Dendrimers Integration in Cancer Imaging and Theranostics

Subjects: Oncology

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Cancer is a result of abnormal cell proliferation. This pathology is a serious health problem since it is a leading cause of death worldwide. Anti-cancer therapies rely on surgery, radiation, and chemotherapy. However, these treatments still present major associated problems, namely the absence of specificity. Nanoparticles, particularly dendrimers, have been paving their way to the front line of cancer treatment, mostly for drug and gene delivery, diagnosis, and disease monitoring. This is mainly derived from their high versatility, which results from their ability to undergo distinct surface functionalization, leading to improved performance.

cancer nanocarriers anticancer dendrimers intracellular targeting theranostics

1. Introduction

All types of human cells may suffer an abnormal proliferation that can lead to cancer cells. Cancer classification/identification is performed according to the tissue and cell type from which the cancer cells arise. Therefore, there are multiple distinct types of cancer, which can vary significantly in their behavior and response to treatments [1]. This disease is a major public health problem and a leading cause of death worldwide in countries of all income levels.

According to the World Health Organization (WHO), the social and economic impact caused by cancer is increasing. In a 2014 report, an annual cost of EUR 1.04 trillion was estimated for global cancer expenses. The report also declared that it is important to continue investing in care and control, which will prevent a considerable number of deaths for years to come. [2]. Extended lifespan associated with environmental factors (e.g., exposure to pollution, carcinogenic agents, radiation, viruses and bacteria) and the low efficacy of available treatments, closely associated with an increased drug resistance, also contributes to cancer development [3].

Currently, the standard cancer treatments used in clinical settings are radiotherapy, surgery, and conventional chemotherapy [4]. However, these therapies present several drawbacks, such as high toxicity, due to insufficient selectivity and unspecific targeting of cancer cells, which leads to increased resistance to anticancer drugs.

It is therefore relevant to find new anticancer agents able to control tumor growth with minimal side effects. In recent years, the use of nanotechnology in cancer treatment has offered some exciting possibilities, including improvement of detection and elimination of cancer cells before tumor development. This includes the use of dendrimer-based nanotherapeutics as a novel strategy for diagnosis and therapy (theranostics) [5][6][7]. Dendrimers

are synthetic 3D polymers with well-defined layered architecture [8]. Owing to their high functionality and loading capacity, as well as their precisely controlled chemical composition and molecular weight, dendrimer-based anticancer therapies offer great advantages over conventional formulations. These include the use of dendrimers as advanced contrast agents (diagnosis) [6], nanocarriers (treatment) [5][9], and theranostic agents (diagnosis, treatment and disease monitoring) [7]. In the quest for new anticancer drugs using dendrimer-based therapies, Shao et al. demonstrated that dendrimers may display innate anticancer activity and anti-metastatic properties without the loading of any therapeutic agent [10].

2. Dendrimer Nanoparticles

Multifunctional nanoparticles display great potential for drug and gene delivery, especially for cancer therapy [11][12]. Dendrimers are a class of hyperbranched synthetic polymers with a very low polydispersity, also known as “cascade polymers” [13]. Biologically, dendrimers are highly biocompatible, with a predictable biodistribution and cell-membrane-interacting features mostly determined by their size and surface charge [14]. They were first synthesized in the late 1970s by Tomalia et al., with the desire to mimic a common pattern in nature with vast potential applications [15]. Due to their hyperbranched structure, dendrimers are extremely versatile macromolecules. Their structure can be defined by three main elements: the inner core, repetitive branching units (dendrons), and terminal groups that provide surface tuning (**Figure 1**) [16].

