect are the effect of charge, terminal functional groups, solubility, generation number, molecular weight and size on the distribution and circulation pattern of dendrimers. Although there have been few studies, the investigations by Duncan and co-workers on PAMAM dendrimers revealed that cationic dendrimers are cleared rapidly from the circulation and accumulate in the liver [16]. Anionic dendrimers did circulate for a longer duration; however, their accumulation in liver was also significant. The investigation of the biological properties of PAMAM dendrimers of third, fifth and seventh generations displayed quite unusual organ distribution profiles, where the third generation was distributed preferentially in kidney, but the fifth- and seventh-generation dendrimers accumulated more in the pancreas [18]. This study also suggested the fast clearance of the dendrimers from the circulation. In a biopharmaceutical evaluation of flurbiprofen formulated with PAMAM dendrimer, the mean residence time and terminal half-life of the dendritic formulation were found to be twofold and threefold higher, coupled with a fivefold greater distribution, compared with free drug [25].

From previous studies, it can be seen that that the surface charge of a dendrimer can determine its biodistribution profile. However, dendrimer composition also plays a major role. An example is provided by a study conducted with neutral/cationic dendrimers and a neutral/cationic dendrimer–gold nanocomposite. The neutral gold nanocomposite showed rapid clearance from all organs except the liver (18.4, 21.7 and 30.0% injected dose (ID)/g organ at 1 h, 1 day and 4 days, respectively) and the spleen (15.9, 18.0, 22.5% ID/g organ at 1 h, 1 day and 4 days, respectively). In contrast, positively charged amine-terminated nanocomposites of dendrimer displayed a lower accumulation in all organs, except kidney (10.9% ID/g after 1 h; 25% ID/g after day 1). However, a contrastingly significant difference was observed with the biodistribution profile of neutral or cationic plain dendrimers versus neutral or cationic dendrimer–gold nanocomposites, with plain neutral dendrimers showing no organ-specific accumulation compared with neutral gold nanocomposites [26]. This study is interesting as it shows that the behavioural

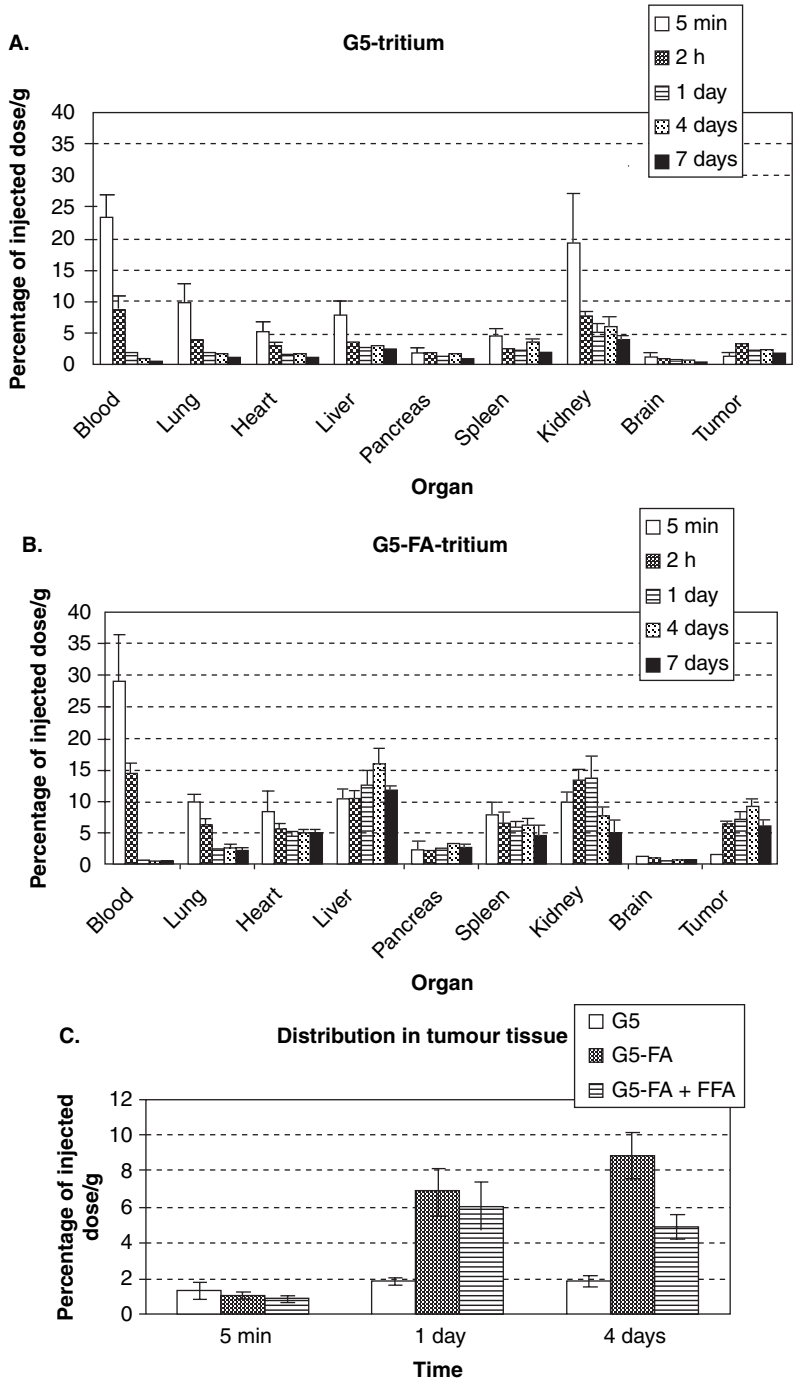

Figure 1. The biodistribution of radiolabelled nontargeted (A) and targeted (B) conjugate in nu/nu mice bearing KB xenograft tumour, depicted as a percentage of the injected dose of dendrimer recovered per gram of organ. The distribution in tumour tissue expressed as a percentage of injected dose with time is shown in C.

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FFA: Free fatty acids.

changes in dendrimers within biological systems depend on their surface charge, as well as their composition. Hence, before speculating about a novel dendritic platform design, it is essential to characterise its *in vivo* parameters.

