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Emerging concepts in dendrimer-based nanomedicine: from design principles to clinical applications

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Abstract. Kannan RM, Nance E, Kannan S, Tomalia DA (Center for Nanomedicine; Johns Hopkins University School of Medicine, Baltimore, MD; NanoSynthons LLC, Mt. Pleasant, MI; University of Pennsylvania, Philadelphia, PA; and Virginia Commonwealth University, VA, USA). Emerging concepts in dendrimer-based nanomedicine: from design principles to clinical applications (Review). *J Intern Med* 2014; **276**: 579–617.

Dendrimers are discrete nanostructures/nanoparticles with 'onion skin-like' branched layers. Beginning with a core, these nanostructures grow in concentric layers to produce stepwise increases in size that are similar to the dimensions of many *in vivo* globular proteins. These branched tree-like concentric layers are referred to as 'generations'. The outer generation of each dendrimer presents a precise number of functional groups that may act as a monodispersed platform for engineering favourable nanoparticle–drug and nanoparticle–tissue interactions. These features have attracted significant attention in medicine as nanocarriers for traditional small drugs, proteins, DNA/RNA and in some instances as intrinsically active nanoscale drugs. Dendrimer-based drugs, as well as diagnostic and imaging agents, are emerging as promising candidates for many nanomedicine applications. First, we will provide a brief survey of recent nanomedicines that are either approved or in the clinical approval process. This will be followed by an introduction to a new 'nanoperiodic' concept which proposes nanoparticle structure control and the engineering of 'critical nanoscale design parameters' (CNDPs) as a strategy for optimizing pharmacokinetics, pharmacodynamics and site-specific targeting of disease. This paradigm has led to the emergence of CNDP-directed nanoperiodic property patterns relating nanoparticle behaviour to critical *in vivo* clinical translation issues such as cellular uptake, transport, elimination, biodistribution, accumulation and nanotoxicology. With a focus on dendrimers, these CNDP-directed nanoperiodic patterns are used as a strategy for designing and optimizing nanoparticles for a variety of drug delivery and imaging applications, including a recent dendrimer-based theranostic nanodevice for imaging and treating cancer. Several emerging preclinical dendrimer-based nanotherapy concepts related to inflammation, neuro-inflammatory disorders, oncology and infectious and ocular diseases are reviewed. Finally we will consider challenges and opportunities anticipated for future clinical translation, nanotoxicology and the commercialization of nanomedicine.

Keywords: critical nanoscale design parameters, dendrimers, drug delivery, nanomedicine, nanoperiodic concept.

Introduction

During the past decade, the very active field of nanomedicine has captured the attention and imagination of some of the most renowned scientists and physicians worldwide. This is evidenced by the emergence within this field of many new high-impact journals, international symposia and medical companies, and an exponential growth in publications, citations, clinical trials [1] and projected global markets (www.transparencymarketresearch.com/nanomedicine-market.html). In particular, the European Nanomedicine Symposia (www.clinam.org) held annually in Basel, Switzerland, since 2007, the American Society of Nanomedicine Symposia (www.amsocnanomed.org) organized each year since 2009 and the seminal 6th Key Symposium on nanomedicine (2009) in Stockholm, Sweden [2], as well as other international research programmes in Europe (http://www.cosmophos-nano.eu/), North America and Asia, have focused on strategic areas of interest in nanomedicine.

As early as 2001, there was a major emphasis towards larger macromolecular structures rather than traditional small molecule pharmaceuticals [3]. Early investigations of macromolecular therapy suggested that new and important benefits were possible, including the ability to engineer and enhance more favourable pharmacokinetic (PK) and pharmacodynamic (PD) properties beyond those of small molecule pharmaceuticals. Recent activities in nanomedicine have been largely inspired by the 'global nanotechnology revolution' and the fact that nanoparticles may now be synthesized reproducibly in highly monodispersed forms with 'critical nanoscale design parameters' (CNDPs) such as size, surface chemistry and shapes that closely mimic important biological entities such as proteins, RNA/DNA, membrane bilayers and viruses. This systematic structural control of synthesized nanoparticle CNDPs has been shown to provide many new potential strategies and modes for delivering therapeutic levels of drugs to specific disease sites whilst minimizing off-target side effects.

The beginning of polymer-based nanomedicine

There are four major known polymer architectures: linear, cross-linked, branched and dendritic types. Within this context, Ringsdorf was the first to propose the use of synthetic linear polymer structures for therapeutic applications [4]. As early as the 1970s, this pioneering work [4] led to diverse activities in a broad biomedical area currently referred to as 'polymeric therapeutics'. During that time, substantial progress was made with regard to preclinical technical challenges, such as (i) polymer synthesis (reproducibly defined structure), (ii) polymer characterization (measurement of CNDPs and purity), (iii) polymer excretion modes (function of molecular weight/chemical functionality), (iv) polymer toxicology and (v) polymer physiology (biodistribution, bioavailability, PKs and PDs). In many instances, there were significant benefits associated with polymer therapeutics leading to a variety of nanosized therapeutic prototypes (i.e. polymer–drug conjugates) which have been reviewed extensively elsewhere [3]. It is noteworthy that the first nanoscale polymer therapeutic was a synthetic linear N-(2-hydroxypropyl) methacrylamide polymer–doxorubicin (anticancer drug) conjugate that entered clinical trials in 1994 [5]. Apart from this, the first polymer-based nanotherapeutics to be approved by the US Food and Drug Administration (FDA) were linear-poly(ethylene glycol) (PEG)–drug conjugates or protein nanoparticle-based structures (e.g. the paclitaxel-containing conjugate Abraxane).

More recently, a number of nanotherapeutics based on organic polymers, dendrimers, proteins and inorganic compositions have been introduced. Many of these candidates have entered Phases II and III clinical trials or have attained regulatory approval as discussed below (Table 1).

The challenges of advancing nanomedicine/therapies into clinical trials and the approval process

The advancement of small molecule pharmaceuticals through the regulatory approval process must involve a thorough understanding of PKs and PDs. Typical PK studies investigate the absorption, distribution, metabolism and excretion (ADME) of the drug. These four parameters and the administered dose determine the concentration of a drug at the site of action and hence the intensity of its effects as a function of time. Developing a nanoparticle-based platform in combination with an approved drug may be expected to have added levels of complexity related to possible changes in the ADME of the drug (Scheme 1). In fact, it is expected that many of these nanomedicines/therapies will be examined by regulatory agencies with new boundaries that span interfaces between pharmaceuticals, medical devices and biological agents [6, 7]. As such, they may be regulated as 'combination products'. The unique features of nanoscale candidates such as their large sizes/surface areas, polyvalent surface chemistry, multicomponent platforms and polydispersity issues compared to small molecule drugs will require additional methods of characterization and new standards [8]. The nature of these unique nanoscale features and their appended ligands suggests the possibility of new interactions with biological cells, tissues, organs and disease sites. Furthermore, because the physicochemical features of nanoparticles are similar to those of proteins, viruses, oligonucleic acids and other nanoscale cellular components, distinctive and highly responsive interactions with cells and tissues might be expected. These interactions may produce either beneficial or negative effects in the presence of therapeutic drugs and must be thoroughly understood. These issues are recognized as a major challenge of regulatory approval.

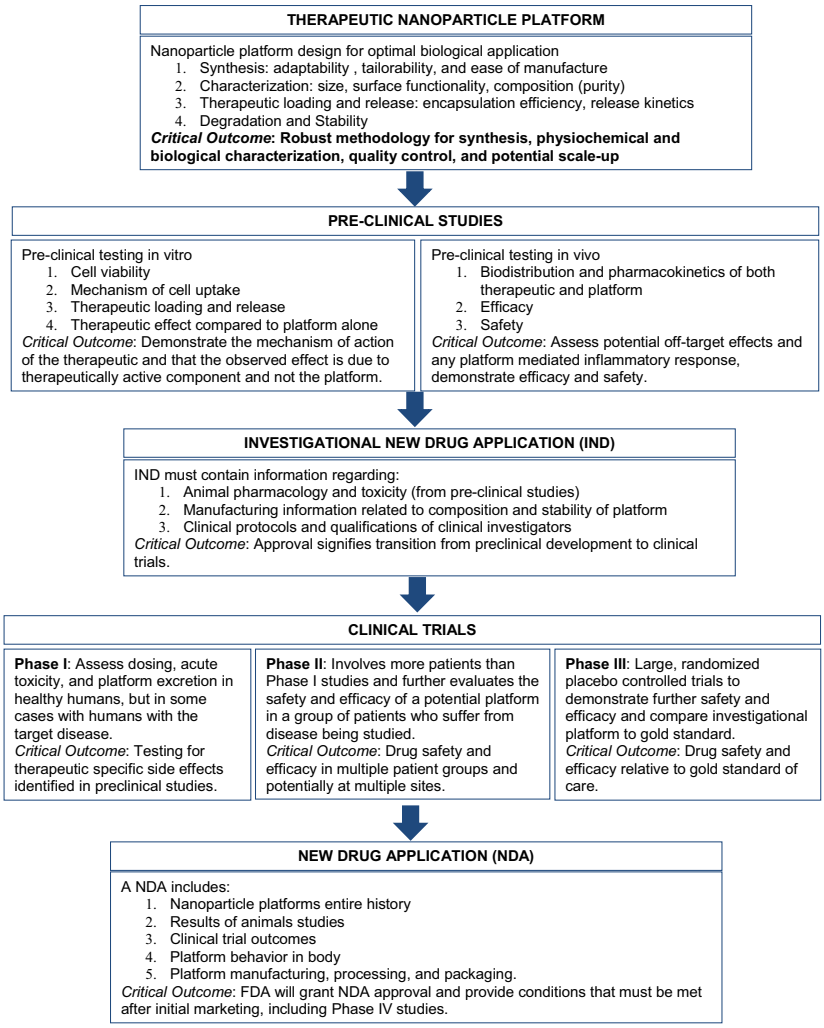
There are great commercial expectations for nanomedicines; at the current annual growth rate of 12.3%, global markets are projected to reach

Table 1 *Recent nanomedicine clinical activity involving various soft and hard nanoplatforms*

Product	Nanoparticle	Condition	Phase	Identifier	Sponsor
Aurimune® CYT-6091	TNF-Bound Colloidal Gold	Solid tumor treatment	1	NCT00356980	National Institutes of Health Clinical Center
Abraxane® ABI-007	Paclitaxel Album in-Stabilized Nanoparticle Formulation	Metastatic breast cancer	2	NCT00733408	University of Washington
Abraxane® ABI-007	Paclitaxel Album in-Stabilized Nanoparticle Formulation	Breast cancer	3	NCT00785291	National Cancer Institute
Abraxane® ABI-007	Albumin-Bound Paclitaxel-Gemcitabine	Metastatic pacreatic cancer	3	NCT00844649	Celgene Corporation
Ferumoxytol	SP Iron Oxide MRI	Pancreatic cancer	4	NCT00920023	Massachusetts General Hospital
Abraxane® ABI-007	Paclitaxel Album in-Stabilized Nanoparticle Formulation	HER2-PosihVe metastatic breast cancer	2	NCT01730833	City of Hope Medical Center
VivaGel® SPL7013	Dendrimer-Based Topical	Treatment of bacterial vaginosis	3	NCT01577537	Starpharma Pty Ltd.
BIND-014	Polymeric Micelle	Non-small cell lung cancer	2	NCT01792479	BIND Therapeutics
BIND-014	Polymeric Micelle	Prostate cancer	2	NCT01812746	BIND Therapeutics
Ferumoxytol	USP Iron Oxide MRI	Cancer of the lymph node	N/A	NCT01815333	M.D. Anderson Cancer Center
Ferumoxytol	SP Iron Oxide MRI	Myocardial infarction inflammation	2	NCT01995799	University of Edinburgh
Abraxane® ABI-007 /AB-Complex	Paclitaxel Albumin-Stabilized Nanoparticle Formulation	Advanced melanoma	1	NCT02020707	Mayo Clinic
DEPTM-Docetaxel DTX-SPL8783	Dendrimer-Based Conjugate	Advanced or metastatic cancer	1	Australian Clinical Trials ACTRN12614000 171617	Starpharma Pty Ltd.

Source: http://www.clinicaltrials.gov. and www.anzctr.org.au.

177.6 billion $US by 2019 (www.transparency-marketresearch.com/nanomedicine-market.html). The major nanomedicine growth areas are predicted to be primarily in neurology, oncology and cardiovascular, anti-inflammatory and anti-infective applications [9].



Scheme 1. *Translation of a typical nanomedicine platform application from the discovery stage to Phases I–III human clinical trials, Food and Drug Administration (FDA) approval and commercialization [6, 7, 10, 11].*

Progress in the translation of nanoplatforms to clinical applications

In the past two decades, there has been a steady increase in the number of nanoparticle-based therapeutics approved for clinical use by the FDA. Many initially approved nanotherapeutics were based on either liposomal drugs or traditional linear (PEG-type) polymer–drug conjugates [3]. Currently, these commercial nanotherapy prototypes are dominated by drug delivery systems and account for more than 75% of total sales of nanotherapeutics [12]. One of the earliest nanomedicines to gain FDA regulatory approval (in 1995) was the product Doxil, which involved encapsulation of doxorubicin in liposomes. In the USA alone since 1990, at least 15 nanomedical-based therapies/applications have been approved for commercial use [13]. Selected examples of several recently approved products and those entering clinical evaluation are shown in Table 1. This clinic activity is too diverse and extensive to discuss in this review; however, it has been reviewed elsewhere [13].

Substantial opportunities remain for the development of future generation nanotherapeutics by enhancing certain recognized nanoparticle limitations associated with present prototypes. Current

nanovector deficiencies (i.e. for liposomes) include lack of (i) sustained drug release, (ii) *in vivo* stability properties required to control kinetic profiles and (iii) drug localization to targeted disease sites. On the other hand, traditional linear polymer/polymeric micelles are more robust with high drug loading capabilities and controlled/triggered drug release features. However, each of these platforms still possesses certain CNDP limitations related to linear, random coil architectures or polymeric micelle properties. These limitations include polydispersities (Fig. 6), lack of persistent shape control, reptation properties, noncontrollable surface chemistry presentations [14] and/or the inability to make systematic size modifications below the 'critical micelle concentrations' exhibited by polymeric micelles, which is currently considered to be around 30–40 nm [15].

Understanding the influence of nanoparticle properties (i.e. CNDPs) on clinical translation issues, such as PKs, PDs, bioaccumulation, excretion modes and toxicology, is of utmost importance. These CNDPs include size, shape, surface chemistry, flexibility/rigidity, architecture and elemental composition. The ability to structure control and engineer CNDPs is undoubtedly a major challenge in the translation of nanoparticle-based therapies into approved clinical applications (see below).

Due largely to the ability to control and engineer CNDPs, a number of dendrimer-based therapy/diagnostic technologies have recently made significant progress towards FDA clinical approval and commercialization. The first reported dendrimer-based nanomedicine applications appeared nearly 20 years ago in an early series of patents by one of the authors [16]. These patents included a wide range of nanomedical applications: (i) targeted delivery for the treatment of cancer and other diseases, (ii) dendrimer-based magnetic resonance imaging (MRI), (iii) DNA/RNA transfection vectors, (iv) delivery of pharmaceuticals and brachiotherapeutic metals by encapsulation and conjugation, (v) dendrimer-based polyvalency for antiviral and antimicrobial applications and (vi) dendrimer-based cardiodiagnostics (e.g. the Stratus analyzer, www.healthcare.siemens.com/point-of-care/cardiac/stratus-cs-acute-care; Dade Behring, now Siemens Healthcare, Germany). These dendrimer-based cardiodiagnostics were approved and have been commercially available since the late 1990s, first from Dade Behring and now from Siemens. Essentially all these earlier patents have either been licensed to Starpharma (Melbourne, Australia) or expired. Based on this early intellectual property and their existing/original patents, Starpharma has successfully developed active, dendrimer-based microbicides under the trademark VivaGel (i.e. SPL7013). In 2012, important Phase I–III trials were initiated using SPL7013 for the treatment of bacterial vaginosis. The lead nanostructure SPL7013 is a poly(l-lysine) dendrimer which presents anionic, naphthalene disulphonate surface groups and exhibits potent activity against bacterial vaginosis when administered as a topical gel. These polyvalent dendrimer constructs are currently the leading approved and clinical stage nanomedicinal product for the prevention/transmission of sexually transmitted infections, including human immunodeficiency virus (HIV) and genital herpes, and the management of bacterial vaginosis. This dendrimer-based SPL7013 has recently been approved in Japan as a protective condom coating and is reported to be 99.9% effective against HIV and genital herpes (www.starpharma.com). Furthermore, a dendrimer-based MRI contrast agent termed Gadomer-17 is currently under investigation in clinical trials (Bayer Schering Pharma AG, Leverkusen, Germany) as a blood pool imaging agent. This imaging agent is similar to the linear-Gd- diethylene triamine pentaacetic acid – (DTPA)-poly(lysine) (PLL) prototype; however, it shows a superior elimination rate, presumably due to the globular nature of the dendrimer component. This dendrimer-based contrast agent also extends the temporal window of dynamic contrast-enhanced MRI and represents a promising diagnostic application for dendrimer nanocarriers in the clinic.