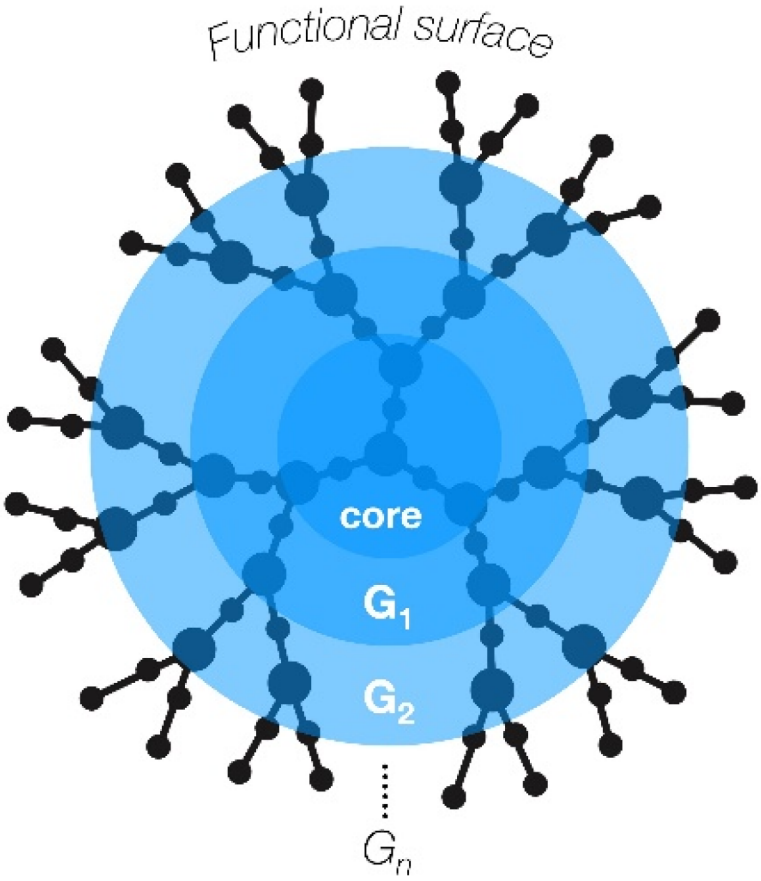

Figure 1. Schematic representation of a dendrimer nanoparticle, showing the core, repetitive branching units (dendrons) that constitute the growing layers (generations, G) and terminal groups (functional surface).

Dendrimers are classically obtained by two main approaches: a divergent or a convergent synthesis. In the first methodology, the dendrimer structure is constructed starting from a core molecule. The core reacts with monomers containing one reactive group and two dormant groups originating the first generation (G1) dendrimer. Then, the periphery of the G1 dendrimer may be activated for reaction with more monomers forming the second-generation dendrimer (G2), and so on. The divergent approach typically originates lower dendrimer generations of open structures and asymmetric shape [16]. In the convergent approach, the individual branches (dendrons) are first synthetized and then attached to a functional core molecule [17]. This methodology minimizes the occurrence of structural problems and facilitates the purification of the product [16]. Later, with the development of “click chemistry” [18], the preparation of dendronized systems became more efficient, requiring minimal purification [19]. She et al. [20], for instance, synthetized G2-poly-L-lysine dendrons that were connected at the core to a heparin sulphate moiety via “click chemistry”, and doxorubicin (DOX) was conjugated to the terminal ends using acyl hydrazine. As a consequence of the dendrimer architecture, the number of peripheral groups increases exponentially with generations, which results in nanosized particles suitable for drug loading and release [21]. However, when a critical branched state is reached, dendrimers cannot grow further because of the steric restriction imposed by the increasing branch density. This phenomenon is known as the “starburst effect” and is usually observed in high generations [16].

Since the discovery of these polymers [15], a variety of dendrimers have been developed, polyamidoamine (PAMAM) being the most studied [22]. PAMAM dendrimers are synthetized by the divergent method, mostly using an ethylenediamine core, and are hydrophilic, biocompatible, and non-immunogenic. Polypropylene imine (PPI) dendrimers, along with PAMAM, have been also widely investigated. PPIs are based on a 1,4-diaminobutane core or similar molecules and grow via double Michael addition reactions [13]. Poly-L-lysine (PLL) dendrimers are amino-acid-based polymers [23] that differ from PAMAM and PPI dendrimers in shape, since they are mostly asymmetrical. They have lysines as branching units and amines as terminal groups [22]. Phosphorous-based dendrimers are another interesting and well-studied class of dendrimers [24]. The potential of phosphorous dendrimers has been largely demonstrated, especially as cancer therapeutics. Similarly, carbosilane dendrimers have been explored as antimetastatic agents when complexed with ruthenium derivatives [25].

The different classes of dendrimers and their chemical structures are summarized in **Table 1**.

Table 1. Summary of different classes of dendrimers depicted in above section.