In another study, a comparative biodistribution profile for radiolabelled targeted and non-targeted G5 PAMAM dendritic conjugates in nu/nu mice bearing KB xenograft tumour was investigated in different organs after 5 min, 2 h,

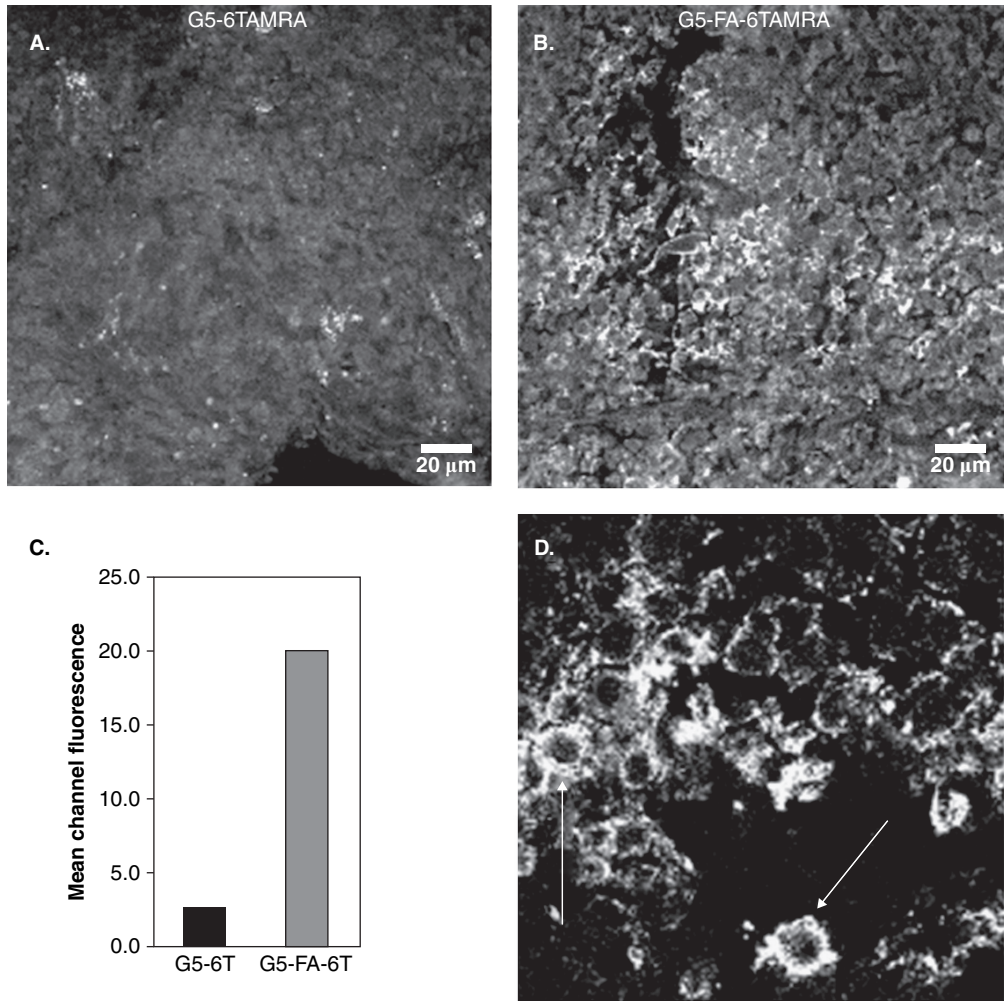

Figure 2. Confocal microscopy analysis of cryosectioned tumour samples from SCID mice that were injected with 10 nmol of either nontargeted G5-6-TAMRA (A) or targeted G5-FA-6-TAMRA conjugate 15 h (B) or 4 days (D) before tumour isolation. Flow cytometry analysis performed on the isolated cells from the same tumour (**B**) concur that tumour cells specifically uptake G5-FA-6-TAMRA compared with G5-6-TAMRA (**C**). The enlargement shows the cells in the medial optical section with significant fluorescence of 6-TAMRA attached to the targeted conjugate (**D**). The arrows in D indicate 6-TAMARA conjugated systems attached to cells.
Reproduced with permission from [27].
SCID: Severe combined immunodeficiency.

1 day, 4 days and 7 days post-administration (**Figure 1**). The inhibition of distribution of targeted conjugate in tumour after injection of mice with 181 nmol free folic acid was observed and compared with the distribution of non-targeted and targeted conjugate at 5 min, 1 day and 4 days. Inhibition was significantly lower on the fourth day (at least $p < 0.05$) [27]. Confocal microscopy and flow cytometry (**Figure 2**) confirmed the internalisation of the drug conjugate within the tumour cells. Any attempt to enhance the drug's concentration at the desired site can profoundly enhance its therapeutic efficacy. Moreover, the study displayed 10-fold higher efficacy and longer survival of the treated animals with the targeted nanoconstruct over free drug [27]. Also, the high cytosolic concentration can overcome multi-drug resistance due to membrane P-glycoprotein in resistant tumours [28]. These findings establish such nanoconstructs as third-order targeting utilities and provide future opportunities for the delivery of genetic materials.

2.3 Dendritic designs for various routes of drug delivery

The drug delivery options offered with various drug delivery designs are diverse. In order to achieve specific objectives in terms of particular disease states or to establish realistic clinical goals, routes and frequencies of administration must be considerations. For example, the intravenous route has been, in general, used in various laboratory practices in initial *in vivo* studies, especially with anticancer drug delivery systems; however, an enhanced frequency of administration during studies and clinical trials is a hindrance. An intratumoural

injection is also not a realistic approach in terms of future clinical practices. The literature describes the widespread application of dendrimer-based systems for different routes of administration, including intravenous targeted anticancer chemotherapy [21], topical [29], oral [30-32], transdermal [33,34] and ocular [35] delivery.

3. Organisation and development of dendritic structures for drug delivery

Dendrimers are, in general, synthesised using either i) a divergent [10,36], or ii) convergent approach [37,38]. In the divergent approach, the synthesis starts at the initiator core and proceeds outwards, towards the higher generations with each addition of repeating monomer units. The convergent method is exactly opposite to that of the divergent approach of dendrimer synthesis.

3.1 Components of the dendritic architecture

Dendrimers consists of three architectural components: a core branch cell, interior branching (or shells) and surface groups (nanoscaffolds). Dendrimer synthesis initiates from a chemical moiety referred to as the initiator core, bearing at least two functional reactive groups. In the first step of synthesis, monomer molecules are conjugated to each of the functional groups of the initiator core. The monomers are conjugated repeatedly, to form repeating monomer units. Thus, the addition of first set of monomers forms a layer of repeating monomers around the initiator core; this is the dendrimer generation. Each functional group, which appears at the periphery of the generation is called the branching point, onto which a second set of repeating monomers are conjugated to produce the second generation. The same reaction sequence is repeated an 'n' number of times to produce the 'nth' generation. Conventionally, the nth generation is represented as Gn. In some dendrimer families, such as PAMAM and PPI, the terms half-generation and full-generation are used. For half-generation dendrimers, the addition of the first set of monomers to the initiator core will produce a half-generation (G0.5), which subsequently yields the first full generation (G1). The addition of a second set of monomers will produce G1.5, which on further treatment will result in a second generation (G2). G3 dendrimers are smaller compared to higher generations like G5 and above and posses open asymmetric structures. A molecular modeling study revealed that the smaller G3 dendrimer maintains a rather open, spread out and asymmetric structure. As the generation number and consequently size of the molecule increases, the structure becomes more dense and spherical [39].

The molecular weight of the dendrimer and the number of peripheral groups increases exponentially with each generation, and the diameter increases more or less linearly. These facts explain the unique physical structures of dendrimers. With each additional generation, the surface density of peripheral moieties enhances. This steric crowding readjusts the conformation of dendrimers developing in a three-dimensional structure (e.g., PAMAM dendrimer; **Table 1**).