Finally, other dendrimer-based *in vitro* cardiodiagnostics (e.g. Stratus), protein diagnostics (i.e. UltraAmp and 3DNA) and gene transfection vectors (i.e. Superfect, Qiagen, Germany and Priofect, Starpharma) have emerged and are currently commercial products (see Fig. 15).

A unifying 'nanoperiodic' paradigm: implications for nanomedicine

Traditional pharmaceutical agents evolved largely around the use of elemental and small sub-nanoscale molecules (e.g. aspirin and arsenic compounds) and the manipulation of their 'critical molecular design parameters' (CMDPs) for the treatment of disease. However, attention is now turning to new opportunities that may be possible with nanoscale pharmaceuticals, diagnostics and

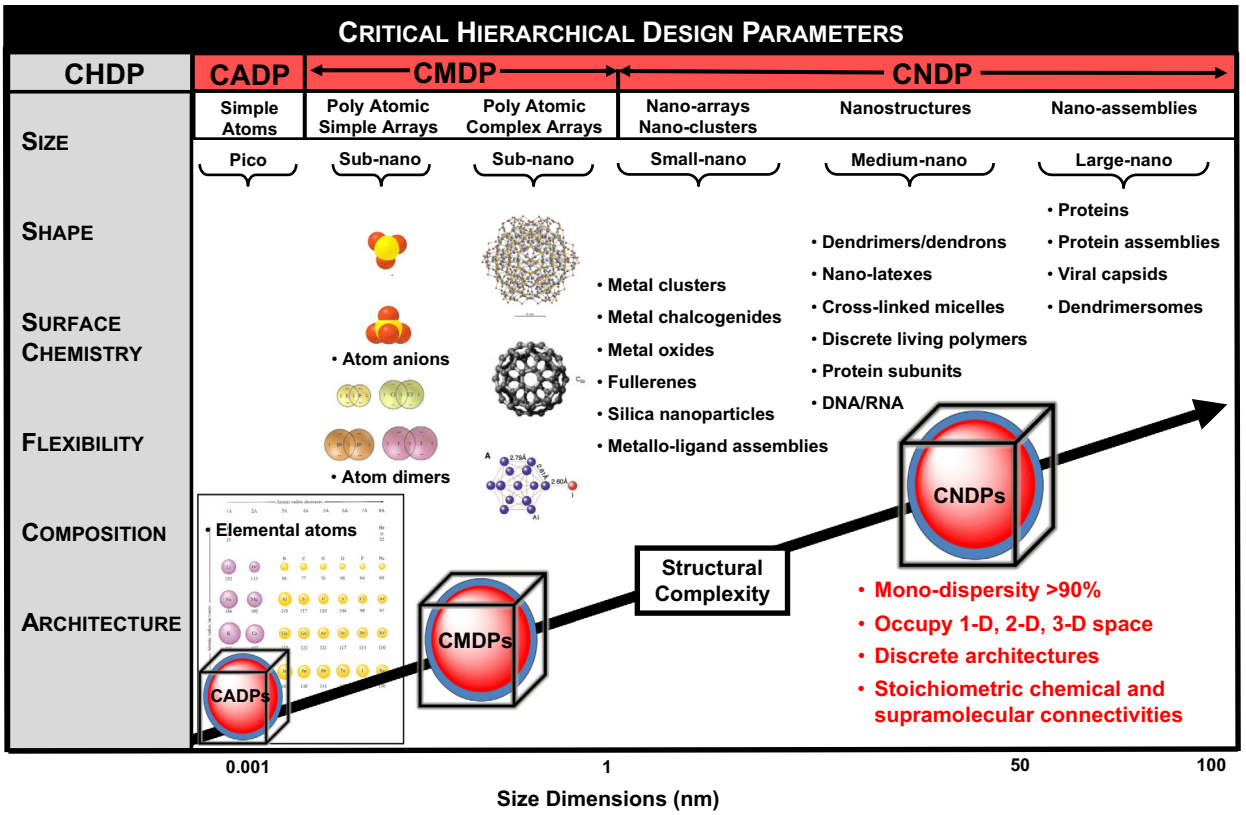


Fig. 1 *Structural control of critical hierarchical design parameters (CHDPs) (i.e. size, shape, surface chemistry, flexibility/rigidity, elemental composition and architecture) required for bottom-up synthesis of higher nanostructural complexity manifesting nanoscale atom mimicry. Abbreviations: Critical atomic design parameters (CADP), critical molecular design parameters (CMDP), critical nanoscale design parameters (CNDP).*

therapies [17] and hence the manipulation of their CNDPs (Fig. 1). In retrospect, it is apparent that the success of traditional pharmaceuticals, diagnostics and therapies would not have been possible without the solid scientific foundation derived from major classical disciplines such as biology, chemistry, physics, engineering and mathematics during the nineteenth and twentieth centuries. Linnaeus-type biological classifications, physical, engineering and mathematical principles, as well as chemistry-based toxicology and physiology insights derived from Mendeleev's periodic table have provided powerful periodic patterns and predictive principles for optimizing the design of traditional pharmaceuticals [18]. As such, it is not surprising that with the recent movement towards nanomedicine, there has been the emergence of new thinking together with demonstrated viability of a comparable unifying nanoperiodic framework. This new paradigm was initially inspired by the structure-controlled synthesis and self-assembly patterns observed in biology and supported by extensive evidence derived from early studies focused on the abiotic, divergent synthesis of dendrimers [19]. This evidence is presented in further detail with extensive discussion elsewhere [20–23].

The existence of a generalized framework for the structural–functional properties of nanomaterials relative to *in vivo* structural–biological interactions would enable a better understanding of important physicochemical characteristics required for successful translation of nanomedicines to clinical applications. Such was the role of Mendeleev's periodic table for atoms/molecules which led to many predictive patterns/rules that now underpin traditional small molecule pharmaceutical development and toxicology. Thus, a new 'nanoperiodic concept' involving structure-

controlled, hard or soft nanoparticles possessing tailorable CNDPs has been introduced recently [21–22]. These CNDPs include size, shape, surface chemistry, flexibility/rigidity, architecture and elemental composition. Many discrete, well-defined nanoparticles are being considered for a wide variety of nanomedicine applications. This concept demonstrates that systematic control and engineering of these CNDPs provides a powerful strategy for optimizing as well as developing *a priori* predictions for ideal function and property designs required for many nanomedicine applications. The ability to control and engineer specific CNDP features in nanostructures was first observed in the 1980–1990s, whilst investigating dendrons and dendrimers, and reported by one of us in 1990 [19]. This work inspired the evolution of a systematic nanoperiodic concept [19–23] for defining and understanding the properties of many discrete hard and soft nanoparticles now used in nanomedicine.

Unifying nanoperiodic paradigm principles

During the last 4.5 billion years of evolution on earth, nature has cleverly devised a system of quantized, critical hierarchical building blocks [20, 21, 23], each of which exhibits a discrete ensemble of physicochemical properties referred to as 'critical hierarchical design parameters' (CHDPs). These CHDPs are unique to each building block, as well as their respective hierarchical status. Therefore, each atomic element has its unique collection of 'critical atomic design parameters (CADPs)', and all stoichiometric molecules manifest well-defined CMDPs. Similarly, discrete, structure-controlled nanomaterials would exhibit their own respective quantized CNDPs. These critical hierarchical building blocks and their respective CHDPs are now recognized to systematically control the transfer of important structural and functional information both within and between hierarchical levels [23]. This important information transfer universally defines the emergence of all new properties and the currently known hierarchal complexity of all biological and abiotic materials (Fig. 1). Beginning with the periodic elements, CHDPs have played a critical role in hierarchical evolution to the complexity associated with life and diseases that threaten human health. More specifically, the minimum dimensions, functionalities and complexities required for the evolution of both life and disease are now recognized to involve certain critical macromolecules, structures and assemblies, all of which exhibit well-defined CNDPs [24–26] at the nanoscale level. As such, the pivotal role of well-defined, quantized, biological nanostructures such as proteins, DNA, RNA and viruses is clearly recognized (Fig. 1). Furthermore, the expected interactions between abiotic synthetic nanoparticles possessing similar CNDPs and these quantized biological entities present clear possibilities, both negative and positive, with important implications for nanomedicine.

An explicit example of a systematic, structure-controlled, CHDP-directed biological assembly involving discrete 'quantized hierarchical building blocks' is illustrated in Fig. 2. By simply invoking a sequence of atomic (CADP), molecular (CMDP) and nanoscale (CNDP) interactions at each hierarchical level, biological tendons can be routinely produced. Whilst considering these exquisite chemical bonding and supramolecular self-assembly patterns, it becomes readily apparent that certain critical universal design parameters determine and control these building block-driven hierarchical assemblies that lead to higher complexity. It is indeed notable that important novel properties are observed for each of the quantized building blocks and their subsequent assemblies as they transition from atoms to tendons. Many of the CNDP-directed nanoperiodic principles, stoichiometries, rules and patterns illustrated in Fig. 2(b) (i.e. the nanoscale region) may be applied to a variety of nanomedicine issues (see below).

As observed for biotic protein-based building blocks (Fig. 2), similar universal CHDP parameters appear to control all interactions, assembly and bonding relationships for analogous abiotic building blocks (Fig. 1). These quantized hierarchical relationships and interactions include supramolecular self-assembly, chemical bonding, mass binding ratios, stoichiometries and transfer of structural information, all of which ultimately determine novel properties from the atomic scale (CADPs) via the molecular scale (CMDPs) to the nanoscale (CNDPs) [27, 28].

Chemists and physicists now agree that certain well-defined, quantized hard and soft nanoscale building blocks mimic elemental atomic building blocks through their stoichiometric relationships and periodic properties; thus, they are referred to as 'superatoms' [29] or nanoelemental categories

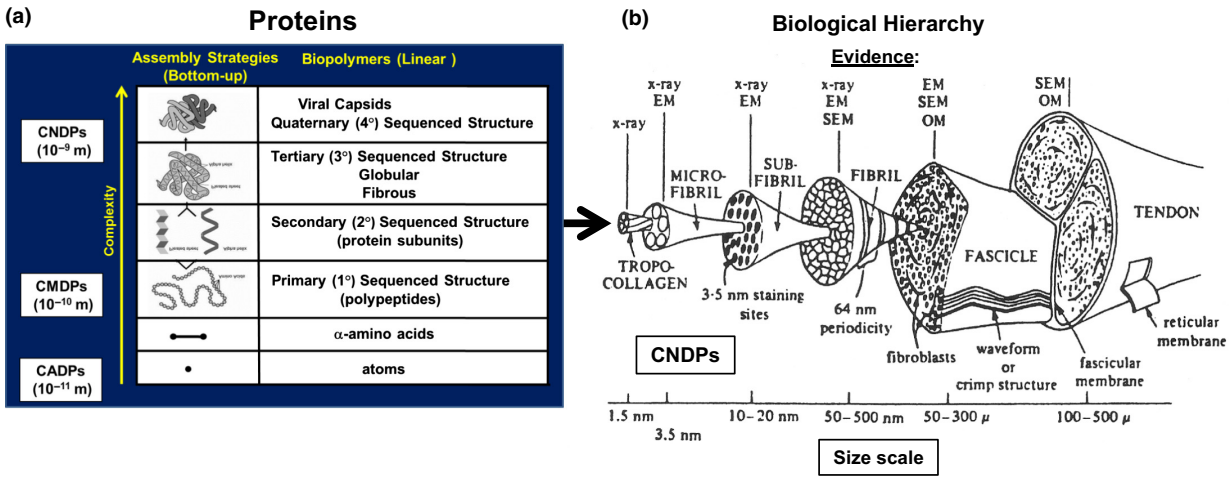


Fig. 2 *(a) Bottom-up synthesis of proteins involving quantized building blocks such as atoms [critical atomic design parameters (CADPs)] and monomers [critical molecular design parameters (CMDPs)] leading to nanoscale, self-organized protein complexity [critical nanoscale design parameters (CNDPs)] [22]; (b) bottom-up assembly through six hierarchical levels involving quantized nanoscale building blocks such as fibrous protein and tropocollagen (CNDPs) to produce novel properties associated with a biological tendon. Quantized building block structures at each level were confirmed by X-ray or electron microscopy [27]. Tendon image courtesy of Prof. Eric Baer, Case Western Reserve University.*

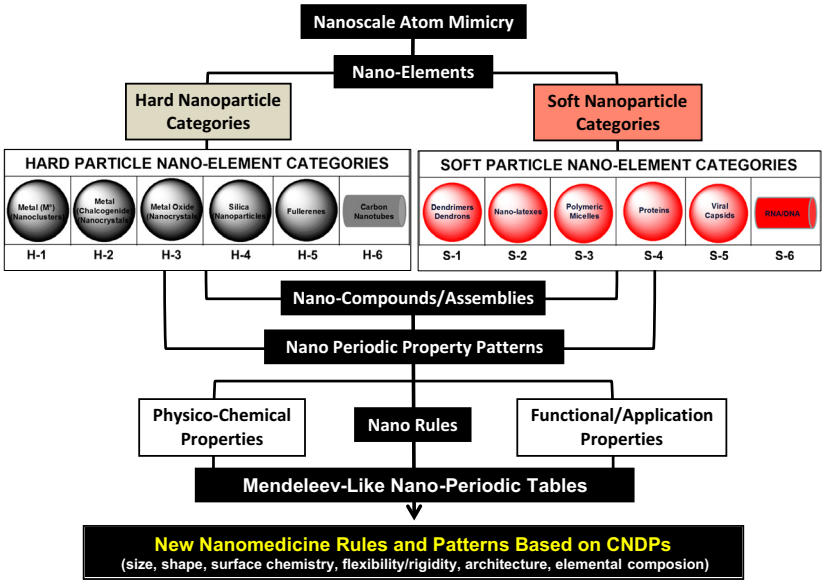


Fig. 3 *A nanoperiodic concept road map based on quantized hard and soft nanoscale building blocks (i.e. nanoelement categories) that exhibit atom mimicry properties by forming stoichiometric nanocompounds/assemblies. These quantized building blocks are >90% monodispersed and possess varying levels of structure-controlled critical nanoscale design parameters (CNDPs). As such, they exhibit well-defined nanoperiodic property patterns, nanoscale rules and Mendeleev-like nanoperiodic tables.*

(Fig. 3) [23, 30]. In all cases, the resulting nanoperiodic rules and property patterns are found to be strictly dependent upon the six CNDPs associated with each of these 12 building block or nanoelement categories: hard particles [H-1] to [H-6] and soft particles [S-1] to [S-6]. In simple terms, this means that, irrespective of the nanoparticle, these six CNDP particle features largely determine their properties, relationships and stoichiometries, and ultimately many applications/end uses that are possible.

Seminal examples of such stoichiometric nanoscale assemblies in the biological world include

many simple and moderately complex viruses. More specifically, Aaron Klug received the Nobel Prize in Chemistry (1982) for the total characterization of the tobacco mosaic virus (TMV), a discrete, well-defined cylindrical nanoobject with a diameter of 18 nm and length of 300 nm and helical symmetry. Using X-ray-confirmed TMV structures and the criteria described in Fig. 3, Klug clearly demonstrated a reproducible, stoichiometric ratio of 1 : 2130 between the single-stranded RNA (ssRNA) core, that is, a soft nanoelement [S-6]-type core, and its protein subunit shell, that is, a soft nanoelement [S-4]-type shell [31, 32]. Thus, TMV is referred to as a stoichiometric, ssRNA core and protein shell nanocompound designated [(S-6):$(S\text{-}4)_{2130}$]. In general, all simple and moderately complex viruses appear to exhibit well-defined stoichiometric relations between their oligonucleic acid cores (i.e. RNA or DNA cores) and their protein subunit shells, as previously described [20–22].

Unique CNDP-driven nanoperiodic interactions to produce stoichiometric relationships between quantized protein building blocks, that is, [S-4]-type nanoelements (Fig. 2), or simple viruses, that is, genomic cores [S-6] and protein subunit shells [S-4], are also observed between other discrete nanoparticles (Fig. 3) [18, 20–22, 27–29]. It should be apparent that this paradigm and its principles have the potential to provide a unifying explanation of important relationships that may exist between discrete engineered soft/hard nanoelements (Fig. 3) and a wide range of biological and toxicological issues of importance to nanomedicine. As will be described below, many of these discrete CNDP-engineered nanoparticles are beginning to produce interesting new predictive patterns/rules as a consequence of their well-defined CNDP-directed relationships with a variety of biological barriers, compartments, fenestrations and functional components (see Figs 4, 8–10).