Classes of Dendrimers	Chemical Structure
PAMAM	Ethylenediamine-based core and terminal groups with primary amines

Classes of Dendrimers	Chemical Structure
PPL	Amino acid lysine-base core and branching units
PPI	1,4-Diaminobutane-based core and terminal groups with primary amines
Phosphorous dendrimers	P-Cl-based core, azabisphosphonates are possible terminal groups
Carbosilane dendrimers	Si-based dendrimer

3. Dendrimers General Role in Cancer

Dendrimers can be used in a vast number of theranostic applications [26][27][28][29]. It is, therefore, important to be aware of the properties they have to display in order to be employed as biomedical devices. Biocompatibility is crucial to preventing undesirable responses from the host, a property that can only be defined depending on specific applications [29]. In order to prevent bioaccumulation and consequent toxicity, biodegradability is a must. Another important aspect for the development of biomedical devices is their pharmacokinetics, namely their fate in the body after administration [29]. Additionally, the water solubility of dendrimer–drug conjugates enhance the bioavailability of poorly soluble drugs [30]. Lastly, polyvalence, i.e., the ability to support versatile surface functionalization and multiple interactions with biological receptors, is a key property for highly versatile platforms [27].

Dendrimers, as shown in several studies, have a high potential to be used as nanocarriers for both diagnostic and therapeutic approaches [31][32]. Dendrimer–drug interactions might occur in many ways and are dependent on multiple factors such as size, charge, or the chemical nature of the dendrimer/drug. The chemistry behind nanocarriers is the same used in diagnostic or therapeutic schemes, with the agent selected for conjugation being the key player. In general, dendrimers could be used as nanocarriers via two major approaches: loading or conjugation at the surface of the drug and/or target molecule. Encapsulation solves solubility problems indicated by many chemotherapeutics and drugs in general. When a drug is entrapped into the dendrimer's cavity, the polymer works as a dendritic box [33][34]; in this case, the dendrimers can cargo the drug of interest by forming structures that are stabilized via non-covalent interactions. In a different context, dendrimers could also be used as gene vectors, especially cationic dendrimers. When the strategy is dendrimer–drug conjugation, systemic effects can be reduced, increasing the efficacy of cellular targeting. This strategy also improves the half-life of the drug. The conjugate linker is also key to understanding release mechanisms. In many cases, ester and amide conjugate linkers are used that allow enzymatic or hydrolytic cleavage [34][35], easier for esters than amides [36]. Dendrimer–drug conjugation may influence the efficacy of drug itself. Importantly, these nano-polymers can cross cellular barriers by transcellular or paracellular pathways.

To use dendrimers as an alternative diagnostic tool or improve the properties of a contrast agent, it is important to guarantee some criteria. Dendrimers offer many advantages to improve the free delivery of contrast agents or drugs, including high solubility and low polydispersity, which are properties of all dendrimer classes (**Table 2**).

Table 2. Suitable properties of dendrimers to be used as delivery systems in different strategies.

Properties	Observations	References
Low polydispersity index	Common to all classes of dendrimers	[37][38]
EPR effect	Size/generation/M_w dependent	[39]
Permeability towards BBB	Already observed for PAMAM dendrimers	[40][41][42][43]
Highly solubility	Common to the majority of dendrimer classes	[44][45]
Multifunctional platform	Common to all classes of dendrimers	[46]
Highly loading capacity	Size/generation/M_w dependent	[47]
Stability	Common to all classes of dendrimers	[39]
Low toxicity and immunogenicity	Size/generation/M_w/charge dependent	[48][49][50]

EPR: enhanced permeability and retention, BBB: blood–brain barrier.

4. Dendrimers Cellular Uptake and Mechanism of Action at Cell Organelle Level

Dendrimers and other nanosized particles naturally accumulate in tumor sites by a possible enhanced permeability and retention (EPR) effect. This happens because the tumor microenvironment is rich in blood vessels due to increased angiogenesis. Using the blood vessels surrounding tumors, the nanoparticles can be driven into the tumors, crossing cellular barriers by transcellular or paracellular pathways [34][51]. The main question at this point is: how does the intracellular transportation of these macromolecules occur? In the case of dendrimers, their entry into