The hallmark of dendrimer chemistry is the ability to synthesise very high molecular weight polymers with narrow molecular weight distributions in a controlled manner. Although the majority of dendrimers reported so far have organic backbones, a variety of dendrimers containing heteroatoms (Si, P, B, Ge, Bi) have been synthesised as well [40-42]. In most of these cases, the heteroatoms are intercalated into the organic skeleton. The only dendrimers with an organic framework reported so far are first- and second-generation silicon derivatives. At the same time, a large variety of 'classical' inorganic polymers are known, and the most widely used ones amongst these include polyphosphazenes, polysiloxanes and polysilanes.

PAMAM dendrimers have been used as platforms for drug transport. Additional functionality, such as targeting and imaging groups needed for selective drug delivery are covalently attached to the particles. The covalently linked clusters of dendrimers, known as tectodendrimers, consist of a dendrimer core surrounded by a lower-generation dendrimer that is covalently linked to the central dendrimer. Tectodendrimer architecture has the ability to make each individual dendrimer perform a separate function. They are composed of a dendritic core, which may or may not contain the therapeutic agent, surrounded by dendrimers. The surrounding dendrimers are of several types, each type designed to perform functions necessary to make it a smart therapeutic nanodevice. These 'smart' therapeutic units bear components that perform many functions such as the recognition of a diseased cell, the diagnosis of a disease state, drug delivery and reporting location in the biosystem [43].

With more advanced approaches, new components are incorporated onto a single G5 PAMAM dendritic molecule, which are also partially acetylated to enhance water solubility. Fluorescein is incorporated onto the dendrimer for the purpose of imaging the molecular therapeutic *in vivo*. Folic acid, followed by active moieties such as taxol and methotrexate (MTX), are conjugated to the dendritic peripheral groups. Atomic force microscopy has been used to characterise the behaviour of this dendrimer complex in biological systems [44]. The modification of dendrimers with PEG chains alters both the biocompatibility (and lack of antigenicity) and toxicity [45].

The modification of the readily available DAN-dendrimer-$(NH_2)_x$ dendrimer series (x = 4, 8, 16, 32, 64) with mono- and diasaccharides, as well as with a cluster trisgalactoside using amide bond formation, has been reported. These high molecular weight compounds were considered as 'neoglycoconjugates', with defined structures that may serve as a platform for multivalent ligands involved in carbohydrate–protein interactions [46].

Besides the covalent functionalisation of dendrimers, a new approach towards non-covalent functionalisation of the dendrimer periphery has been designed, known as the 'clicking' concept, where a fifth-generation urea adamantyl dendrimer host can form a complex with 32 guest molecules by electrostatic and hydrogen bonding interactions. This new approach of non-covalent functionalisation of the dendrimer periphery

Table 1. The effect on the conformational make up of PAMAM dendrimers of doubling the generation number.

Generation	Number of peripheral groups	Diameter	Conformation	Surface density	Comment
G1	8	–	Open, floppy	–	Non-spheroidal; no diametric measurement
G2	16	–	Open, flat	–	–
G4	64	4.5 nm	Spheroidal	1.0 nm^2	The accommodation of 64 peripheral groups is only possible if the 4.5 nm diameter becomes spheroidal
G8	1024	9 nm	Spheroidal	3.5 nm^2	The diameter is only doubled (i.e., the average spacing between terminal groups is reduced with increasing generation)

is ideal for the formation of multivalent bioconjugates. Biologically interesting molecules such as amino acids, DNA bases or sugar derivatives can be assembled at the dendrimer periphery in this way. As it is a dynamic system, it is possible to assemble mixtures of molecules with different properties onto the dendrimer. Sugar derivatives such as *N*-acetyl-galactosamine-terminated glycoproteins (specifically recognised by the ASGP-R receptor localised on liver cells) play an important role in molecular recognition inside the human body. Sugars can be clicked in as a targeting device for drug delivery [47,48].

3.2 Dendrimers or their constructs as carriers for the delivery of biologically active molecules

The host–guest-molecule interactions can occur within the internal cavities of the dendrimer core (endoreceptors) or at the multivalent periphery (exoreceptors). Hence, dendrimer-based drug delivery can be achieved by coupling a drug to the system through one of two approaches (**Table 2**). Hydrophobic drugs can be complexed within the hydrophobic dendrimer interior to make them water soluble, or drugs can be covalently coupled onto the surface of the dendrimer.

3.2.1 Non-covalent interactions

It is important for the drug or any biologically active molecule to be in free form when being delivered by the carrier to the site of action, in order for it to realise its full therapeutic value and, hence, it is always preferable to avoid any covalent attachment with the carrier. The non-covalent encapsulation of drug molecules is initiated by using dendrimers as unimolecular micelles and 'dendritic boxes'.

Dendrimers acting as hosts, using various non-covalent interactions, have been reported [49]; the mechanisms used are detailed below:

- Hydrophobic interactions, exemplified by π–π interactions between electron-rich aryl ether and the aromatic guest molecule. The enhanced ability to entrap the electron-deficient aromatic guest molecules is an interesting issue.
- Polar interactions leading to pH-based host–guest complex formation via acid–base interactions and hydrogen bonding between tertiary amines and acidic substrates is a useful concept used by many scientific groups to define the role of dendrimers in drug delivery.
- Dendritic scaffolds are highly defined structures of dendrimers, which can be used as artificial hosts by molecular imprinting inside a polymeric network of dendrimers [50].

An example in this area is from gene delivery applications, where DNA is complexed with PAMAM dendrimers [51]. In addition, hydrophobic drugs and dye molecules have been incorporated into various dendrimer cores [52-54]. An advantage of using dendritic unimolecular micelles rather than conventional polymeric micelles is that the micellar structure is maintained at all concentrations because the hydrophobic segments are covalently connected. However, this approach suffers from a general drawback in that it is difficult to control the release of molecules from the dendrimer core. In some cases, harsh conditions are required [53], whereas in others the encapsulated drug is not well retained and the molecules are rapidly released [55,56].

Research into stabilising PEG or polyethylene oxide (PEO) chains on the dendrimer periphery has brought favourable results. PEG-linked systems have been used to incorporate anti-cancer drugs such as 5-FU [45], MTX and adriamycin [56] in an attempt to retard the drug release rates in these systems. A promising new approach to control the release of drugs from the encapsulating micellar compartment involves the use of hybrids of PEO and dendrimers with pH-sensitive hydrophobic acetal groups on the dendrimer periphery [57]. Loss of the hydrophobic groups upon acetal hydrolysis at mildly acidic pH triggers the disruption of the micelle and releases the payload [58].

Hydrophobic drugs research has further explored the feasibility of using water-soluble dendrimers for drug delivery by developing surface-modified dendritic units. An important study in this area was the synthesis of neutral dendrimers with the inclusion of a small acidic hydrophobic agent, benzoic acid. The synthesis of water-soluble dendrimers was achieved by

Table 2. Dendrimers in drug delivery.