Developing an optimum nanotherapeutic platform based on intrinsic structure-controlled, tunable nanoparticle CNDPs

Implicit in the regulatory approval process for all nanomedicine applications is the need to deliver a patient-compliant nanoplatform with good safety margins and high therapeutic efficacy at an acceptable cost. As described in Scheme 1, the regulatory pathway through the stages of preclinical investigational new drug and Phases I–III clinical evaluation for approval of a nanomedicine therapy is complex and challenging. As for a traditional small pharmaceutical medicine, it is important to understand strategies and optimize all critical parameters required for selecting the nanoscale candidates with the highest probability of success [14, 33, 34]. Traditionally, pharmaceutical scientists routinely use quantitative structure–activity relationship (SAR) analyses for defining and optimizing the best small molecule structures as part of the preclinical evaluation process. This strategy has been highly successful for developing predictive patterns and identifying many optimal traditional pharmaceutical candidates. For example, CMDPs such as molecular size, surface chemistry and flexibility were found to determine valuable and predictable patterns for optimizing transport of small molecule pharmaceuticals across the blood–brain barrier (BBB) [35].

Many of these 'quantitative SAR-like principles' based on traditional pharmaceuticals are currently being applied to nanoparticle CNDPs and their role in various nanomedicine applications [14, 33, 34]. For example, dendrimer CNDPs are being examined systematically as a function of their interactions with drugs, lipid membranes, proteins [36] and genetic material [37] in an effort to define behaviour patterns for their use as delivery vectors in nanomedicine [38]. It is notable that Nel's group has defined nanoparticle 'size' followed by 'shape' as perhaps the two most important physical properties of a nanomaterial [39, 40]. These two CNDPs determine many cellular uptake, transport and accumulation events that occur *in vivo*. All biological organisms depend on highly tuned and controlled uptake/transport activities that are strictly regulated by size and shape. For example, all cellular membrane bilayers are approximately 4–10 nm thick. On the other hand, vertebrate nuclear pore complexes are approximately 80–120 nm in diameter [41]. Therefore, these size-regulated biostructures exercise very important barrier functions as nanoparticles enter and exit. These size-/shape-controlled sites determine nanoparticle interactions, for example, with biological membranes and organelles that in turn directly influence cellular uptake, metabolism and excretion, bioavailability, tissue accumulation and organ toxicity. Undoubtedly other CNDPs such as surface chemistry, flexibility/rigidity, elemental composition and architecture will also exert their own intrinsic influence [42]. Finally, it is also well known that nanoparticle interactions with functional barrier/porosity parameters related to

disease pathology, such as BBB impairment, enhanced permeation and retention (EPR) effect, inflammation and infection, may have to be engineered based on CNDPs.

Thus, a therapy road map describing possible pathways for observed excretion, extravasation and certain biodistribution modes for traditional drugs and nanoparticles might be considered, as shown in Fig. 4 and suggested by others [43].

More specifically, this flowchart allows examination of the dependency of therapeutic size on intrinsic *in vivo* behaviour patterns for traditional small molecule CMDPs compared to larger nanoparticle CNDPs. Irrespective of the administration mode (e.g. oral, parenteral or inhalation), *in vivo* clearance will be expected to follow certain pathways that are now known to be largely directed by either CMDP or CNDP features, respectively.

These CNDP-dependent *in vivo* barriers must be considered in the total design and engineering of all nanoscale imaging, therapies and diagnostic strategies and devices. Nevertheless, after systemic administration, subtle nanoscale size differences will determine many features including (i) the rate of extravasation from the circulatory system, (ii) the mode of excretion (i.e. kidney vs. liver or spleen), (iii) the blood circulation time, (iv) the propensity for passive targeting via the EPR effect [44], (v) organ selectivity, (vi) disease site (e.g. tumour) or organ penetration and (vii) receptor-mediated recognition space or cell uptake behaviour. For example, systemic administration of small molecule drugs (<1 nm) typically leads to short circulation times due to fast kidney excretion modes and whole-body permeability due to rapid extravasation from the circulatory system. On the other hand, larger nanoscale therapeutics (>4 nm) exhibit longer circulatory residency times, size-selective excretion modes and permeability patterns associated with their nanoscale size and surface chemistry as shown in Fig. 4 [45–48]. These remarkable nanosize-dependent excretion modes are some of the first examples of CNDP-dependent nanoperiodic property patterns observed *in vivo* [45].

It is noteworthy that, in addition to size, the other five CNDP features that define important *in vivo* nanoperiodic property patterns should also be considered as potential design parameters for 'predictively' engineering and optimizing nanomedicines/devices. For example, in addition to nanoparticle size, other CNDPs such as surface chemistry, shape and flexibility [43] can directly influence *in vivo* complement activation [49], protein opsonization [50], bilayer disruption and red blood cell/platelet lysis [51], as illustrated above (Fig. 4). Although a growing number of CNDP-dependent properties have been reported, they have been described only as qualitative, empirical observations [14, 33, 34] and not as useful, quantitative nanoperiodic property patterns within the context of a unifying paradigm. Only a few selected examples of both empirical and quantitative observations based on the six CNDPs will be discussed below.

Nanostructure control and engineering CNDPs: implications for nanomedicine

Size

Incremental nanometric size differences as small as 1–3 nm may completely alter the excretion mode of a nanoparticle. We [20–22, 45] and others, including Kobayashi *et al.* [48], have reported that *in vivo* administered poly(amidoamine) (PAMAM) dendrimer-based MRI contrast agents exhibit very selective blood pool, organ affinity and renal/liver excretion properties as a function of their nanoscale dimensions. Similarly, Bawendi *et al.* [46] and Burns *et al.* [47] observed corroborating kidney and liver size excretion patterns, respectively, for proteins, quantum dots and silica nanoparticles. Of importance, epidermal penetration properties were also found to be dependent on both size and surface chemistry as shown in Fig. 9. In the case of nanoscale gold particles, sizes < 1–2 nm are alleged to exhibit nanotoxicity, whereas larger sizes (>15 nm) appear to present little risk of side effects [52]. It is widely recognized that tumour penetration and intratumoural distribution by nanoparticles is highly size dependent relative to tumour porosity. As such, smaller nanoparticle dimensions for therapy vectors are observed to be more effective against tumours than larger particles [53–59]. Thus, heterogeneity in particle size (i.e. with liposomes) can also significantly influence biodistribution and targeting [60].

Shape

Control of persistent three-dimensional size and shape of soft therapeutic nanoparticles is important for predicting *in vivo* biodistribution, cellular uptake, tumour uptake [61] and excretion modes. It is known that traditional, random coil, linear

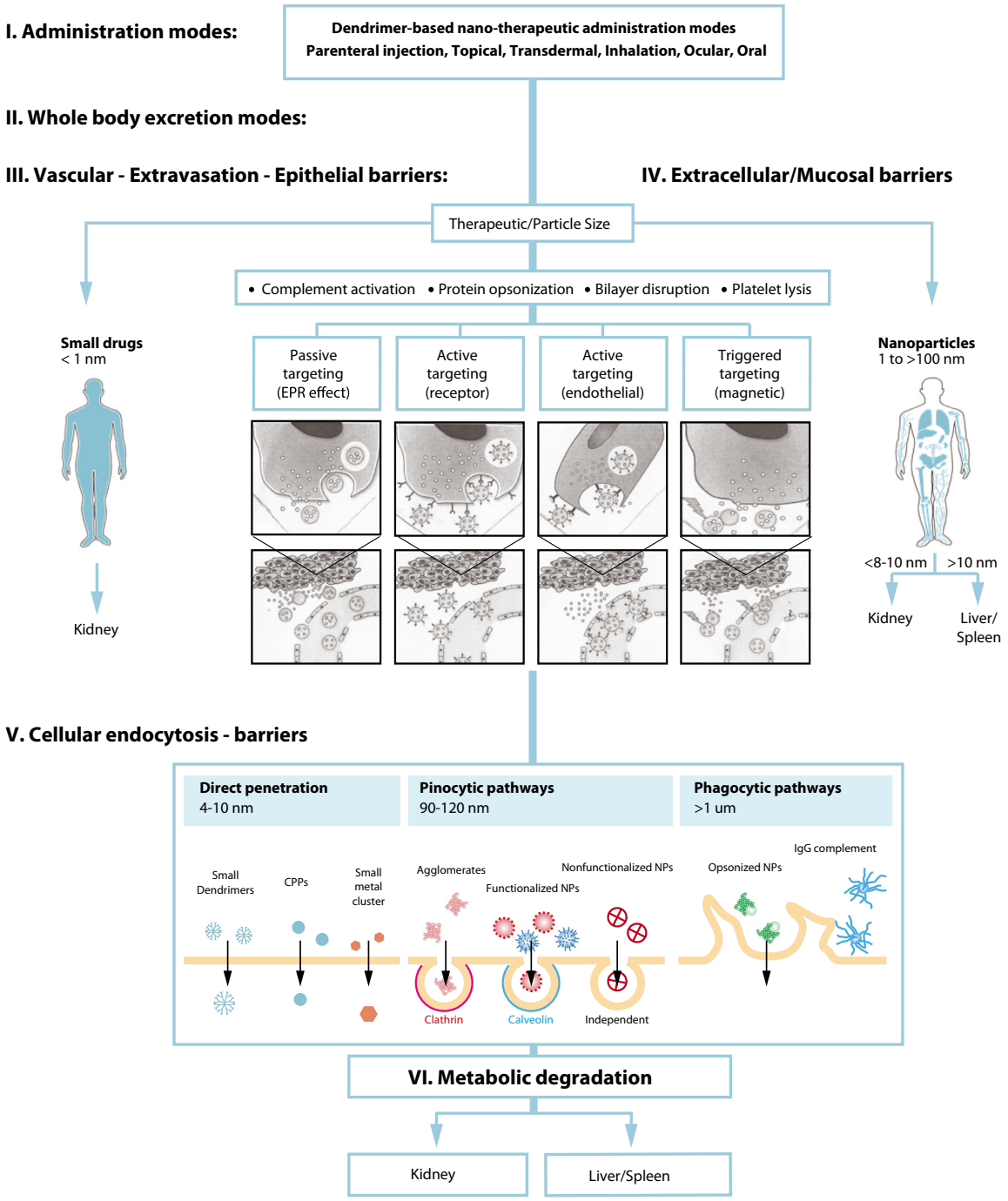


Fig. 4 *A schematic road map depicting in vivo barrier and processing differences (I–VI) experienced by small molecule drugs (< 1 nm) versus nanoparticle therapies (approximately 1–100 nm). These barrier/processing differences are examined in the context of administration modes and several popular targeting strategies: passive (i.e. EPR effect), active receptor uptake as well as image and magnetically directed triggered release mechanisms. It is apparent that systematic manipulation of nanoparticle critical nanoscale design parameters provides unique and important strategies for optimizing/controlling the desired therapeutic goals.*

polymers (Fig. 6) and certain rod-like nanostructures may reptate and undergo extravasation through very small vascular pores even <2–3 nm [62]. In addition, it has been shown by Kostarelos [63] that cylindrical carbon nanotubes demonstrate simple kidney excretion properties largely based on shape [64]. Furthermore, many nanotherapeutic applications for gold nanoparticles are shape dependent [62, 65]. The importance of rod versus spherical shapes in drug delivery is becoming widely recognized [66–69].

Surface/interior chemistry

Surface chemistry including charge and hydrophobicity/hydrophilicity has a vital role in tissue/cell interactions and toxicity (Fig. 13). Exo-presentations of targeting ligands from shape-persistent nanoparticle scaffolding (i.e. dendritic or rigid nanoarchitectures) have better receptor recognition properties compared to linear, random coil scaffolding presentations that may invert or become buried in the flexible random coil configurations [14]. Stealth-like surface chemistry (i.e. PEG) is used routinely to avoid adverse *in vivo* nanoparticle–protein interactions (e.g. opsonization) leading to aggregation, precipitation and inactivation. Pioneering work by Moghimi's group [43, 49, 70] demonstrated the importance of appropriate surface chemistry to avoid deleterious complement activation events. Generally neutral/anionic charged, hydrophilic nanoparticle surface chemistry is more desirable for biocompatibility and lower toxicity compared to cationic/hydrophobic nanoparticle surfaces which lead to *in vivo* lysing of blood platelets and/or cell membrane disruption [71]. However, recent *in vivo* results by Shcharbin *et al.* [72] appear to contradict earlier *in vitro* results. Both surface/interior dendrimer chemistries are reported to influence excretion modes, circulatory residency times and tumour penetration properties. These specific CNDPs appear to demonstrate important nanoperiodic property patterns relative to passive tumour targeting (EPR effect) and penetration of subcutaneous mammary tumours (4T1) in mice models [73].

Polyvalent dendrimer surface chemistry presentations of arginine–glycine–aspartic acid (RGD)-type linear polypeptides from PAMAM dendrimers have been used to characterize unique cell adhesion properties based on nanopatterning [74]. Modification of PAMAM dendrimer surface chemistry by PEGylation, followed by conjugation of 4-thiobutylamidine (dramatically reduced cytotoxicity whilst improving the ability to modulate P-glycoprotein efflux and tight junction integrity). These properties combined with CNDP-directed size and surface charge, as reported by Sadekar and Ghandehari [75], are providing important predictive nanoperiodic property patterns for optimizing dendrimers as oral drug delivery vectors. In addition, changing surface functionality alone can change the mechanism of dendrimer uptake into cells [76] and the efficacy of conjugated drugs [77].

Flexibility/rigidity

The importance of nanostructural flexibility for the enhancement of dendrimer-based transfection properties was first suggested by Tang *et al.* [78]. The significance of this CNDP was subsequently exploited by Peng *et al.* [79]; these authors reported that using dendrimer vectors with more flexible cores led to enhanced transfection activity. Furthermore, flexibility of the dendritic branching motif in dendrimers has been directly implicated in the release properties of dendrimer encapsulated drugs [80]. An in-depth model describing the physics behind the functional need for particle flexibility in biological systems has been reported previously [81]. According to Habib *et al.* [82], a PAMAM dendrimer possessing a Peng-type flexible core is critical in the transfection of small activating RNA (saRNA) which successfully upregulated albumin and regressed liver cancer tumour volumes.

Elemental composition

In general, soft [S-n]-type organic nanoparticles (Fig. 3) are considered to be more biocompatible, more flexible and less toxic than hard [H-n]-type inorganic nanoparticles which tend to form rigid less biocompatible assemblies. On the other hand, hard nanoparticles such as [H-2]-type cadmium chalcogenide (quantum-dots) are considered to be much less biocompatible and have higher *in vivo* toxicity. An exception may be [H-1]-type gold nanoclusters with dimensions >15 nm [65].

Based largely on many of these compelling structure-controlled CNDPs described above, dendrimers/dendrons have become an area of interest for the development of nanotherapies and devices. The intrinsic structural control and tunable CNDPs observed with dendrimers present significant and previously unavailable opportunities in medicine [83]. Optimizing these CNDPs for a chosen application should be expected to enhance therapeutic efficacies and improve diagnostic/imaging capabil-

ities (Fig. 4). These issues are described in greater detail in the following section.

Dendrimers: structure-controlled, CNDP-engineerable nanoparticles

The CNDP-driven nanoperiodic paradigm is used to examine intrinsic dendrimer features as well as their unique CNDP-tunable nanoperiodic patterns. These features are often referred to collectively as 'dendritic effects' [84]. The structure-controlled CNDPs may be exploited for optimizing functions involving PKs, PDs, drug delivery strategies, excretion modes, biodistribution patterns, biocompatibility and nanotoxicology [43]. This CNDP-driven approach may be viewed as a powerful tool for predicting, synthesizing and optimizing a wide range of nanoparticle–drug constructs. Applicable to all monodispersed nanoparticles, this strategy should be expected to accelerate and enhance the probability of successful preclinical investigations, clinical trials and FDA approvals.

Presently, dendrimers are the only known synthetic nanoparticle category that allows mathematically defined synthetic control and systematic engineering of a nanostructure with total control over all six dendrimer CNDPs. As a consequence, many unique CNDP-directed nanoperiodic property patterns or dendritic effects have been reported [84]. Furthermore, as noted by Mignani *et al.* [83], dendrimer–drug conjugates are generally considered to be more adaptable to a greater range of nanomedicine administration routes (Fig. 4) and targeting strategies (Figs 7 and 10) than traditional polymer–drug conjugates and essentially all other nanoparticle categories.