Dendrimer	Drug	Ref.
Non-covalent encapsulation		
PAMAM	Ibuprofen	[64]
PEGylated PAMAM	Methotrexate	[56]
PAMAM	Ibuprofen	[70]
PAMAM	Indometacin	[33]
PAMAM-OH	Benzoic acid 3-Amino 1,5-dibromophenol Iodine Salicylic acid 2,6-dibromo 4-nitrophenol	[14]
PEG polyether dendrimers	Indometacin	[55]
Polyglycerol dendrimer	Paclitaxel	[30]
PEGylated PAMAM dendrimer	5-Fluorouracil	[45]
PAMAM	Flurbiprofen	[25]
PAMAM	Doxorubicin HCl	[63]
Covalent conjugation		
PAMAM	5-Fluorouracil	[69]
PAMAM-OH	Ibuprofen	[99]
Multifunctional PAMAM	Methotrexate	[74,75]
Polyester dendrimer	Doxorubicin	[94]
PAMAM	Propranolol	[31]
Multifunctional PAMAM	Paclitaxel	[86]

PAMAM: Polyamidoamine.

treating PAMAM G1.5 and G2.5 dendrimers with tris(hydroxymethyl) amino methane. The acidic guest molecules were bound within these neutral dendrimers, and no other small non-polar molecules (e.g., tioconazole) could be retained within the dendrimer [14]. The drug–dendrimer complexes were found to be unstable at pH < 7, and the bound substrate started precipitating after only 10 min at pH 6. This suggested that nitrogens within the dendrimer might be behind the binding of guest molecules. Tertiary nitrogens become protonated near pH 6, and the dendrimer loses its ability to bind and retain the acidic guest. Therefore, binding is a consequence of hydrophobic interactions plus ion pairing. On addition of phosphate-buffered water (pH 7), a complex of infinite water solubility was formed. These complexes where shown to remain stable at neutral pH, even after storage for several weeks. In summary, this study reported that only acidic guest molecules bind within the interior of the dendrimers and the internal tertiary amines of PAMAM dendrimers are available for acid–base interactions and hydrogen bonding (**Figure 3**), as well as for other non-covalent interactions with encapsulated guest molecules. This makes these polymers effective agents for solubilising hydrophobic drugs.

PEGylated dendritic systems have been reported to encapsulate up to 6.5 adriamycin molecules and up to 26 MTX molecules. Thus, the methoxy polyethylene glycol (M-PEG)-attached dendrimers displayed the encapsulation of greater amounts of acidic drugs (e.g., MTX) than adriamycin. Furthermore, the affinity of these drugs with dendrimers was observed to be weak, as they released rapidly in isotonic solution. However, the effect of generation and surface functionality can have a profound impact on the characteristics of encapsulation and release of a biologically active molecule [56]. With another hydrophobic drug, nifedipine, lower generation (< G3) PAMAM dendrimers were shown to have significantly increased solubility [59]. Solubility was further enhanced in the presence of half-generation, ester-terminated dendrimers at pH 7, as the tertiary amines of these half-generation dendrimers are less susceptible to protonation compared with full-generation dendrimers [60]. Hence, the size, generation type and pH dependence of dendrimers have a major impact on the solubility of drugs in aqueous PAMAM dendrimer solution. The protonation of the tertiary amines at pH 4 in full-generation dendrimers enhances the polarity and, thus, increases the bulk of water molecules inside the dendritic cavity, thereby reducing the chances of accomodating poorly soluble drugs. The nanoenvironment in dendritic cavities is considerably less polar than that in the bulk aqueous phase [61]. Thus, poorly soluble biologically active molecules in a aqueous medium can become solubilised only in those low-polarity channels or at an essentially deprotonated state. In addition, the tertiary amines in the internal cavities of the PAMAM dendrimers act as hydrogen bond donors [62]. One study reported higher loading of doxorubicin in the dendrimer (at a drug:dendrimer molar ratio of 3:1) with 97% incorporation in TES (*N*-tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid) buffer at pH 7.5, compared with 68.9% in acetate buffer (pH 4.5) [63]. The greater hydrophobic interaction between PAMAM and doxorubicin at pH 7.5 compared with the lower pH is an interesting feature to note; however, in contrast to earlier studies [56], the release rate of doxorubicin in this study was slow, and only < 10% release was observed at 37°C in TES and acetate buffer, which increased to 11 and 13.9%, respectively, at 25°C for a drug:dendrimer 3:1 molar ratio in 48 h. A few other examples involve electrostatic interaction between the dendrimer and ibuprofen [64] or flurbiprofen [25], using the terminal free amines and tertiary nitrogen of the dendrimer to interact with the carbonyl moiety of these drugs, and display pH-dependent complex formation. It is been further stated that the pH-dependent conformations of dendrimers may contribute to interference with complex formation, as these conformational changes decrease with lower generation [62].

3.2.2 Covalent interactions or coupling

Dendritic systems are perfect candidates for delivery because their low polydispersity can assure the reproducibility of pharmacokinetic behaviour, and the presence of modifiable

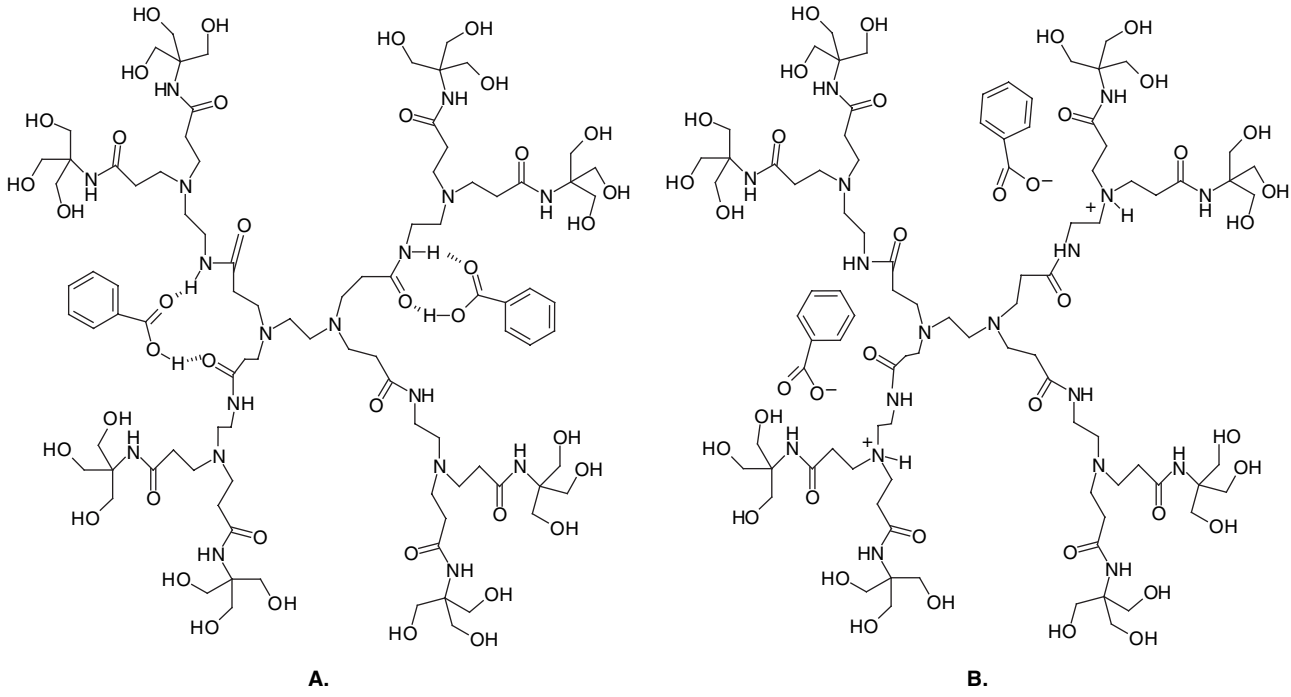

Figure 3. A representation of the hydrogen bonding interaction between PAMAM dendrimer and the guest molecule (A); and the probable ion pairing interaction (B).

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PAMAM: Polyamidoamine.

terminal groups allows improved water solubility and the non-statistical attachment of drug molecules. The covalent scaffold also provides a stable structure for the internal encapsulation of a drug, which is not based upon thermodynamics and physical factors [65]. Furthermore, a poly(arylether) dendrimer has been designed with two different surface functionalities that are able to covalently bind model drugs and solubilising groups. PEG was chosen as the solubilising group as a result of its high water solubility and biocompatibility. Different kinds of hydrolytically labile linkages were investigated for drug conjugation, including carbonate, carbamate and ester linkages, and the drugs used were cholesterol and two amino acids: phenylalanine and tryptophan [66]. The stepwise synthesis and the controlled multivalency of dendrimers allow the chemical coupling of bioactive molecules to form nanoconstructs for drug delivery. The preparation of multivalent folic acid conjugates of dendrimers [67] has important implications in targeting tumour cells. The multivalent character of dendrimers form the platform for the attachment of diagnostic and therapeutic molecules, or combinations of these agents. The dendrimer generation and the degradable links for attachment of drugs to its periphery can be meticulously organised to control the loading capacity and release rates, respectively. A cisplatin–PAMAM dendrimer conjugate is one such example, which allows the targeting of the otherwise non-specific action of the drug, as well as overcoming its poor aqueous solubility, thereby enhancing the overall therapeutic efficacy of the conjugated active molecule compared with the free form [21,68].

In another study, the anticancer drug 5-FU was conjugated to the periphery of PAMAM dendrimers [69]. These dendrimers, which were based on a cyclic tetra-amine core, showed potential as a carrier for antitumour drugs, and the controlled release profile of this potent anticancer drug using a multivalent dendritic periphery represented a promising development. As discussed in the previous section, the PEG-linked PAMAM dendrimer was also used to incorporate 5-FU [45] (**Figure 4**). Similarly, high drug payloads up to 58 molecules of ibuprofen covalently conjugated to one molecule of G4 PAMAM-OH dendrimer has displayed appreciable *in vitro* and *in vivo* response [70]. The chemical moiety conjugated onto the dendritic periphery is similar to a new chemical molecule, with distinct properties from the parent molecule, especially in terms of the solubilisation behaviour of the conjugated bioactive. Both hydrophobic model drugs and hydrophilic PEO moieties have been attached to the dendrimer periphery in a controlled manner with the help of two different chain end functionalities. The aqueous solubility of polyaryl ether dendrimers increased with the help of PEO chains on the dendritic periphery [66].

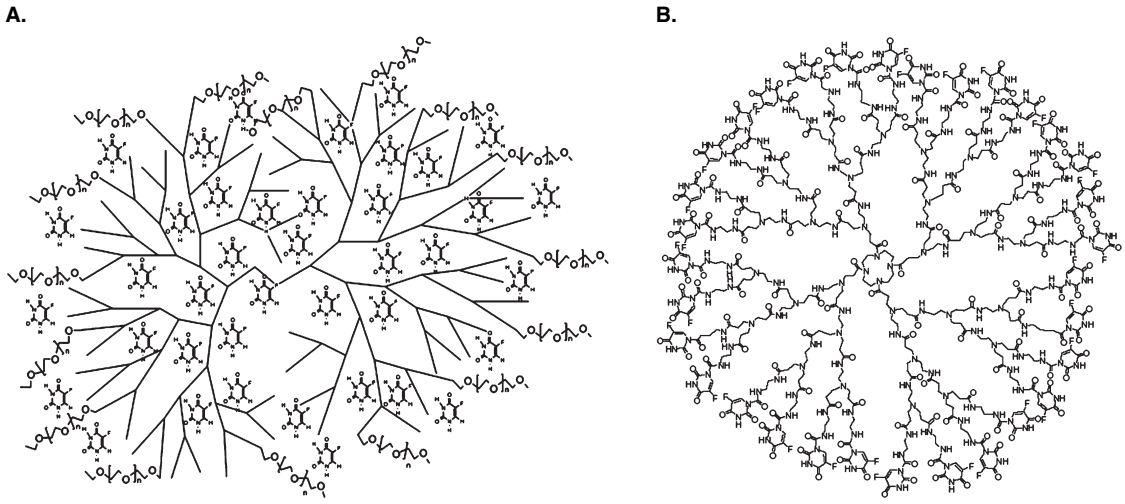

Figure 4. The molecular structures of dendritic polymers and drugs for rationalising their (A) encapsulation or (B) conjugation with the dendrimer.

Earlier it was observed that the potency of free MTX responsible for the induction of cytotoxicity relies on its polyglutamation, which leads to entrapment and accumulation of the drug in the cell [71]. The concepts of multivalency introduced by Whitesides and co-workers [72] opened a new pathway for the development of multivalent dendrimers conjugating folate and MTX moieties via amide and ester links [67,73,98]. The use of folate as a ligand, coupled with an antifolate drug (MTX), has emerged as an interesting feature of dendritic nanoconstructs (**Figure 5**) for cellular uptake and selective action against folate receptor-positive cell lines *in vitro*. In a separate study, the benefit of covalent conjugation of MTX to the dendrimer periphery (trifunctional G5 dendrimer conjugate of fluorescein isothiocyanate, folic acid and MTX) was indicated with respect to the conjugate's retention in the cell, which was found to be independent of polyglutamation, leading to the overcoming, to some extent, of the drug resistance caused by the free drug [28,74].

Another study indicated that MTX as a dendrimer inclusion complex was released rapidly compared with the covalent conjugate. Covalently coupled MTX dendrimer conjugates were stable under identical conditions in water and buffered saline [75]. Thus, the slow intracellular release of the anticancer drug can overcome the multiple drug resistance, coupled with the reduced potency, of the free drug.