This CNDP-driven approach may be viewed as a powerful tool for predicting and synthesizing a wide range of nanoparticle–drug constructs with optimized function for nanoparticle pharmaceutical activity, gene transfection applications, enhanced drug solubilization, excipient properties and *in vivo* imaging/targeting nanodevice design. Application of these CNDP-directed engineering strategies to essentially any monodispersed, organic (soft) or inorganic (hard), quantized nanoparticle category (Fig. 3) is expected to accelerate and enhance the probability of successful preclinical investigations, clinical trials and FDA approvals.

More specifically, we examine intrinsic features as well as certain CNDP-directed nanoperiodic property patterns associated with dendrimers. As members of a fourth new major polymer class, dendrimers are referred to generically as 'dendritic polymers', after the three major traditional architectural types: linear, cross-linked and branched polymers [22]. We discovered these precise nanostructures in 1979 as a new class of macromolecules and later coined 'dendrimers' by Tomalia *et al.* [85, 86]. Dendrimers may be derived from virtually any soft or hard atomic element found in the periodic table [18, 22, 28] and have been synthesized with dozens of different elemental compositions. They are broadly divided into two major categories: covalent (Fig. 5) or supramolecular [87] types. However, we will focus only on covalent-type dendrimers in this review.

Amongst the most widely studied dendrimer compositions (i.e. families) of interest to nanomedicine are the Denkewalter-type PLL (Starpharma), Tomalia-type PAMAM (NanoSynthons LLC, Mt. Pleasant, MI, USA and Dendritech, Midland, MI, USA), Hult-type poly(ester) (bis-MPA) (Polymer Factory, Stockholm, Sweden), Majoral/Caminade-type phosphorous-based (Biodendrimers, Vic en Bigorre, France), Vögtle/Meijer/Multhaupt-type poly(propyleneimine) (PPI) (SyMO-Chem BV, Eindhoven, the Netherlands), Simanek-type triazine-based [88] and Jayaraman/Jain-type poly(propyletherimine) (PETIM) dendrimers [89]. All of these types of dendrimers are commercially available with the exception of the latter two.

All covalent dendrimers possess three major architectural components: (i) a central core, (ii) an interior of concentric branch cells derived from tree-like dendrons and (iii) peripheral/terminal surface groups. The tethered covalent connectivity between these three architectural components governs the overall physicochemical features of a dendrimer. Dendrimers are derived from conven-

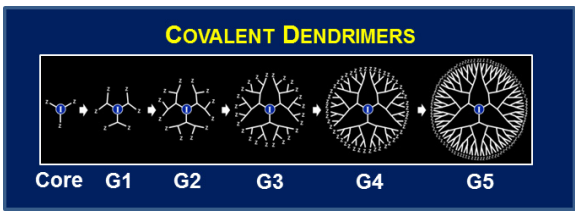


Fig. 5 *Divergent synthesis of a covalent dendrimer series [generation (G) = 0–5] with core multiplicity of 3 and branch cell multiplicity of 2.*

tional monomers, but differ from classical polymers by growing around a core in concentric 'onion skin-like' branched layers. These concentric branched layers are termed 'generations'. Each successive generation (G) increases the dendrimer mass (approximately twofold) and precisely amplifies terminal functional groups geometrically (i.e. 2^G) whilst systematically increasing the diameter approximately 1 nm per generation. These highly amplified surface groups provide mathematically defined polyvalent surface sites from which to conjugate drugs, stealth moieties, targeting groups or modify nanoparticle zeta potentials. More than 100 different dendrimer compositions and 1000 different surface modifications have been reported. This generational growth produces discrete, three-dimensional, highly branched nanostructures with precise elemental compositions, molecular weights, monodispersities, nanometric sizes and structural control of CNDPs approaching that of biological proteins. Referred to as 'artificial proteins' [90, 91], dendrimers and dendritic polymers have received considerable attention worldwide with over 16 000 patent and literature citations in the past decade alone [22].

The iterative construction of covalent dendrimers involves either divergent or convergent synthesis strategies. These strategies generally use orthogonal [92], protect–deprotect [93, 94] or click chemistry [95, 96] protocols for producing the covalent connectivity. Each dendrimer in a generational series (G = 0–5) (Fig. 5) is a discrete compositional entity with precise molecular formulas, elemental compositions, molecular weights, number of surface groups and nanometric sizes (nm). As such, a discrete single macromolecular structure may be obtained at each generation with a precise molecular weight and polydispersity of 1, in the same way as for a traditional organic structure (Fig. 6a) or a protein (Fig. 6, right column). This is in contrast to polydispersed traditional linear polymers such as PEGs which have been used for polymeric therapy as shown in Fig. 6(b). Most

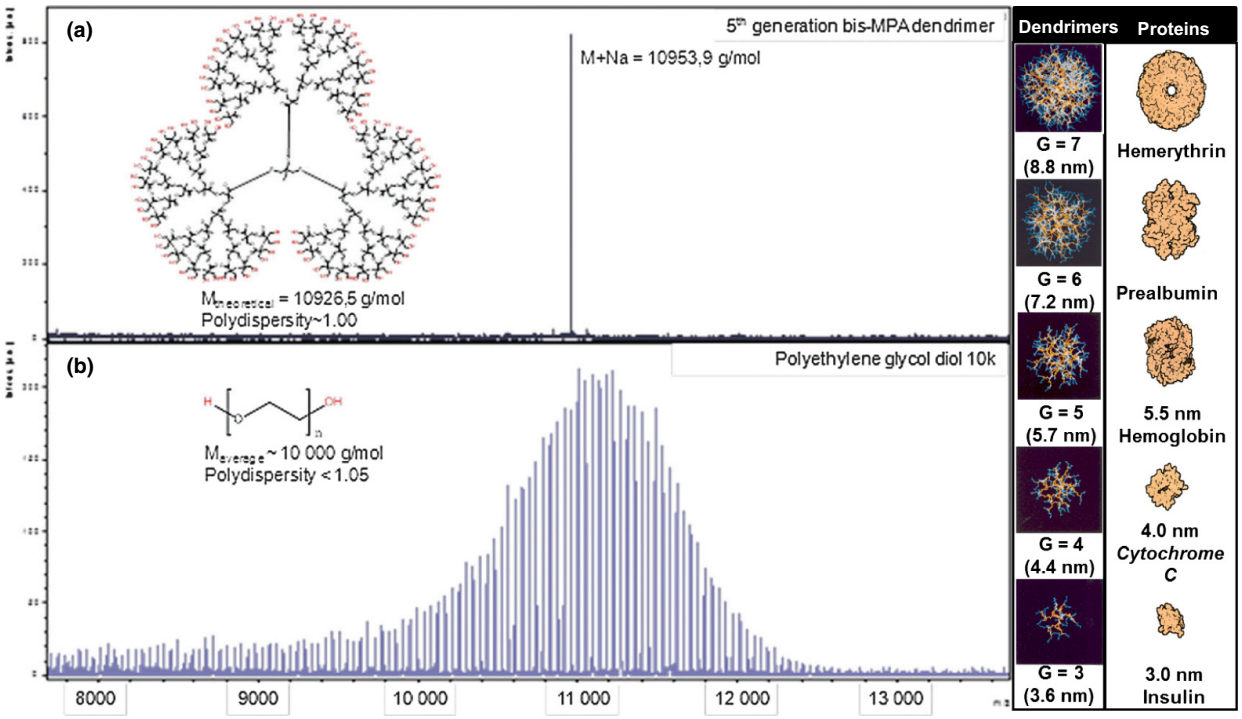


Fig. 6 *(a) Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) spectra of a monodisperse generation-five 2,2,-bis(hydroxymethyl) propionic acid-based dendrimer (top) and (b) well-defined linear polyethylene glycol polymer (bottom), reproduced in part from Ref. [98] with permission from The Royal Society of Chemistry. Right column, a comparison of poly(amidoamine) dendrimer dimensions with comparable protein dimensions illustrating the potential for dendrimer-based protein mimicry related to nanometric size and shape [99].*

notable is the ability to engineer dendrimer sizes as a function of generation to actually mimic many well-known proteins as shown in Fig. 6 (right column) [97].

It is clear that covalent dendrimers may be viewed as quantized nanoscale building blocks that show a high level of atom mimicry by forming unlimited stoichiometric, covalent nanocompounds or supramolecular assemblies when combined with other soft or hard nanoelement categories, as illustrated in Fig. 3.

Intrinsic dendrimer characteristics: tunable CNDPs for optimized nanomedicine applications

Due to their unique macromolecular properties and well-defined relationships with a wide range of therapeutic drugs (Fig. 7A), and their adaptability to a variety of drug targeting applications in nanomedicine (Fig. 7B), dendrimers have attracted considerable attention. Many of these dendrimer-based strategies are proposed for *in vivo* targeted delivery of traditional drugs, imaging and diagnostics, and as active polyvalent nanoscale pharmaceuticals for the treatment of infectious diseases (see below). This activity is largely attributed to a growing number of novel properties, driven by dendritic architecture as well as the ability to control and engineer certain CNDPs for these nanostructures, including the following.

- Precise synthetic control over nanoscale CNDPs to produce discrete macromolecules that scale closely to the dimensions of proteins, yet do not exhibit immunogenic responses. Such monodisperse size control is not currently possible with traditional polymer architectures (Fig. 6b) [100].
- Versatile, well-defined polyvalent surface chemistry and interior nanocontainer properties that may be engineered to deliver therapeutic levels (20–40% w/w) of encapsulated, conjugated drugs or complexed drugs.
- Dendrimer–drug conjugates with dimensions <10 nm versus polymeric micelles which are limited to >30 nm [53, 101]. These smaller dendrimer conjugate dimensions (i.e. <8 nm) provide deeper tumour penetration and renal excretion features.
- Dendrimer–drug conjugates may be engineered and targeted to deliver drugs selectively to disease sites thus avoiding off-target delivery of toxic drugs to sensitive organs (for example, reducing exposure to cardiotoxic drugs).
- Dendrimer–drug conjugates may be readily freeze-dried to produce water-soluble powders with enhanced drug solubility thus avoiding potential toxic side effects due to formulation solubilizers.

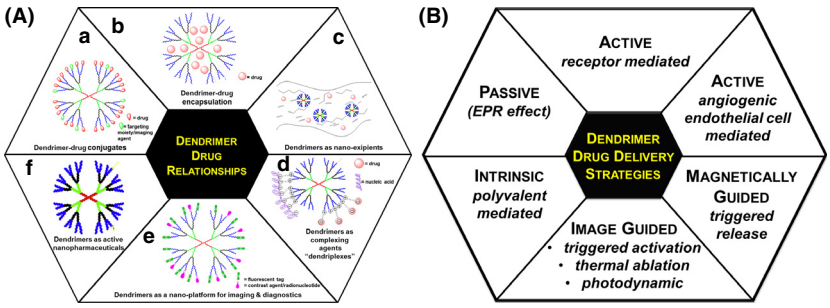


Fig. 7 *Dendrimer–drug relationships based on structure-controlled critical nanoscale design parameters that may be readily engineered and various nanomedicine drug delivery strategies. (A) Dendrimers exhibit at least six unique relationships with traditional drugs including dendrimer–drug conjugates (a), encapsulation of drugs in dendrimers (b), dendrimers as nanoexcipients for drugs (i.e. oral delivery) (c), dendrimer–drug complexes (d), dendrimer imaging/ablation agent conjugates (e) and dendrimers as intrinsically active nanopharmaceuticals (f). (B) The diversity of well-defined dendrimer–drug relationships provides at least six demonstrated drug targeting strategies: passive, active (receptor mediated), active (angiogenic endothelial cell mediated), magnetically guided, image guided and intrinsic (polyvalent mediated).*

- Dendrimers are stable monodisperse covalent structures in contrast to liposomes which are metastable and may rearrange to various sizes and shapes.
- Precise size-calibrated nanostructures that may be decorated with engineered ratios of targeting-, imaging- or stimuli-responsive moieties [102] suitable for *in vivo* theranostic applications [103].
- Well-defined nanoscale compositions that are degradable (i.e. bis-MPA dendrimers) [98], self-immolative [104], biodegradable (i.e. PLL dendrimers that break down to natural L-lysine and are readily metabolized) [105] or suitable for desirable excretion and biodistribution modes.

Importance of dendrimer-based CNDP property patterns related to nanomedicine

Extensive dendrimer-based nanoperiodic property patterns related to their intrinsic physicochemical, functional and application properties have been reported. In general, these patterns are reported as disconnected empirical observations with no relation to a unified perspective. These CNDP-directed nanoperiodic property patterns have been observed since the discovery of dendrimers in the1980s [85]. Nanoperiodic patterns or 'dendritic effects' [84] have been routinely used in the past for *a priori* predictions of generation-dependent dendrimer properties. Within the context of the nanoperiodic framework (Fig. 3), it has been noted that many of these patterns occur as a function of incremental changes in CNDPs and thus may be used for establishing critical periodic patterns and rules of importance to nanomedicine. Although too extensive to review in detail here, these nanoperiodic property patterns are now recognized as powerful tools for structural optimization of many nanomedicine applications including drug delivery, vector design and nanotoxicology as well as for directing important functions such as excretion and biodistribution modes and gene transfection efficacy. Significant examples of dendrimer-based nanoperiodic patterns related to nanomedicine include CNDPs such as nanoparticle size and surface chemistry. As described below, these two parameters may directly influence excretion modes, organ selectivity, EPR or skin penetration.

For example, important *in vivo* organ-specific biodistribution and excretion patterns based on real-time MRI studies have been observed. It is noteworthy that these patterns were found to be strictly dependent on dendrimer size and surface/interior chemistry [45, 48]. Furthermore, many of these excretion and biodistribution property patterns were found to be sensitive to incremental size changes as small as 1–3 nm. Unlike most other nanoparticle features, such finely tuned incremental nanoparticle sizes can be readily achieved with dendrimers as a function of generation. As illustrated in Fig. 8, specific organ targeting, prevention of nanoparticle extravasation from the circulatory system and kidney versus liver excretion may be controlled as a function of only several nanometer differences in nanoparticle size. It should also be noted, as shown in Fig. 8, that the rigidity/flexibility as well as the dendrimer surface congestion may be controlled as a function of generation level. These generation-dependent features directly determine dendrimer drug encapsulation properties (i.e. $G = 4–6$) which may be exploited for engineering optimum drug delivery devices (see Fig. 10).

As another example, these CNDP-directed nanoperiodic property patterns may be used to optimize the design/engineering of dendrimer-based transdermal delivery vectors for a variety of therapies (Fig. 9). As reported by Yang *et al.* [106], very small dendrimer size differentials (approximately 2 nm) as well as tunable surface chemistries have been found to play pivotal roles in determining the degree of epidermal penetration for either transdermal or topical nanomedicine applications. Engineering these simple dendrimer-based CNDPs (i.e. size and surface chemistry) provides a powerful paradigm for optimizing suitable epidermal penetration properties for transdermal drug delivery applications. Alternatively, these CNDP-directed property patterns have been used to demonstrate nonepidermal penetrating drug delivery properties; the dendrimer may be used as an active polyvalent nanoscale topical microbicide such as with the commercial dendrimer-based product VivaGel.