3.2.3 *Nanoscale dendritic drug delivery systems*

Dendrimers (5 – 20 nm) have found a place as a unimolecular system or conjugate among the other greatly explored nanoscale carriers, such as polymeric micelles and liposomes (80 – 100 nm). As per recent reports, SPL7013 gel (VivaGel™; Starpharma) for HIV/AIDS, which uses topical dendritic drug delivery, is undergoing an expanded Phase I randomised placebo-controlled trial to assess safety and tolerability in healthy young women, with twice-daily administration of for 14 days [101]. An important study in this direction revealed the molecular size and molecular weight dependent extravasation of a series of PAMAM dendrimers across the microvascular network endothelium. The diffusion of hydrophilic units followed 'restricted diffusion', which follows the rule that as the size of the diffusion molecule increases, the exerted viscous drag on it, together with its degree of exclusion from the membrane pores, also increases. Hence, an increased extravasation time was observed with an increasing PAMAM dendrimer diameter from 14 – 15 Å, due to its enhanced exclusion from the endothelial pores. On the other hand, PEG, with a larger hydrodynamic volume than any of the PAMAM dendrimers, took a relatively longer duration compared with dendrimers for extravasation across the microvessel endothelium into intestinal tissue [76]. Dendriplexes, a compact complex formed due to the interaction of DNA with partial dendrimers (dendritic polylysine with seven lysine and eight terminal amino groups with or without a lipidic core), have been shown to be suitable, in terms of their physiochemical and biological properties as gene carriers and delivery units. The nanometeric size range (60 – 70 nm) and DNA protection from nuclease degradation offered by these dendrimer-based carriers suggests their potential for biological applications [77] (**Table 3**).

The delivery of potent biologically active agents, such as anticancer drugs, in general, faces two prevalent obstacles: a lack of specific localisation of the drug, and hydrophobicity related administration problems [78,79]. A drug's pharmacokinetic parameters leads to its distribution to both affected and unaffected areas. This can have serious implications in terms of drug toxicity. The second issue impedes the delivery of such drugs in large volumes of aqueous media, delivered concomitantly with co-solvents, or chemically modified prodrugs; all of these approaches can potentially reduce the

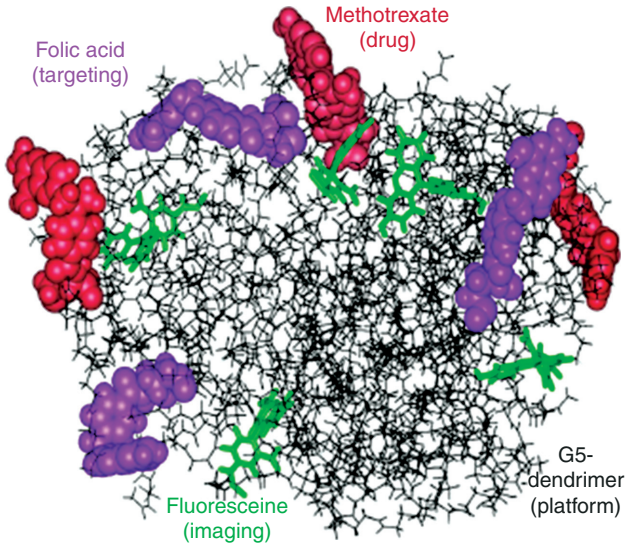

Figure 5. This simplified computer model of the U-M 'Trojan horse' nanoparticle shows the dendrimer's branching structure and how molecules and drugs are attached. U-M scientists use folic acid as 'bait' to achieve internalisation of methotrexate inside tumour cells.
Reproduced with permission from Jolanta Kukowska-Latallo, Michigan Nanotechnology Institute for Medicine and Biological Sciences [102].
U-M: University of Michigan.

Table 3. Dendrimers as nanoscale units.

Systems in therapeutic trials	Size range (nm)
Polymeric drug or sequestrant (e.g., dendrimers)	3 – 20
Polymer–protein conjugate	20
Polyplex–polymer–DNA conjugate	40 – 60
Polymer–drug conjugate	5 – 15
Polymeric micelles	60 – 100

Table 4. Synthesis of folate-[G2]-OH and folate-[G2]2-[C] conjugates.

Conjugate	The ratio of folic acid/dendrimer in reaction mixture	Folate residues per conjugate
	8.0	3.2
Folate-[G2]-OH	15.2	7.3
	24.0	8.2
Folate-[G2]2-[C]	48.0	12.6

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efficacy of the drug or even result in detrimental side effects [81]. Dendrimers have shown the ability to overcome these challenges posed by the biological system and drug candidate, due to the nanometric size and macromolecular characteristics of dendrimers [80,82]. They can also be attractive targeting systems due to their easy passage and retention through hyperpermeable vasculature and defective lymphatic drainage, such as in tumours. Apart from this, their hydrophilic periphery and nanoscale size places them in an important category among other carries that are capable of overcoming the major obstacle of drug targeting: reticuloendothelial system uptake. This unique disposition characteristic has been exploited for effective tumour targeting via enhanced permeability and retention (EPR) effect. The approach is quite similar to polymer–drug conjugates, which passively target a site due to the polymer's characteristics, and allows prolonged circulation time in the bloodstream. *N*-(2-hydroxy propyl) methacrylamide (HPMA) copolymer-conjugated doxorubicin is one such example that reached Phase I clinical trials [83].

3.2.4 Surface-engineered dendritic drug delivery systems

The surface engineering of nanocarriers could overcome numerous barriers to the targeting cells or tissues. This can be achieved by modifying the surface of nanoparticles by coating or conjugation with hydrophilic polymers, such as poloxamine, poloxamer block copolymers or polyethylene oxide; by PEGylation, or by anchoring or coating various ligands and antibodies to long-circulating carriers. Furthermore, potential nanoscale macromolecules in drug delivery meet certain criteria, such as water solubility, biocompatibility, biodegradability, scaffolding properties with a high drug capacity, site-specific drug delivery, controlled or triggered drug release, isolation at the molecular level and protection of the drug against inactivation during transit to the target cells, minimal non-specific cellular and protein interactions and appropriate cellular adhesion, cellular uptake, and intracellular trafficking [73]. Ringsdorf in 1975 provided a rational model for pharmacologically active macromolecules, specifically the utilisation of polymers as macromolecular carriers of drugs [84]. Five components described by him were the polymeric backbone, the drug, the spacer, the targeting moiety and the solubilising agent.

A few earlier works have engineered and developed dendritic platforms to serve as drug-conjugated multifunctional nanoconstructs based on the Ringsdorf model. In this context, Frechet and co-workers [67] designed a surface-engineered dendritic device with hydrazide-terminated polyarylethyl dendritic units, with MTX or folic acid conjugated to the exterior of the dendritic structure (**Table 4**), with the objective of internalising the systems within cells via endocytosis [85]. The design and conjugation of paclitaxel with multifunctional PAMAM dendrimers is another example displaying the potential of dendrimers in designing nanoconstructs for

anticancer drug delivery. Functional molecules such as imaging agents (fluorescein isothiocyanate), ligand (folic acid) and drug were conjugated to the dendritic periphery [86]. In yet another investigation, a PAMAM dendrimer-based multifunctional engineered nanodevice was developed, conjugating a ligand, an imaging agent and the anticancer drug, MTX, through peripheral functional groups. The trifunctional G5 dendrimer (conjugating fluorescein isothiocyanate, folic acid and MTX) was reported to interact with folic acid receptor-expressing KB cells and displayed time- and dose-dependent inhibition of KB cell growth and the intracellular localisation of the conjugate [74]. Furthermore, evaluation of these dendritic nanoconstructs [87] after intravenous administration showed enhanced therapeutic efficacy in immunodeficient mice bearing human KB tumours overexpressing the folic acid receptor, and appreciably reduced toxicity compared with free drug [27]. Also, the specificity of folic acid-conjugated dendrimers in the MTX–folic acid–dendrimer can be seen from this study, showing similar uptake of folic acid–dendrimer conjugates, and 10% less uptake for non-folate-conjugated free dendrimers. The multifunctional periphery of dendrimers can be further used to modulate the release profile of biologically active molecules. Drug conjugated to dendrimer has been shown to display a controlled release profile, compared with that of the drug in the form of an inclusion complex, which showed that it can be retained until it is delivered to the desired site [75]. Bonding of the carboxyl groups of MTX to dendrimer peripheral groups (either amine or hydroxyl terminated) via amide or ester linkages is an example of such a dendrimer conjugate. Furthermore a conjugate with an ester linkage, when hydrolysed, has been shown to release the active agent at the lower pH state of endosomes, and was found to be fourfold more cytotoxic to tumour cells than free MTX. However, its counterpart, an amide conjugate, was less toxic, suggesting that the higher cytotoxicity of the ester conjugate resulted from intracellular drug delivery and release [88]. The activity of dendritic nanoconstructs against cancer cells has displayed promise for the development of a well-controlled pH-triggered system. Surface modification of dendrimers by covalent coupling with PEG [45], ligands such as peptide [89] and carbohydrate (mannose) [90], has shown promising outcomes in the field of drug targeting or site-specific drug delivery. These reports highlight the many possibilities with these dendritic systems for drug delivery, including site-specific targeting, prolonged circulation time, intracellular delivery, and enhancing the therapeutic efficacy of biologically active molecules.

3.2.5 *Drug carrying co-polymeric dendritic nanocontructs*

Co-polymeric dendritic nanoconstructs is a term used here to refer any design using linear polymers, such as PEG or PEO, coupled to dendrimers to provide essential modulations to achieve drug delivery. Jain and co-workers have developed a PEG–lysine-based dendrimeric nanoparticulate carrier that controls the release rate of the antimalarial drug, artemether. In this example, the problems of a short biological half-life, low aqueous solubility, non-availability of a compatible aqueous base for formulation and its intravenous delivery were addressed. The additional benefits of chondrointin sulfate coating on these dendrimers were observed, with reduced haemolysis, decreased macrophage uptake and enhanced mean residence time [89]. Polyvalent sialic acid-conjugated dendrimer is another example that inhibits the action of influenza A virus through occupation of cellular receptors responsible for accommodating the virus [91]. The high concentration of PEG can offer enhanced solubility to the poorly soluble drugs; however, these PEG-containing drug formulations, on dilution post-administration into the gastrointestinal tract or intravenous injection into the blood, can profoundly reduce the solubility of these biologically active molecules. A new dendritic architecture with graft and star-shaped graft polymers bearing a large number of ethylene glycol molecules has been shown to increase the water solubility of paclitaxel by upto fourfold. Such systems offer an opportunity to overcome the hindrance of both oral and parentral delivery of the drug [30]. **Figure 6** shows the cumulative release profile of paclitaxel from a binary solution of dendrimers (**Figure 6A**), poly ((oligoethyleneglycol methacrylate [OEGMA])) and star poly(OEGMA) (**Figure 6B**). Furthermore, it was concluded that dendrimers were more effective than PEG 400 in enhancing the drug's solubility, and this was attributed to the increased local density of ethyleneglycol units. Dendritic unimolecular micelles with a hydrophobic core surrounded by a hydrophilic shell have also been prepared by coupling dendritic hypercores with PEG–mesilate. Entrapment of the model drug indometacin in these dendritic micelles was achieved with 11 wt% loading, and preliminary *in vitro* release tests suggested a sustained release profile [55].

The degradable nature of the polymeric backbone of any carrier can provide aid in controlling drug delivery, as well as its clearance from the biosystem. In this context, Grinsstaff and co-workers designed a polyether-ester dendrimers and used dendrimers composed of succinic acid and glycerol for the encapsulation of the anticancer agent 10-hydroxycamptothecin [92,93]. The drug conjugates on polyester dendrimers composed of 2,2-*bis*(hydroxymethyl)propanoic acid monomers were studied for their biodegradation characteristics [12,94]. It was observed that the PEO star G3 dendrimer conjugate, with an acid-labile hydrazone linkage conjugating doxorubicin, allowed controlled release under the acidic environments of endosomes and lysosomes, with a pH in range of 4 – 6. *In vivo* data revealed a prolonged terminal half-life of ~ 72 min for doxorubicin–PEO, relative to the 8-h half-lifes for G4 polymer and M-PEG capped versions. In addition, these systems displayed an improved distribution pattern relative to other systems studied, with no significant accumulation in any vital organ. Also, at pH 7.0, drug release was observed, and 70% drug was released after 20 h at pH 4.5.

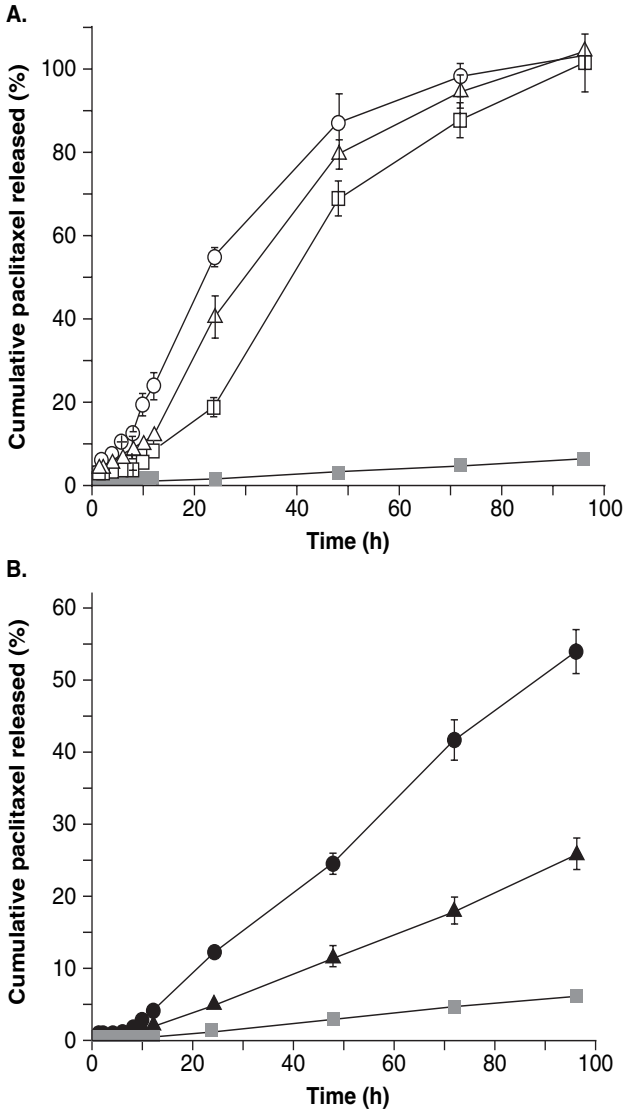

Figure 6. The cumulative release profiles of paclitaxel that was solubilised in aqueous polymer solutions. A. 80 wt% of the G5 dendrimer (open circle), 80 wt% of the G4 dendrimer (open triangle), 80 wt% of the G3 dendrimer (open square) and paclitaxel particles (closed and grey-coloured square); **B.** 10 wt% of the five-arm star poly(OEGMA) (closed circle), 10 wt% of the poly(OEGMA) (closed triangle) and paclitaxel powder (closed and grey-coloured square).