Engineering CNDPs for optimized dendrimer-based drug delivery strategies

Undoubtedly, structure-controlled CNDPs are amongst the most important features of dendrimers for nanomedical applications. As illustrated

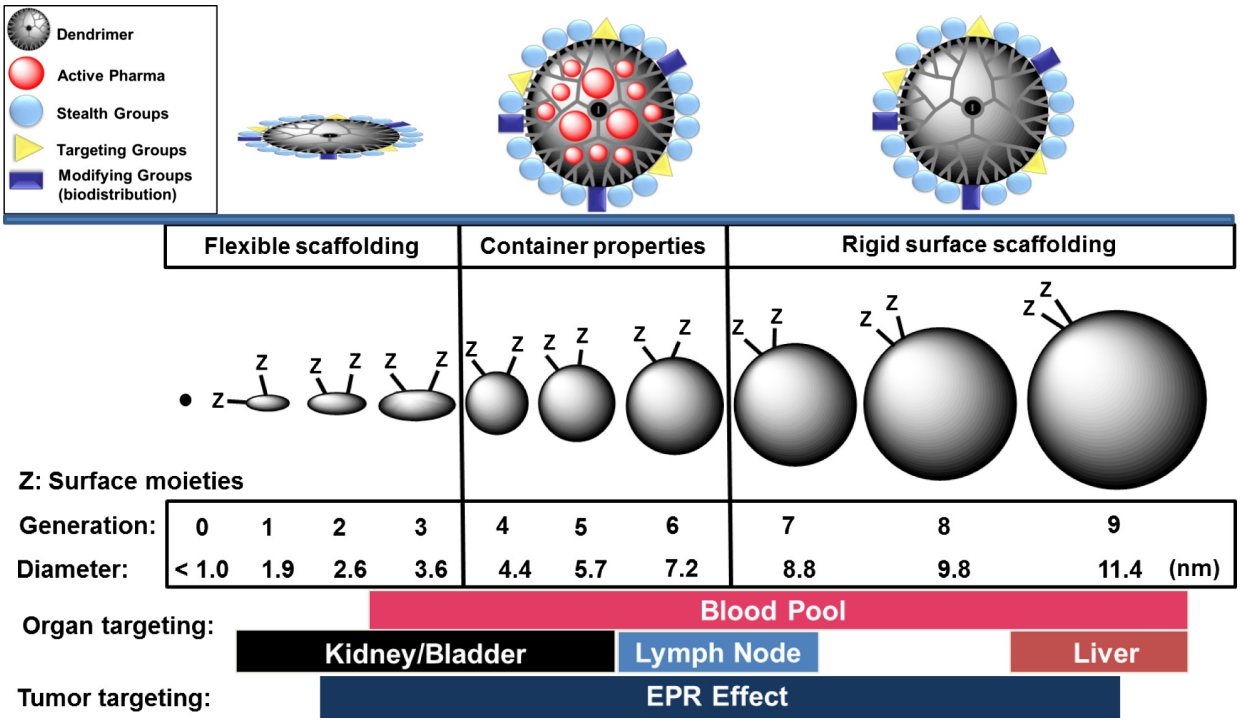


Fig. 8 *Nanoperiodic drug encapsulation and organ-/tumour-targeting patterns observed for poly(amidoamine) dendrimers-based on critical nanoscale design parameters such as size, interior/surface chemistry and flexibility/rigidity [45].*

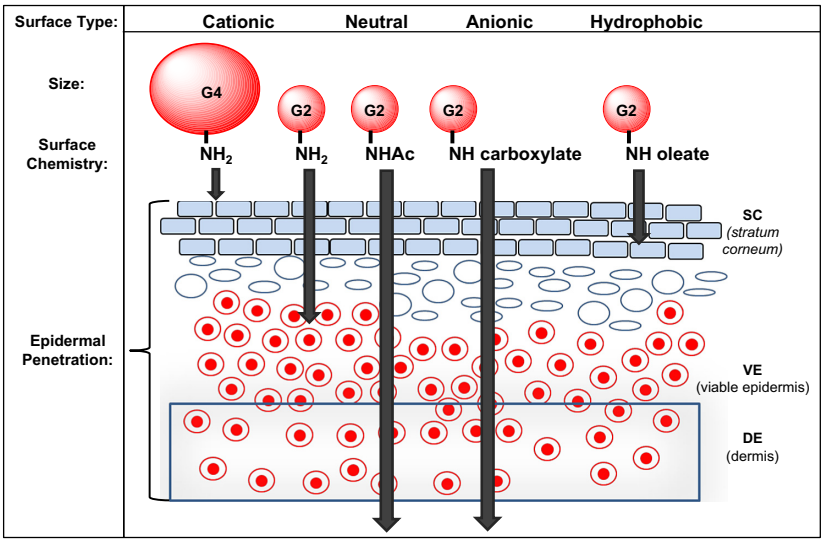


Fig. 9 *A comparison of critical nanoscale design parameter (i.e. size and surface chemistry)-directed patterns associated with epidermal penetration by poly(amidoamine) dendrimers. These quantized epidermal penetration patterns are useful for designing and engineering optimized dendrimer prototypes for use as topical microbicides or transdermal drug delivery vectors. Reproduced and adapted with permission [106]. Copyright: 2012 American Chemical Society.*

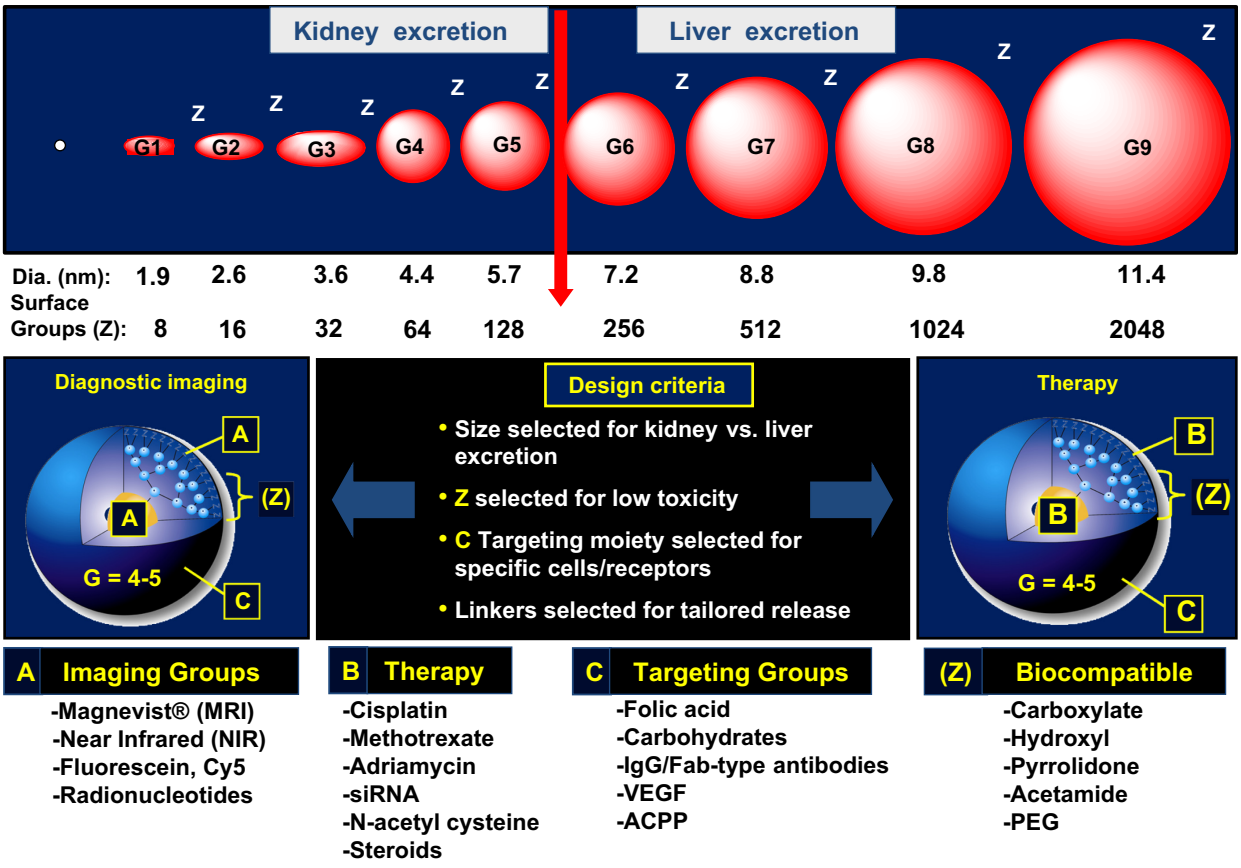


Fig. 10 *A schematic illustration of dendrimer critical nanoscale design parameter control and engineering for optimizing prototypes suitable for various nanomedical applications. (i) Size control (approximately 1 nm per generation) with mathematically defined polyvalent surface functionality; (ii) polyvalent dendrimer surface chemistry can be chemically partitioned into imaging groups (A), therapy with cleavable linkers (B), targeting groups (C) and biocompatible or circulatory enhancement groups (Z) [45].*

in Fig. 10, these structure- controlled CNDPs may be systematically designed and modified to exhibit desired excretion modes as a function of size. On the other hand, the polyvalent dendrimer surface chemistry may be mathematically designed and engineered to introduce desirable ratios of imaging, therapy, targeting or biocompatibilizing groups for a single-molecule nanodevice. These CNDP features may be optimized for a desired drug administration mode, targeting strategy or to overcome various *in vivo* barriers as described above (Fig. 4). Nevertheless, it is very important to properly design and utilize appropriate cleavable linkers when conjugating therapeutic components (Fig. 10) to ensure appropriate release of active therapy at the disease site [107].

Many of these CNDP-based principles were used by Tsein *et al.* [108] to design a multifunctional dendrimer-based targeting nanodevice to image primary and metastatic cancer sites in real time, as described below (Fig. 11). This targeting strategy was suitable for either delivering therapy or assisting surgeons in the resection of primary tumours by more effectively defining tumour boundaries.

Designing a successful dendrimer-based theranostic platform

The earliest dendrimer–drug conjugates (Fig. 15) generally involved simple associations, complexations or encapsulations with small molecule pharmaceuticals [83]. The primary objective was to increase *in vivo* residence time, modify biodistribu-

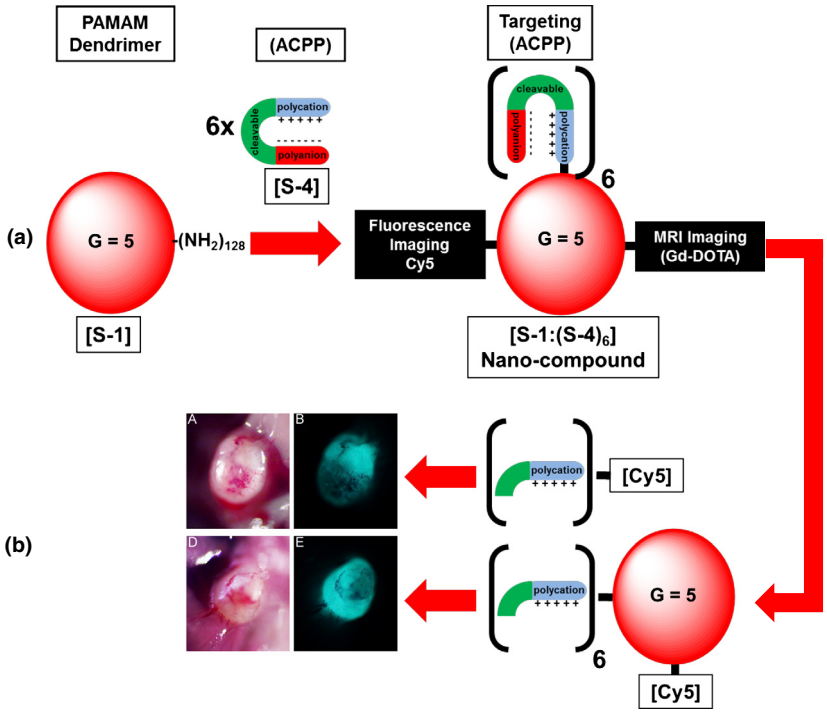


Fig. 11 *(a) An amine-terminated PAMAM dendrimer (G = 5), that is, an [S-1]-type nanoelement, conjugated with six activated cell-penetrating peptides (ACPPs), that is, 6x-[S-4]-type nanoelements, as targeting moieties, followed by conjugation of either Cy5 fluorescent image moieties or Gd-DOTA imaging groups to produce a dendrimer-based theranostic device with tumour-specific targeting features to tumours bearing metalloproteases, that is, a stoichiometric nanocompound designated [S-1:(S-4)$_6$]. (b) a tumour targeted/imaged with an (ACPP)-Cy5, that is, an [S-4]-Cy5-type conjugate, compared to a tumour targeted/imaged with the dendrimer-based theranostic, that is, [S-1:(S-4)$_6$], adapted from [117], and the mouse xenograft is reprinted with copyright permission from PNAS* [118].

tion profiles or target therapy to disease sites with concurrent enhancements of PKs and PDs compared to naked drug administration. Attention is now turning towards more complex platforms that integrate real-time diagnostic, imaging, targeting and therapy delivery features into a single-molecule nanodevice. These multifunctional, integrated nanodevices are broadly referred to as 'theranostics' [109–111]. In some instances, targeted multimodal imaging [112], radiation-induced photodynamic ablation [113] or protease-triggered drug release in combination with imaging [114, 115] features have been designed into these devices. This area has been extensively reviewed recently [116].

However, an effective PAMAM dendrimer-based theranostic platform for detecting, imaging and delivering cancer therapy to both primary and metastatic cancer sites has been reported recently by the group of Chemistry Nobel Laureate, Roger Tsien [108, 117, 118]. This theranostic device is essentially a stoichiometric nanocompound obtained by conjugating six activatable cell-penetrating hairpin peptides (ACPPs), that is, 6X-[S-6]-type peptides, onto a single $G = 5$, PAMAM dendrimer, that is, [S-1] soft nanoelement (Fig. 11a). This [S-1:(S-4)$_6$]-type nanocompound is then further modified with either Cy5 fluorescent or Gd-based MRI imaging components. In animal models, this PAMAM dendrimer theranostic device was shown to successfully enhance primary tumour boundaries for ACPP-targeted/image-guided surgical resection, thus leading to substantially higher cancer survival rates. Recent work has shown that these ACPP sequences may also be modified to detect atherosclerotic plaques by targeting associated thrombin activity [119].

Finally, it is pleasing to note that recent investigations have used our earlier nanoperiodic property patterns derived from *in vivo* MRI studies [45] as a model for designing dendrimer-based theranostics

for the detection, imaging and treatment of prostate cancer [111].

Current applications of dendrimers in preclinical in vivo efficacy studies

It is now becoming widely recognized that dendrimer-based *in vivo* tissue interactions such as complement activation, protein interactions (i.e. opsonization), excretion modes, cellular uptake, cell cytotoxicity and *in vivo* biodistribution patterns are strongly influenced by their CNDPs (Fig. 4). Many of these issues have been extensively reviewed and reported as empirical observations [120–123]. However, they remain to be quantified in the context of their CNDP-dependent nanoperiodic property patterns to provide a unified perspective.

A growing awareness of these *in vivo* CNDP-dependent nanoperiodic property patterns in combination with the many unique dendrimer–drug relationships (Fig. 7A), a wide range of drug administration/targeting modes (Fig. 7B) and new intrinsic dendrimer-targeting properties (i.e. inflammation targeting), have led to dramatically enhanced activity in emerging preclinical nanomedicine applications. A brief overview of selected preclinical studies involving dendrimer-based *in vivo* therapies is provided in Table 2, followed by a discussion of recent nanomedicine activities in several areas, including inflammation and neuroinflammatory disorders, infection, oncology and ocular diseases.

Inflammation

Inflammation is an increasingly important clinical target for nanomedicine. It is associated with many major diseases including arthritis, cancer, neurodegenerative disorders and cardiovascular plaque formation. Substantial evidence suggests that appropriate attenuation of inflammation may produce a broad range of beneficial outcomes. As such, it is noteworthy that dendrimers have been used successfully not only as delivery vectors for traditional anti-inflammatory drugs but also directly as active nanostructures due to their intrinsic anti-inflammatory properties.

Initial investigations with PAMAM dendrimers demonstrated their effective roles as delivery vectors for a variety of nonsteroidal and steroidal anti-inflammatory drugs [159]. However, subsequently we showed that naked, unmodified PAMAM dendrimers exhibited unexpected anti-inflammatory properties in three different rat models of arthritis [129]. In this study, we compared the anti-inflammatory activity of intraperitoneal administration of naked, unmodified PAMAM-G4/4.5 dendrimers presenting simple surface chemistries, such as amine, hydroxyl and carboxylic acid terminal groups, with that of the traditional anti-inflammatory agent indomethacin. All simple unmodified dendrimers showed dose-dependent effects that were similar to those of free indomethacin. The amine-terminated dendrimer ($G = 4\text{-}NH_2$) showed significant efficacy at moderate doses (approximately 8 mg kg^{-1}, intraperitoneal administration) up to 8 h after administration. Sustained attenuation of inflammation was shown with daily dosing of the dendrimer up to 14 days. A subsequent investigation by Kakkar *et al.* [160] extended this work by showing that even lower-generation PAMAM dendrimers (i.e. $G = 0–1$) possessing hydroxyl groups showed similar anti-inflammatory properties. These authors proposed that the simple hydroxylated PAMAM dendrimers were functioning as cyclooxygenase-2 and inducible nitric oxide synthase inhibitors based on enzyme inhibition and molecular simulation studies. It is noteworthy that acetylation of $G = 5$; amine-terminated PAMAM dendrimers to produce corresponding terminal acetamido groups appeared to eliminate this anti-inflammatory activity. On the one hand, $G = 5$; PAMAM dendrimers, possessing acetamide terminal groups in concert with conjugated folic acid and methotrexate targeted activated macrophages expressing folate receptors were shown to reduce inflammation parameters in a collagen-induced arthritis rat model [124]. These dendrimer–folic acid–methotrexate conjugates were systemically delivered at a high methotrexate dose (approximately 15 mg kg $week^{-1}$) compared to that of free methotrexate. In related studies, it was shown that dendrimer–folic acid conjugates could be used to enhance targeting of inflammatory tissue in a chlamydia-induced reactive arthritis model [125, 126].