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Poly(OEGMA): Poly(oligoethyleneglycol methacrylate).

3.2.6 *Dendrimers in gene delivery*

Initial work using dendrimers for gene delivery applications is exemplified by the delivery of DNA complexed with PAMAM dendrimers [51]. Here, the effect of polyamidoamine cascade polymers on transfection efficiency was evaluated. Array-designed antisense oligodeoxynucleotide (ODN) was covalently conjugated to a novel anionic dendrimer using a pentaerythritol-based phosphoroamidite synthon. The ability of this conjugate to effectively deliver and downregulate EGFR expression in cancer cells was also evaluated. The cellular uptake of the ODN–dendrimer conjugates was up to 100-fold greater than mannitol, and up to 4-fold greater than naked ODN in cancer cells. *In vitro* RNase H-mediated cleavage assays confirmed that covalently conjugated antisense ODNs in the dendrimer conjugate were able to hybridise and cleave the array-defined hybridisation target site within the EGFR mRNA, without the need for ODN dissociation from the conjugate. In cell culture, ODN–dendrimer conjugates were effective in inhibiting cancer cell growth, which correlated with a marked knockdown in EGFR protein expression. These data highlight a novel anionic dendrimer delivery system for gene-silencing ODNs, which improved their biological stability, cellular delivery and antisense activity in cultured cancer cells [95]. Another example is of PAMAM G5 dendrimer conjugated to a cell-penetrating peptide, Tat peptide, in search of an efficient cellular delivery vehicle for antisense and siRNA oligonucleotides. Both antisense and siRNA readily formed complexes with the synthesised dendrimer–peptide conjugate. These were introduced into NIH 3T3 MDR cells, and primarily accumulated in intracellular vesicles. The results revealed that the dendrimer–oligonucleotide complexes were moderately effective for the delivery of antisense RNA, and only poorly effective for the delivery of siRNA. Conjugation of the dendrimer with the Tat cell-penetrating peptide failed to further enhance the effectiveness of the dendrimer [96].

Another important study investigated the potential use of the starburst PAMAM dendrimer (G3) conjugate with α-cyclodextrin, bearing siRNA, as a carrier for RNA interference. The major obstacle for siRNA delivery is the difficulty in crossing the biological membrane due to its hydrophilicity and high molecular weight. The ternary complex composed of the pGL2 control vector (pDNA)–pGL2 siRNA–α-CDE conjugate showed a greater degree of pGL2 siRNA sequence-specific gene silencing, without off-target effects, than those of commercial transfection reagents, such as Lipofectamine™2000 (Invitrogen), TransFast™ (Promega Corp.) and Lipofectin™ (Invitrogen). These results suggest that the dendrimer–α-cyclodextrin conjugate has the potential to be a novel carrier for siRNA [97].

4. Conclusion

The basis of the potential of dendrimers relies broadly on their nanoscale size, biodegradable nature and non-immunogenic characteristics. The multifunctional periphery and hollow interior channels can be modified and used much more broadly than other macromolecular polymers or vesicular carriers. The many possibilities of the dendritic artifacts for

site-specific drug targeting, prolonged circulation time, biocompatibility and intracellular delivery may lead to the enhanced therapeutic efficacy of highly potent biologically active molecules.

5. Expert opinion

In last two decades, the applications of dendrimers in the areas of chemistry, physics, imaging and drug delivery have witnessed rapid growth. Their unique architecture, well-defined structure, size and vastly diverse properties are useful tools to address numerous fundamental issues in drug delivery. At present, there are many basic objectives yet to be fulfilled for many existing and new drug molecules, such as solubility, administration and targeting. Dendritic nanotechnology may enable the construction of clearly defined three-dimensional structures on inorganic, organic or biological templates to address many of these issues. One particularly interesting feature of the dendrimer is its multi-dimensionality, where size-based modifications are easily performed, and are reproducible. This offers an advantage over many nanoscale systems, and makes the system suitable for more widespread drug delivery objectives, such as controlling drug payload, solubilisation, and release kinetics *in vitro* and *in vivo*. However, the release of drug from dendrimer may vary with modified conditions and with the chemistry of different drugs, which will always need to be optimised. Furthermore, the advantage of making possible the encapsulation or conjugation of drug molecules is a unique phenomenon of dendritic systems. The presence of high-density peripheral groups provides an opportunity to attach ligands, spacers for drug or ligand, and groups that modulate the solubility and toxicity. These surface-engineered constructs may resolve many new research goals. The efforts in this direction offer an appreciable opportunity for the precise and well-controlled delivery of anticancer [63,74,98], antimalarial [89], antitubercular [90], anti-inflammatory [33,99] agents, and many other potent biologically active molecules. These are a few examples that present the multi-dimensional and multi-functional characteristics of dendrimers. Although these dendritic architectures pose a distinct advantage over their linear analogues, the steric hindrance that results due to the dense globular architecture remains the major challenge in designing more useful enzymatic- or pH-triggered and bio-friendly conjugates. Consequently, the polymer's ability to formulate a number of model drugs for delivery is due to the dynamic structure of the dendrimer. Hence, these materials are becoming a favourite topic of research, and it has been suggested that, in the twenty-first century, they might become new building blocks, in a similar fashion to the uses of alloys, plastics and semiconductors in twentieth century.

One of the most important challenges at present in the field of medicine is the proper distribution and targeting of therapeutic moieties within the patient's body. Improving the efficiency and delivery of therapeutic molecules to their final target cells is, therefore, a priority for the pharmaceutical industry. A large amount of research is being undertaken to develop novel 'advanced' materials capable of incorporating biologically active substances, transporting them to the right place and releasing them in a controlled way. In this way, biocompatibility issues concerning the potent and non-specific actions of the majority of drugs may be resolved. Numerous such dendritic systems are already under extensive research, with a pool of published data on a few well-known polymeric structures such as PPI, PAMAM, diamino butane and melamine. The major benefit and expected outcome of the development of novel nanostructured materials and their modifications is the strengthening of molecular medicine.

We must not let the fascinating applications of dendrimers in drug delivery be marred by insufficient evidence on their safety and toxicity. More extensive and exclusive research efforts are, therefore, needed so as to establish the pharmacokinetic and pharmacodynamic data of dendrimers to achieve regulatory success. The functionalisation approach can be helpful in addressing toxicity related issues via the controlled release of an incorporated drug. The optimisation of cancer chemotherapy may emerge as the most outstanding attribute of dendrimer applications. Molecular modelling of the conceptualised dendrimer nanoconstructs is likely to emerge as a highly rewarding strategy.

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