It is noteworthy that this anti-inflammatory activity is not dependent solely on the PAMAM dendrimer interior composition. In addition to Tomalia-type PAMAM dendrimers, it has now been shown that Majoral-type phosphorus interior-based dendrimers with aminobisphosphonate surface groups exhibited anti-inflammatory properties [127].

Table 2 *Preclinical studies using dendrimer-based nanomedicine for* in vivo *therapy*

Application	Platform	Active agent	Remarks/Clinical status	References
Inflammation				
Drug delivery	PAMAM (G5)	Methotrexate	Collagen-induced arthritis	[124]
	PAMAM (G4)	Naked dendrimer	Chlamydia-induced reactive arthritis	[125, 126]
	Phosphorus ABP terminated	Naked dendrimer	Rheumatoid arthritis	[127]
	PAMAM (G4)	Naked indomethacin	Improved drug solubility and increased accumulation in inflammatory regions in arthritic rats	[128, 129]
	PAMAM	Methyl-prednisolone	Allergen-induced lung inflammation	[130]
	Mannose	Mannose-grafted dendrimer	Acute lung inflammation prevention	[131]
	PAMAM (G4)	Fluocinolone acetonide	Retinal degeneration model	[132]
	PAMAM (G4)	N-acetyl cysteine (NAC)	Single systemic administration of D-NAC on day 1 of life showed improvement in myelination and motor function in rabbit model of inflammation-induced cerebral palsy	[133]
Cancer				
Drug delivery or intrinsic dendrimer surface chemistry	PAMAM (G3.5)	Cisplatin	Increased MTD and bioavailability of cisplatin, prolonged survival of mice with melanoma	[134]
	PEG-PLL	Doxorubicin	Greater antitumour activity in breast cancer compared to liposomal formulation	[135]
	PEG-PAMAM	Methotrexate	Greater antitumour activity compared to non-PEG dendrimer	[136]
	PEG-PAMAM	Doxorubicin	RGD-conjugated PAMAM dendrimer showed greater tumour accumulation with PEG than without, and improved survival in animals with gliomas	[137]
	PPI	Surfactants	Greater antitumour activity in brain tumours	[138]
	PEG-PAMAM	5-fluorouracil and folate	Haemolytic toxicity was reduced with PEG–folic acid dendrimers, and accumulated in tumours compared to non-PEG dendrimers	[139, 140]
	PEG-PLL	Camptothecin	Enhanced survival in human colon carcinoma models	[141]
Gene delivery	PAMAM	Oligo-DNA	Dendrimers could effectively deliver ^{111}In-labelled oligo-DNAs to tumour	[142]
	PAMAM	*Angiostatin* gene	Intratumoural administration of dendrimers with the *Angiostatin* gene effectively inhibited tumour growth	[143]
	PPI	DNA	Intravenous administration of transferrin-bearing PPI dendrimers led to rapid and sustained tumour regression	[144]

Table 2 (*Continued*)

Application	Platform	Active agent	Remarks/Clinical status	References
Infectious disease				
Drug delivery or intrinsic dendrimer surface chemistry	PETIM-DG		Minimizes bacterial invasion in Shigella-induced gut wall damage in rabbits	[145]
	PAMAM-OH		Prevention of *Escherichia coli* infection in pregnant guinea pig chorioamnionitis model	[146]
	PAMAM	Nacetyl-neuraminic acid	Inhibition of HA-mediated adhesion in influenza model	[147]
	PEG-PPI	RIF	Tuberculosis treatment	[148]
	PLL (VivaGel)		Protects against HIV and HSV infections in humans after vaginal administration; clinically approved	[149]
Gene delivery	PAMAM	siRNA	Suppressed HIV infection in humanized mouse model	[150]
Diagnostic				
MR contrast	PAMAM G4, G6	Gd	Enhanced retention, reduced renal clearance	[45, 151–153]
CT/SPECT	PAMAM G4	Triiodinated and chelated ^{99}Tc	Increases blood circulation time and provides effective simultaneous contrast enhancement in both CT and SPECT	[154]
PET	Peptide dendrimer conjugate	Cyclic 9-amino acid peptide (LyP-1)	Ability to detect atherosclerotic plaques using *in vivo* PET imaging	[155]
CEST	PPI, PAMAM G2, G5	DOTAM, MRI contrast agents	Used for pH mapping, to detect gliomas at very low agent concentrations	[156–158]

PAMAM, poly(amido amine); ABP, azabisphosphonate; G, generation; MTD, maximum tolerated dose; PPI, polypropylenimine; RGD, arginine-glycine-aspartic acid; PEG, poly(ethylene glycol); PLL, poly-l-lysine; PETIM, poly(propyl ether imine); DG, dendrimer glucosamine; HA, hemagglutinin; RIF, rifampicin; HIV, human immunodeficiency virus; HSV, herpes simplex virus; siRNA, short interfering ribonucleic acid, CT, computed tomography; SPECT, single-photon emission computed tomography; MR, magnetic resonance; MRI, magnetic resonance imaging; LyP-1, cyclic 9-amino acid peptide; PET, positron emission tomography; DOTAM, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracarboxy amide; CEST, chemical exchange saturation transfer.

Hayder *et al.* recently demonstrated that intravenous injection of these phosphorous-based dendrimers presenting aminobisphosphonate surface groups inhibits the secretion of proinflammatory cytokines and osteoclastogenesis. These two fundamental monocyte-dependent processes are associated with inflammation and bone erosion in rheumatoid arthritis [127].

In addition to effects on systemic inflammatory diseases, it has been reported that conjugates of PAMAM and the corticosteroid methylprednisolone reduced allergen-induced lung inflammation by providing improved residence time in the lung following intranasal administration [130]. More recently, the inhalation of dendrimer grafted with mannose units was reported to inhibit neutrophil recruitment thus preventing ongoing acute lung inflammation [131]. It is noteworthy that certain dendrimer–glucosamine conjugates used for the treatment of infectious diarrhoeal diseases (see below) may also exhibit specific anti-inflammatory activity [145].

Neuroinflammation

Both acute and chronic neuroinflammation are important in the pathogenesis of many neurological disorders including cerebral palsy, autism, multiple sclerosis and Alzheimer's disease. Activation of microglia and astrocytes is acknowledged to play a pivotal role in the regulation and immune defence of the central nervous system (CNS) and is directly implicated in the neuroinflammatory process. Therefore, attenuation of inflammation by targeting these cells can be expected to provide significant therapeutic benefits. Nevertheless, targeting diffuse neuroinflammation in the CNS and delivering therapeutics to attenuate these conditions remains a serious challenge. Recently we have reported that intravitreal administration of PAMAM dendrimers demonstrated novel, intrinsic targeting properties by selectively localizing in activated microglia and astrocytes in the brain and retina [132, 133, 161]. These PAMAM dendrimer–drug conjugates have shown promising preclinical efficacy for alleviating neuroinflammation associated with both ocular and brain diseases. When a hydroxyl terminated; G4; PAMAM dendrimer was administered into the vitreous chamber of a healthy rat, the dendrimer was readily cleared and there was minimal retinal retention or cellular uptake over 24 h. However, when administered to a Royal College of Surgeons rat model of retinal degeneration, there was significant retinal uptake into activated microglia, photoreceptors and the retinal pigment epithelium. This pathology-dependent uptake mechanism was utilized for targeted drug therapy. A clinically used steroid, fluocinolone acetonide (FA), was conjugated to the hydroxyl-functionalized; G4; PAMAM dendrimer, producing a nanodevice with sustained drug release for up to 90 days. It is remarkable that a single intravitreal administration of 1 μg FA conjugated to 6 μg dendrimer (D-FA) showed significant neuroprotection, preservation of photoreceptor nuclear cell counts and attenuation of inflammation for 1 month [132]. This D-FA nanodevice compared favourably to a commercial implant over a 1-month period in the same animal model. However, further toxicity and large animal studies are required to enable clinical translation.

Advances in the application of therapeutic dendrimers have indicated that they may also be used to treat neuroinflammatory disorders of the brain, where most therapeutics must cross the highly selective BBB and reach a diffused area of disease-affected cells and tissue to achieve efficacy (Fig. 12). Following subarachnoid administration, PAMAM dendrimers localized in inflammation-associated cells such as microglia and astrocytes, even in regions far from the site of injection, for example, in a rabbit model of maternal inflammation-induced cerebral palsy [162]. Such site-specific localization into cells associated with neuroinflammation was observed even upon systemic administration to rabbits with cerebral palsy. Transport across the BBB and brain parenchyma and the subsequent cellular localization of this dendrimer are contrary to expectations; the requirements for transport into the brain are typically thought to involve molecular weights smaller than 500 Da (dendrimers are approximately 15 kDa), lipophilicity (dendrimers are hydrophilic) and long circulation times (these dendrimers have relatively short circulation times). Thus, it was hypothesized that impairment of the BBB induced by the neuroinflammatory process enabled dendrimer uptake into the brain. However, unlike typical nanoparticles and small drugs that remain near the leaky blood vessels, these dendrimers appear to diffuse well within the brain parenchyma, allowing greater distribution within the brain tissue. More importantly, a single systemic dose of hydroxyl terminated; PAMAM-G4 conjugated to N-acetyl cysteine (D-NAC) produced dramatic improvements in motor function, neuronal counts and myelination, and reduced neuroinflammation. The D-NAC conjugate was more efficacious when compared to tenfold higher amounts of free drug in a challenging paediatric neurological disorder for which there is no effective cure. Detailed mechanistic studies suggest that these improvements may be largely due to the intrinsic dendrimer-targeting features that selectively delivered these drugs to targeted cells in the brain [133]. Recent studies, the first of their kind for PAMAM dendrimers, have built on these findings to develop combination therapy approaches for treating brain injury following hypothermic cardiac arrest in a large animal (canine) model. As in the case of rabbits with cerebral palsy, the dendrimers were transported to the sites of injury in the brain, selectively localizing in activated microglia and injured neurons [161]. The ability of PAMAM dendrimers to localize only in the injured areas of the brain has significant implications for reducing the neurotoxicity of CNS drugs whilst increasing selective CNS delivery of drugs that are not generally transported across the BBB. This coupled with the fact that the dendrimers are

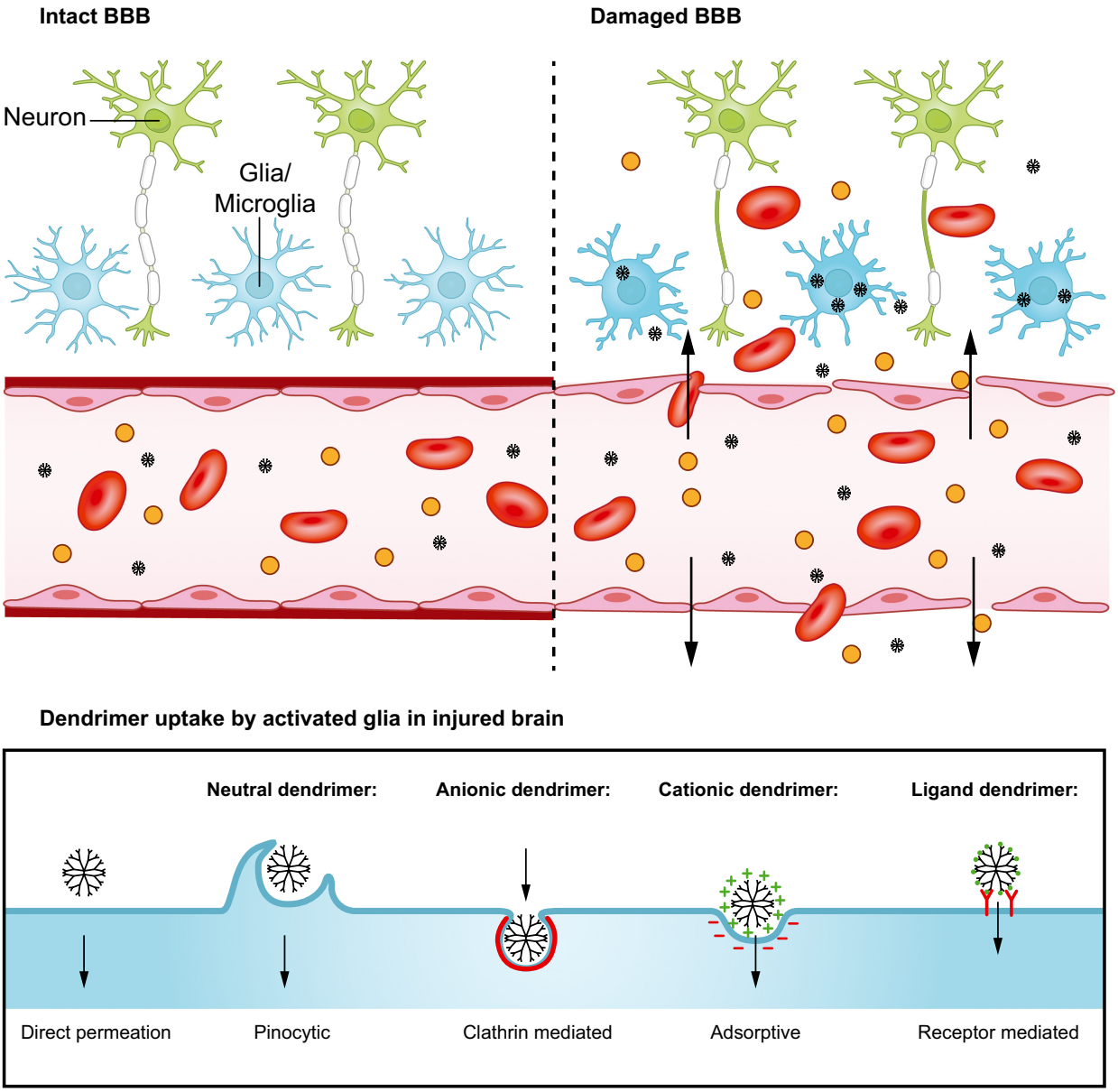


Fig. 12 *Therapeutic dendrimer-based platform (G4; hydroxyl-terminated PAMAM) for targeting neuroinflammation. Dendrimer–drug conjugates traffic into the brain through the blood–brain barrier impaired due to disease, leading to selective internalization in activated microglia. Given the small size (approximately 4 nm) of the G = 4; hydroxyl-terminated dendrimer, clearance occurs via the kidney with a half-life of several hours.*

cleared relatively rapidly from off-target organs may have significant implications for reducing the side effects of many drugs.

These extraordinary dendrimer intrinsic targeting properties and promising distribution patterns in the CNS were further corroborated and shown to be largely dependent upon the dendrimer surface chemistry [163].

Infectious diseases

Until recently, common infectious diseases caused by pathogenic microorganisms, including bacteria,

viruses, parasites and fungi, have been reasonably well controlled by a broad range of antimicrobial and antibiotic agents. Unfortunately the prevalent and indiscrete use of these agents has led to the emergence of many new, resistant, mutated, pathogenic strains [164]. Clearly a critical and universal need for new broad-spectrum antibacterial and antimicrobial agents has arisen. As such, attention has focused on a number of unique dendrimer-based nanostructures that have exhibited both antiviral and antimicrobial activity [165]. Significant work by Chen and Cooper [166], Mintzer and Grinstaff [167] and Balogh *et al.* [168] have demonstrated broad antipathogenic activity for dendrimers. Largely due to their readily modifiable polyvalent surface chemistry, dendrimers have shown potential as both antibacterial and antimicrobial medicines. This appears to be due to interference with pathogenic adhesion to eukaryotic cells, targeting bacterial membranes, imitating detergent activity, mimicking antimicrobial peptides and inhibiting biofilm formation [169]. Their antipathogenic properties have been determined by their low cytotoxicity to eukaryotic cells, and they are being investigated as novel drugs for various infectious diseases, especially those that are persistent, marked by a high mortality rate or untreatable [170].

Recent work by Choi *et al.* [171] has shown that polyvalency associated with dendrimer-based vancomycin conjugates increased avidity to Gram-positive bacteria, including in vancomycin resistant models, by four to five orders of magnitude compared to free vancomycin. Shaunak *et al.* [145] have explored the use of PETIM dendrimer–glucosamine conjugates against a broad spectrum of infectious diarrhoeal diseases including *E. coli,* enterotoxigenic *E. coli,* salmonella, typhoid and *Clostridium difficile*. They demonstrated the inhibitory properties of PETIM-DG against Shigella-induced epithelial gut wall damage in rabbits by minimizing bacterial invasion and reducing local cytokine expression [172]. On the other hand, PAMAM dendrimers have also been used to prevent preterm delivery induced by *E. coli* infection in a pregnant guinea pig chorioamnionitis model. Application of hydroxyl terminated; PAMAM dendrimers into the cervix prevented *E. coli* from ascending into the uterus and reaching the foetus, thus avoiding preterm delivery [146]. Both PAMAM and carbosilane dendrimers have shown the ability to protect against infection in a murine influenza pneumonitis model. Inhibition of the HA-mediated adhesion of the virus strain A/NIB/44/90M (H3N2) has been examined using PAMAM dendrimers conjugated to N-acetylneuraminic acid (Neu5Ac). These dendrimer conjugates were shown to be more effective inhibitors than monomeric Neu5Ac. These results were confirmed by *in vivo* tests using a murine influenza pneumonitis model in which mice were protected from H3N2 subtype infection using a PAMAM dendrimer–sialic acid conjugate [147].

Other recent studies have demonstrated the use of a PEGylated–PPI dendrimer conjugate containing rifampicin as a controlled delivery nanodevice for treating tuberculosis [148]. On the other hand, PEG–PLL dendrimer conjugates and chondroitin sulphate-coated dendrimers exhibited very efficient encapsulation of the antimalarial drug chloroquine; antimalarial activity was comparable to that of free chloroquine [173]. Similarly, a commercial PLL-based dendrimer SPL7013 has been shown to effectively protect against genital HSV or simian/human immunodeficiency virus (SHIV) in mice, guinea pigs and macaques via intravaginal administration [174, 175]. After successful testing in monkeys, SPL7013 has exhibited potent inhibitory activity against HIV-1 and HSV-2 in humans [149]. Recently, the clinical relevance and efficacy of these dendrimer-based therapies has been demonstrated by their advance into Phases II and III human trials.

Similarly, Stanley Prusiner, the Nobel Laureate for Physiology or Medicine in 1997 who was recognized for the discovery of infectious prion diseases, was the first to report the successful use of PAMAM dendrimers for reversing infectious prion plaques (i.e. aberrantly folded isoforms of normal glycoproteins) [176]. Recent studies by McCarthy *et al.* [177, 178] have highlighted the nature of various dendrimers (PEI/PAMAM-type) and their interactions with prion plaque. Insights into the role of dendrimers for the treatment of prion diseases have been reviewed recently by McCarthy *et al.* [177, 178]. It is noteworthy that several dendrimer-based CNDPs discussed above (size, shape and surface chemistry) are intimately involved in these processes. It is clear that dendrimers may be engineered to detect, and eliminate *in vitro,* many prion stains, and therefore, they are currently being examined for *in vivo* prion applications [179, 180].

Finally, dendrimers have been used as a delivery vector in a variety of other applications against infectious diseases. More specifically, Zhou *et al.* [150] reported promising results using a flexible core, cationic, $G = 5$; PAMAM dendrimer as a vector for delivering siRNA (i.e. as a 'dendriplex') in humanized mouse models for the treatment of HIV-1 infection. These dendrimer–siRNA based dendriplexes suppressed HIV-1 infection by several orders of magnitude compared to free siRNA mixtures and were able to suppress infection repeatedly [150].

Cancer

Cancer remains a major area of focus for nanomedicine largely due to the many intrinsic physicochemical advantages associated with nanoparticles, as well as unprecedented new cancer therapy strategies based on nanoparticles [9], including photodynamic therapy, thermal ablation or antitumour vaccines [181]. Amongst these intrinsic nanoparticle properties are tunable CNDPs such as larger controlled nanometric sizes, the ability to reduce drug toxicity by encapsulation or conjugation and polyvalent surface chemistry [181]. These features permit passive targeting (i.e. the EPR effect) or active receptor-mediated tumour targeting in concert with imaging/diagnostic components, as described by Tsien's group [108]. These engineered combinations of functionalized nanoparticle components (i.e. nanoparticle-based theranostics) promise new avenues for targeted delivery of large therapeutic dosages to specific disease sites with concurrent imaging/diagnostic function whilst reducing potential side effects to healthy tissue. These options are not possible when administering traditional small molecule anticancer drugs which require attenuated dosages due to the inability to localize at tumour sites and consequently whole-body toxicity issues.

As discussed above, the ability to control, engineer and tune nanoparticle CNDPs provides very important strategies for designing and optimizing many new nanomedical therapies for the treatment of cancer. As early as 1999, it was shown by Malik *et al.* [134] that properly engineering the surface chemistry of a PAMAM dendrimer (e.g. sodium carboxylate terminal groups) provided an optimal, monodispersed scaffolding for encapsulating the anticancer drug cisplatin. This simple 'guest–host' construct provided an intravenous injectable dendrimer-based protocol that significantly enhanced the maximum tolerated dose, bioavailability, and prolonged the survival of mice bearing melanomas. Recent work has shown that similar dendrimer surface engineering of PLL dendrimers by PEGylation [135, 182] reduced cardiotoxicity compared to liposomal or solution doxorubicin formulations [53], whereas PEGylation of methotrexate-conjugated PAMAM dendrimers exhibited significantly prolonged residence in the blood and enhanced antitumour activity compared to non-PEGylated dendrimer conjugate or the drug alone [136]. Additional selected examples are shown in Table 1 and have been reviewed extensively by us [22] and others [183–185].

Over the past decade, dendrimers have been examined as effective gene delivery vectors for a variety of anticancer therapies involving DNA, antisense DNA, siRNA and more recently saRNA [82]. For example, intraperitoneal administration of three different oligo-DNA–PAMAM dendrimer complexes in mice bearing human ovarian tumours showed high levels of tumour accumulation of oligo complexes [142]. Intratumoural administration of *angiostatin* gene delivery using PAMAM dendrimers inhibited tumour growth and reduced tumour-associated vascularization [143]. Both PAMAM and PPI dendrimers have shown tumour-homing abilities, allowing for tumour-specific gene expression following intravenous administration [186]. The innate targeting of PPI dendrimers was combined with transferrin ligand targeting to demonstrate the complete regression of tumours in mice using a therapeutic plasmid DNA–PPI dendrimer conjugate [144].

Very recently, we [82] have reported the use of a unique dendriplex formed from a novel saRNA [187] and flexible core, PAMAM dendrimer vectors against multifocal liver tumours in a cirrhotic rat model. Intravenous administration of this dendriplex substantially reduced advanced liver tumour masses (up to 80%) whilst enhancing the production of albumin to near healthy levels within weeks after several injections. Advancing these encouraging preclinical studies to human clinical trials has been proposed pending appropriate preclinical toxicology studies. More comprehensive reviews focused on the entire field of dendrimer-based nanomedicine in oncology have been published previously [183, 188].

Ocular diseases and wound healing

A variety of diverse activities ranging from antimicrobial effects [169], wound healing [189–193], gene transfection [194], regenerative medicine [195] and targeted drug delivery to anti-inflammatory sites for treatment of both eye and brain diseases [132, 133, 161] have been reported with the use of dendrimers in a variety of ocular nanomedical applications.

Recently, a novel hybrid PAMAM dendrimer hydrogel/poly(lactic-co-glycolic acid) platform has been developed, which contains two antiglaucoma drugs for enhanced bioavailability and sustained effective reduction in intraocular pressure following a single topical administration in adult male rabbits [196]. More extensive reviews of dendrimer-based preclinical applications related to ocular diseases have been published recently [197, 198].

In summary, an articulate 'big picture perspective' for CNDP-directed patterns has been provided recently [61] for many of these preclinical efforts and relative to *in vivo* physiological barriers as described above (Fig. 4). These empirical insights corroborate the need for quantifying CNDP-dependent property patterns as part of a powerful predictive strategy for developing future nanomedicines.

Dendrimer and general hard/soft nanoparticle toxicology

Any platform under consideration for nanomedicine applications is expected to demonstrate low acute/chronic toxicity profiles, low accumulation/acceptable excretionary pathways and nonimmunogenic features. Present predictive toxicology for traditional pharmaceutical agents originated with the emergence of inorganic/organic chemistry. Simple observations by 19th century chemists involved direct human exposure; they often reported, for example, the taste, smell and texture for newly synthesized compounds. Similar predictive nanotoxicology challenges exist in the twenty-first century; however, such methods with their associated risks and consequences are not acceptable. Thus, the safety and toxicology of new nanomaterials is of current concern. This has led to many studies to evaluate acute nanotoxicology at the cellular level, in animal models (i.e. at the organ and cellular levels) and eventually in humans including long-term chronic ecological and life cycle investigations [199, 200]. Although the specific building blocks of many nanomaterials may be considered safe, the nanoscale products derived from these precursors may lead to novel nanoscale effects/properties (e.g. large surface area/mass) and present new toxicology issues. As discussed above, unprecedented physicochemical properties (Fig. 2) are expected to emerge for all quantized building blocks as a function of their respective CHDPs (i.e. CADPs, CMDPs and CNDPs) [23, 201].

At the nanoscale level, these effects must be clearly elucidated and quantified. A number of recent studies have evaluated the role of CNDPs on the PK and toxicological characteristics of not only dendrimers but also other nanoparticles [43, 120, 202, 203]. Although evidence was initially anecdotal, it is now apparent that CNDPs may indeed provide a strong common set of parameters/principles for understanding nanotoxicology. Here, we highlight only the major aspects. In general, amongst the six CNDPs, size and surface chemistry appear to play the major roles in *in vivo* toxicity [22, 204]. This is believed to be largely attributable to size-dependent charge–charge interactions between nanoparticle surfaces (i.e. high surface area/mass) and blood/tissue constituents, as well as surfaces of the vasculature.

Dendrimer-based in vivo biodistribution and nanotoxicology patterns

Dendrimer-based, CNDP-directed nanoperiodic property patterns related to *in vivo* biodistribution and nanotoxicology are emerging from our laboratory [205] and elsewhere [203]. The first reported examples involved Gd-chelated dendrimer-based MRI contrast agents and related to real-time excretion modes and organ targeting [45, 72, 151, 206]. The two most extensively studied CNDPs, dendrimer size and surface chemistry, are briefly summarized in Fig. 13. It has generally been assumed that dendrimer interior compositions do not play a dominant role in biodistributions and nanotoxicology, except at lower generations where their intrinsic structural flexibility may expose differences in interior chemistry [48] or in specific cases dendrimer degradation products (i.e. PLL dendrimers) might stimulate other *in vivo* metabolic pathways [105]. *In vivo* dendrimer-based biodistribution patterns, PKs and nanotoxicology have been reviewed more comprehensively elsewhere [72, 207]. To date, Shcharbin *et al.* [72] have published the most comprehensive overview of this area. Focusing on a wide range of dendrimer and

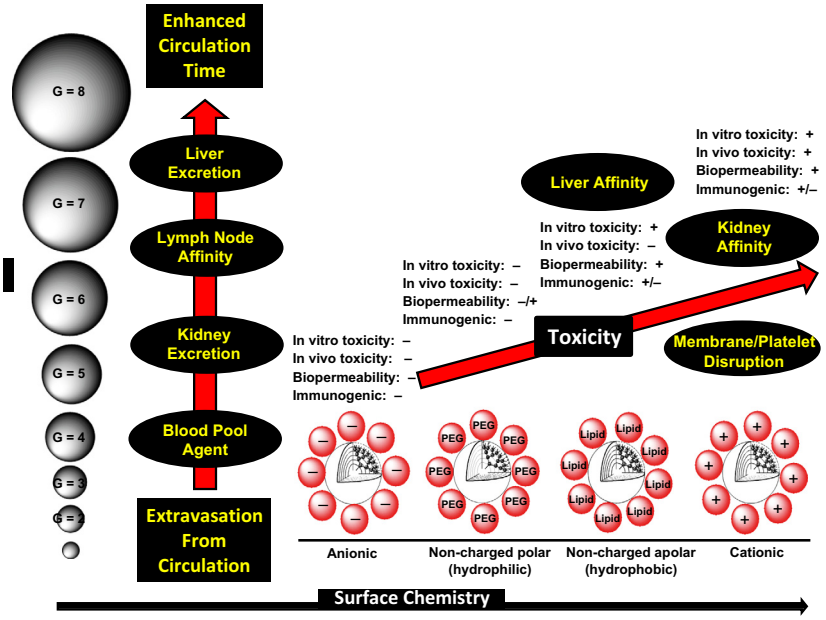


Fig. 13 *Dependency of biodistribution, toxicity, biopermeability, immunogenicity and membrane/platelet disruption on dendrimer size (i.e. generation) and surface chemistry.*

dendriplex compositions, these authors conclude that *in vivo* nanotoxicology of dendrimers is substantially more optimistic than suggested by earlier *in vitro* evaluations. Only a brief overview will be presented here.

In vivo dendrimer clearance is mainly dependent on size (i.e. generation level) and surface chemistry [208]. Systematic increases in dendrimer size usually result in prolonged blood retention and reduced renal excretion as demonstrated with PAMAM G5–G8 [45, 209]. However, excessive increases in size (>10 nm) or presentation of inappropriate surface chemistry may not increase blood retention, but rather may cause protein opsonization and rapid elimination due to reticuloendothelial system (RES) (liver and spleen) recognition (Fig. 13).

Similar qualitative observations have been reported concerning subtle differences in dendrimer interior chemistry. More specifically, PPI dendrimers with an equivalent generation size and surface functionality [208], but with a more hydrophobic interior chemistry, cleared more rapidly from the circulation (with twofold higher accumulation in the liver) compared to PAMAM dendrimers with more hydrophilic interiors. As such, blood clearance appears to be not only size dependent but also affected by dendrimer interior and surface chemistry. Similarly, cationic dendrimers exhibit fast clearance leading to low blood levels accompanied by a high degree of nonspecific accumulation in the liver, kidney, spleen, lung and pancreas [210, 211]. Therefore, an important feature associated with lower-generation dendrimers ($G < 6$), in contrast to larger nanoparticles, is their relatively rapid clearance from the blood and off-target organs [203]. This suggests that if therapeutic concentrations at the target tissue can be attained with lower generations ($<G = 6$), the side effects of dendrimers and dendrimer–drug conjugates can be reduced.

In general, amine surface functionality consistently exhibits greater toxicity at high doses compared to neutral or anionic surfaces such as hydroxyl, carboxyl, acetamido or PEGylated groups [72, 120, 202, 203]. However, it has been shown that the cytotoxicity of cationic, amine-terminated PAMAM dendrimers is increased at higher generations ($>G = 5$). Recent studies by Grainger and coworkers have examined the interactions between high-generation PAMAM dendrimers ($G = 7$) and blood to understand the mechanism of potential toxicity [212, 213]. These amine-terminated dendrimers (at a concentration of 100 μg mL^{-1}) induced *in vitro* fibrinogen and albumin aggregation leading to blood coagulation and platelet disruption. These findings also correlated broadly with observed *in vivo* toxicity for higher amine-terminated dendrimers. By contrast, hydroxyl and carboxylate dendrimers ($<G = 7$) did not induce blood toxicity [212, 213] and were nontoxic *in vivo*

(oral administration, up to 500 mg kg^{-1}) in mouse models [214]. Finally, in contrast to dendrimers presenting cationic, amine-terminated chemistry, those possessing neutral moieties did not affect BBB integrity or activate microglia at high concentrations [163].

It has been found with essentially all hard/soft nanoparticles that avoiding/reducing cationic amine surface chemistry and using neutral/anionic, hydroxyl, carboxyl or acetamido surface groups substantially reduces adverse effects at high doses. For example, the biosafety of hydroxyl terminated; $G = 4$ PAMAM dendrimers was assessed in healthy neonatal rabbits [133]. Essentially, no changes in renal or hepatic functions or neurobehaviour were noted compared to healthy kits treated with phosphate-buffered saline. Liver enzymes remained normal indicating that there was no hepatocellular injury due to dendrimer administration. When this dendrimer was injected into the cerebrospinal fluid of a healthy newborn rabbit, no accumulation was seen in the brain 24 h later suggesting rapid clearance from the brain in healthy animals and minimal neurotoxicity. When injected into neonatal rabbit kits with cerebral palsy, accumulation was observed only in activated microglia and astrocytes in the injured brain region, and not in other cells. Therefore, targeted delivery combined with the intrinsic CNDP-driven rapid clearance of mid- and lower-generation dendrimers may help minimize toxicity. This is consistent with the findings of other recent toxicity studies which suggested that hydroxyl-terminated PAMAM dendrimers are noncytotoxic even up to 500 mg kg^{-1} and do not cause protein opsonization or haemolysis [214]. These results were further confirmed in a recent study in a canine model; no adverse effects were observed at all therapeutically relevant dose levels [161]. It should be noted that extensive evaluations by Shcharbin *et al.* [72] have shown that *in vivo* administration of dendrimers appears to produce substantially lower cytotoxicity than would be expected from earlier *in vitro* assays.

As described above, anionic and neutral dendrimers show substantially higher blood levels accompanied by lower liver accumulation and significant urinary excretion within 24 h after administration. In general, PAMAM dendrimers possessing neutral surface chemistry exhibit size-dependent excretion modes; lower molecular weight structures ($G = 1$–5) rapidly accumulate in the kidney via glomerular filtration [45, 209] and larger structures ($>G = 6$) partition to the liver. It is notable that radiolabelled higher-generation ($G = 5$–7), Hult-type, poly(ester) dendrimers, possessing terminal hydroxyl groups, exhibited complete clearance through the kidneys within 15 min [215]. On the other hand, dendrimer surface PEGylation has been shown to increase blood half-life and decrease accumulation in the liver, spleen and kidney; however, these biodistributions appear to be dependent on the molecular weight of PEG and dendrimer flexibility [216, 217].

Finally, it was pleasing to note that other soft/hard nanoparticle categories (Fig. 3) are reported to exhibit size-dependent kidney excretion patterns that essentially parallel those observed for dendrimers (Figs 8, 10 and 13) [46, 47]. Analogous size-dependent patterns for renal versus liver excretion have also been reported for other soft nanoparticles such as [S-4]-type proteins [46], as well as hard nanoparticles including [H-2]-type cadmium chalcogenides (quantum dots) and [H-4]-type silica nanoparticles [47]. However, related studies of nonspherical, high aspect ratio, rod-like carbon nanotubes have shown that excretion modes are dramatically affected by both size and shape [63].

General CNDP-directed, hard/soft nanoparticle biodistribution, PK and toxicology patterns

The focus of nanotoxicology during the past decade has progressed from simple protocols based largely on traditional small molecule toxicology perspectives to significantly expanded criteria. These expanded criteria are now generating novel assays that will properly evaluate emerging properties, as well as risks/benefits associated with nanoscale materials. In an effort to assess the toxicological and biological impact of these new properties, Nel *et al.* [39, 218, 219] and others [220–222] have proposed many new nanomaterial assessments, including measurements of redox activity, reactive oxygen species cationic toxicity, complement activation, photo activation, membrane disruption and dissolution shedding of toxic ions. In parallel with these activities, there is growing consensus that new toxicology protocols must be enhanced to include high-throughput screening that enables quantitative, pathway-based, mechanistic evaluation of nano-SAR patterns with a transition from qualitative animal models to quantitative testing in human cells or cell lines. It is proposed that this will involve high-throughput screening protocols for assessing large compositional/combinatorial

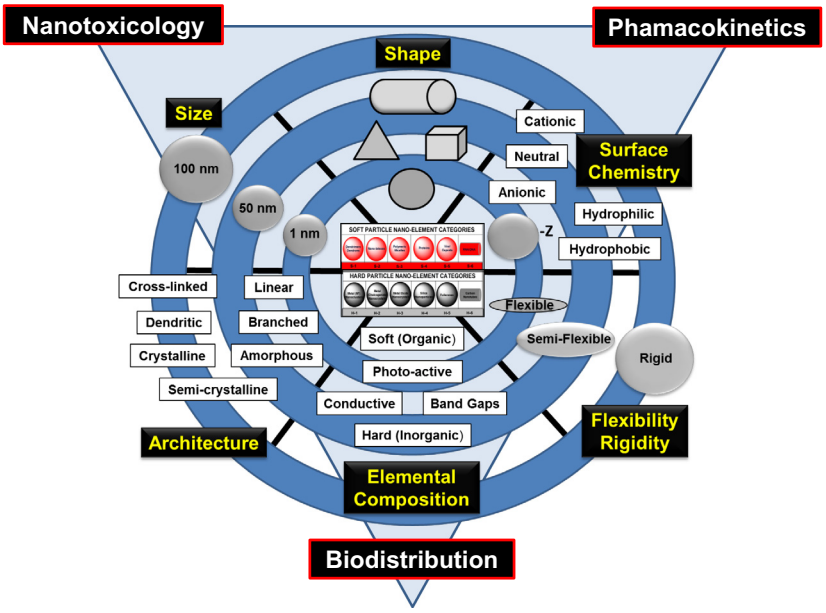


Fig. 14 *Soft and hard nanoelement categories (centre). A schematic overview of six critical nanoscale design parameters that may significantly affect nanotoxicology, pharmacokinetics and biodistribution parameters for all soft and hard nanoelement categories in various nanomedical applications.*

libraries of engineered nanomaterials as a means for developing critical hazard rankings and nano-SAR patterns for predicting *in vivo* injury/toxicological outcomes.

The ultimate objective is to develop a unifying nanotoxicology paradigm that will provide reliable and quantitative *a priori* predictions for the interaction of all soft/hard nanoparticles with living systems. To accomplish this goal, many researchers active in this area are advocating in-depth, nano-SAR-type evaluations of the same CNDPs that constitute the central dogma of the nanoperiodic paradigm described herein (see below).

In our opinion, a systematic approach to evaluate the quantitative impact of soft/hard nanoparticle CNDPs such as size, shape, surface chemistry, flexibility/rigidity, architecture and elemental composition on living systems will yield very important nanoperiodic patterns. These patterns should provide critical predictive paradigms not only for nanotoxicology but also for nano-PKs and nanobiodistributions (Fig. 14), as well as many other issues of high importance to nanomedicine and nanoscience in general.

Emergence of dendrimer-based products/therapeutics in the past decade

Dendrimer-based nanomedical products were first introduced in the late 1990s. These commercial products based on our licensed patents [16] included cardiac diagnostics (Stratus) and DNA gene vectors (Superfect, Qiagen, Hilden, Germany). An exception to those based on our patents was a diagnostic system referred to as UltraAmp, 3DNA dendrimer technology (i.e. protein detection amplifiers) developed by Genisphere, Inc. (Hatfield, PA, USA). Essentially all commercial activity before the year 2000 focused on simple dendrimer compositions; applications relied on their structure-controlled nanoscale sizes and surface chemistry.

As shown in Fig. 15, several dendrimer-based products emerging from the period 2000–2010 included (i) organic light-emitting diodes (Cambridge Display/Sumitomo, Tokyo, Japan); (ii) the antiviral topical nanopharmaceutical SPL7013 (Starpharma), which is presently in Phases II–III clinical trials as a microbicide for treatment of bacterial vaginosis; (iii) MRI agents (Gadomer-17, Bayer Schering Pharma AG); (iv) siRNA delivery vector (Priofect, Merck KGaA, Darmstadt, Germany); (iv) cardiac diagnostics (Stratus); (v) protein detection amplifiers (UltraAmp, Genisphere, Inc.); and (vi) ocular/surgical adhesives (OcuSeal/Adherus, HyperBranch Medical Technology, Inc., Durham, NC, USA).

More complex dendrimer-based nanodevices have emerged (Fig. 15), focused on critical nanomedicine challenges such as targeted delivery of cancer therapies and applications in inflammation and

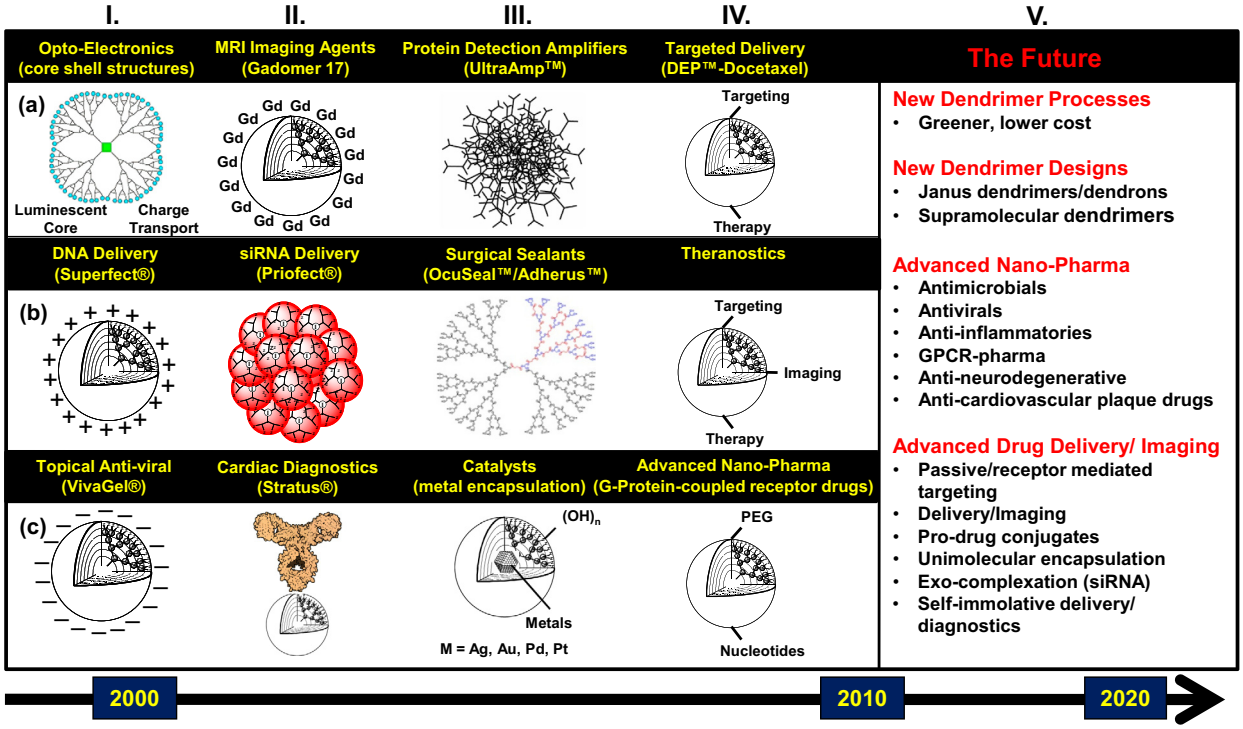


Fig. 15 *Dendrimer-based commercial products developed from 2000 to 2010 (columns I–III). Dendrimer-based nanomedical products currently under development; DEP-docetaxel (Starpharma) is entering Phase I clinical trials (column IV). Future dendrimer-based developments and applications predicted to occur before 2020 (column V).*

infection. Other applications include targeted delivery of cancer therapies with concurrent imaging capability, as well as advanced polyvalent nanopharma prototypes referred to as G-protein-coupled receptors [223]; both of these are currently under development (by the US National Institutes of Health and the private sector) but are not yet at a commercial stage.

The most commercially advanced dendrimer-based nanotherapies use either PLL dendrimers SPL7013 as active topical microbicides against bacterial vaginosis or drug delivery vectors referred to generically as DEP dendrimer drug delivery technology. Currently DEP-docetaxel is under active development by Starpharma and AstraZeneca as a tumour-targeting nanotherapy which has exhibited enhanced effectiveness against breast, prostate, lung and ovarian cancer compared to docetaxel alone.

A variety of future dendrimer-based developments and applications that are predicted to emerge by 2020 are shown in Fig. 15:

- Advances in the development of new dendrimer processes that do not depend on traditional excess reagents or protect–deprotect strategies.
- Recent developments with Janus dendrons/dendrimers leading to supramolecular dendrimers/dendrimersomes are expected to define a new area comparable to present covalent dendrimers [85].
- New intrinsically active dendrimer nanopharmaceuticals based on polyvalency.
- New dendrimer-based *in vivo* 'theranostic devices' suitable for real-time imaging, treatment and diagnosis of various diseases and personalized medicine.
- The first dendrimer-based, FDA-approved nanomedicines and nanotherapeutics are expected as nanoscale microbicides or drug delivery/transfection vectors.

At present, several hard and soft nanoplatforms, including dendrimers, are under investigation as potential advanced nanomedical devices for the ablation of cardiovascular plaque (CosmoPHOS-Nano Project effort in Europe: http://www.cosmophos-nano.eu/).

Despite substantial progress in the development of nanomaterials for nanomedicine, there still remain many important challenges that if resolved would facilitate progress in the approval process. Such challenges, which in many cases involve the engineering of nanoparticle CNDPs, include the following requirements:

- Well-defined, better characterized soft/hard nanoparticles that may be synthesized reproducibly by standardized methodologies.
- Stealthier nanoparticle surface chemistry beyond PEGylation to present fewer side effects and avoid complement activation [224].
- Well-defined, size-controlled, monodispersed hard and soft nanoparticle categories, with well understood aggregation and protein corona features.
- Nanoparticles with suitable surface chemistry for reproducible stochastic or quantized attachment of drugs, targeting groups, imaging moieties or stealth coatings [224].
- Smaller nanoparticles sizes (<10 nm) or degradation features that enable desirable kidney excretion modes (versus liver, etc.) and more effective tumour penetration properties.
- Shelf stable, water-soluble, single-component nanomedicine formulations with simple administration protocols.
- Noninvasive patient-friendly administration modes (e.g. oral or transdermal).
- Intrinsic features/surface chemistry to ensure longer circulation times.
- Elimination/excretion properties to ensure no long-term accumulation.

Conclusions: the future of nanomedicine

Here, we have reviewed several key nanoparticle platforms in preclinical, clinical and FDA-approved development phases with a special focus on the active role of dendrimers in nanomedicine. This activity was described within the context of a new nanoperiodic paradigm based on structurally controlled CNDPs possessed by all well-defined soft/hard nanoparticles. As discussed, the six CNDPs (size, shape, surface chemistry, flexibility/rigidity, architecture and elemental composition) may be systematically controlled and engineered to provide important design principles for optimizing benefits and reducing risks when properly applied to a wide range of nanomedicine applications.

Following an introduction to polymeric therapeutics in nanomedicine, a regulatory road map from initial platform identification to final approved nanomedicine products, and discussion of current candidates that are in the Phases I–III clinical testing pipeline, a new CNDP-based, nanoperiodic paradigm was introduced that may be used as a unified perspective for analysing a variety of critical *in vivo* challenges encountered in the advance of all nanoplatforms through the regulatory approval process. We then focused on the structure-controlled properties of dendrimers that have led to systematic CNDP-directed, nanoperiodic relationship patterns that allow *a priori* prediction of excretion modes, biodistribution features, EPR effects and epidermal penetration properties by systematically adjusting dendrimer CNDPs such as nanoscale size and surface chemistry. All six CNDPs may be readily engineered to provide a wide range of unique dendrimer–drug platforms, therapy administration modes, drug targeting and imaging and diagnostic strategies of importance to nanomedicine. These engineered CNDP features have been successfully combined to provide a working example of a dendrimer-based theranostic platform for imaging and treating cancer. Both intrinsic and engineered dendrimer features have led to many active preclinical investigations that have been reviewed in the areas of inflammation including neuroinflammation, infectious diseases, oncology and ocular disease/wound healing. Finally, important CNDP (i.e. size and surface chemistry)-directed nanotoxicology patterns were reviewed with respect to dendrimers. A growing

consensus on the critical need for a universal 'predictive nanotoxicology paradigm' suitable for all well-defined soft/hard nanoparticles was described.

It is noteworthy that many key soft/hard nanoparticle features considered in the quest for a predictive nanotoxicology paradigm [39, 42, 43, 204, 218–222, 225] are in fact the same parameters that constitute the CNDP-driven central dogma underpinning the unifying nanoperiodic concept described in this review. We hope that important first steps may begin towards a quantitative evaluation of many anticipated CNDP-based nanoperiodic property patterns which are expected to produce new predictive paradigms for optimizing/managing both benefits and risks in nanomedicine.

In conclusion, there appears to be considerable promise and benefits for many current nanomedicines and nanotherapies, including enhanced PKs and reduced off-target side effects, compared to whole-body exposure associated with traditional pharmaceuticals [43, 226]. This promise is clearly related to a universal quest for improved 'quality of life' medical care, new strategies for managing untreatable diseases and resistant pathogens, anticipated growth in global medical needs and social pressure for more cost-effective health care. It is clear that these objectives must be met without compromising safety margins whilst offering effective, less invasive, 'compliance-friendly' therapies at costs acceptable to current global healthcare criteria. Issues associated with new nanomaterial properties and unknown toxicities remain some of the greatest challenges in the field of nanomedicine These are high-priority issues that are driving a strong need for developing a 'paradigm for predictive nanotoxicology', as suggested by Nel *et al.* [39, 204, 218], Moghimi *et al.* [227], Fadeel [225] and others [220, 221]. We hope that the systematic nanoperiodic concept and CNDP-directed nanoperiodic property/toxicology patterns described herein will inspire important first steps towards such a 'predictive paradigm' for better optimized nanotherapy design, as well as a further quantitative understanding of nanotoxicology in general.

Conflict of interest statement

DAT is the founder and Chief Executive Officer of NanoSynthons LLC, which manufactures PAMAM dendrimers and several conjugates for nanomedical applications, and is a minority shareholder in Starpharma.

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