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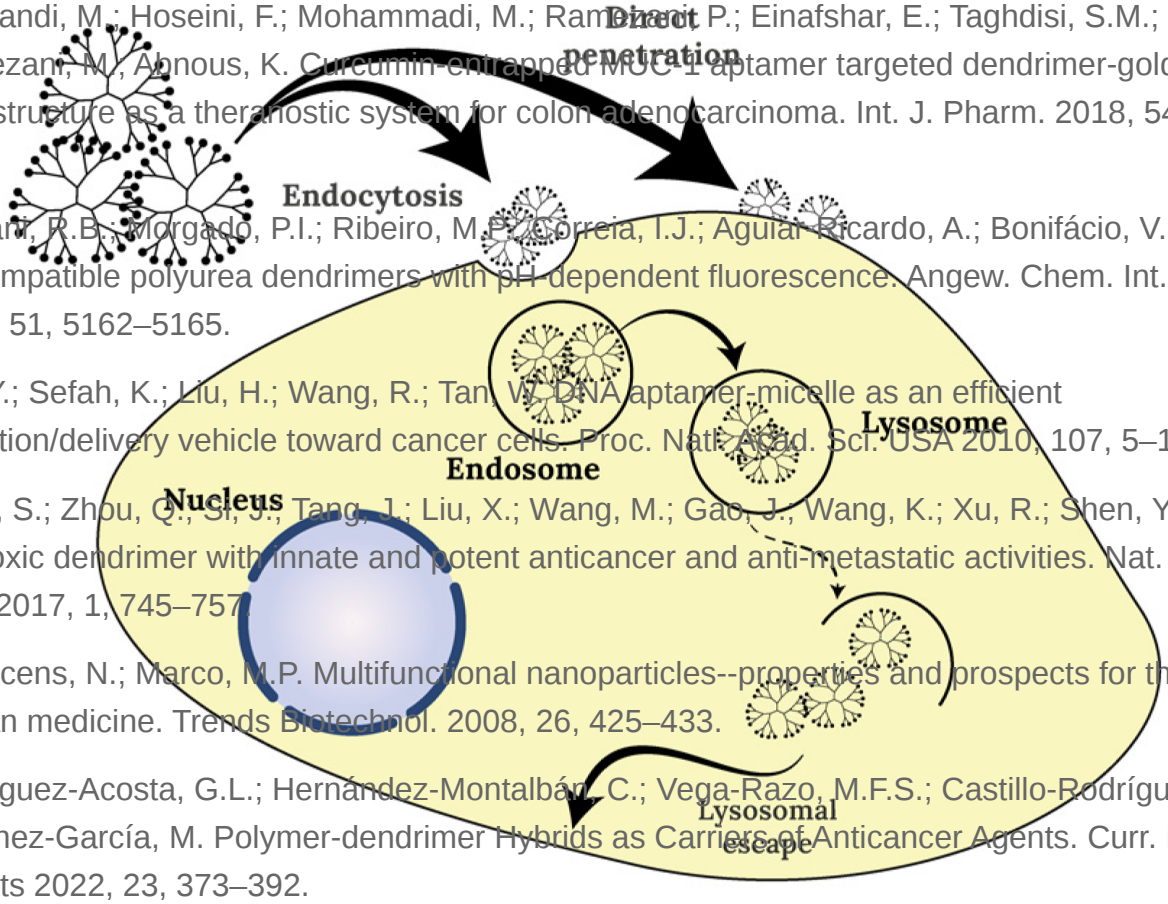

Figure 2. Schematic representation of dendrimer intracellular transportation. In general, dendrimers enter the target cells via direct penetration or endocytosis pathways. At cell level, dendrimers that follow the endocytic pathway are released from endosomes and migrate to lysosomes. Then these macromolecules can be released from lysosomes through a "proton sponge" effect, as shown for poly(propylene imine) dendrimers. Adapted from [51].

target cells via direct penetration or endocytosis pathways [52] (Figure 2). Importantly, the biomacromolecules, the future of the nanotherapeutics. Eur. J. Pharm. Biopharm. 2015, 93, 52–79.

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properties significantly depend on size and uptake efficiencies [53]. NPs possess biomimetic features due to their size, which is in the same range of biomolecules such as antibodies, nucleic acids, proteins, and membrane receptors. Cellular uptake, toxicity, targeting, and intracellular trafficking of NPs can be optimized by tuning physicochemical properties of NP such as size, shape, and surface properties [54]. These properties determine their uptake into mammalian cells, normally achieved by endocytosis, a form of active transport that can be classified into two major categories: phagocytosis and pinocytosis [55].

Briefly, phagocytosis occurs in specialized cells, designated phagocytes, and consists of the internalization of large particles, such as debris, bacteria, or other large-size solutes. In this process, the target particle is coated with specific molecules (opsonins) that promote its internalization and is then ingested by the cell and compartmentalized to a phagosome (plasma-membrane-derived vesicle). In the intracellular space, the phagosome fuses with the

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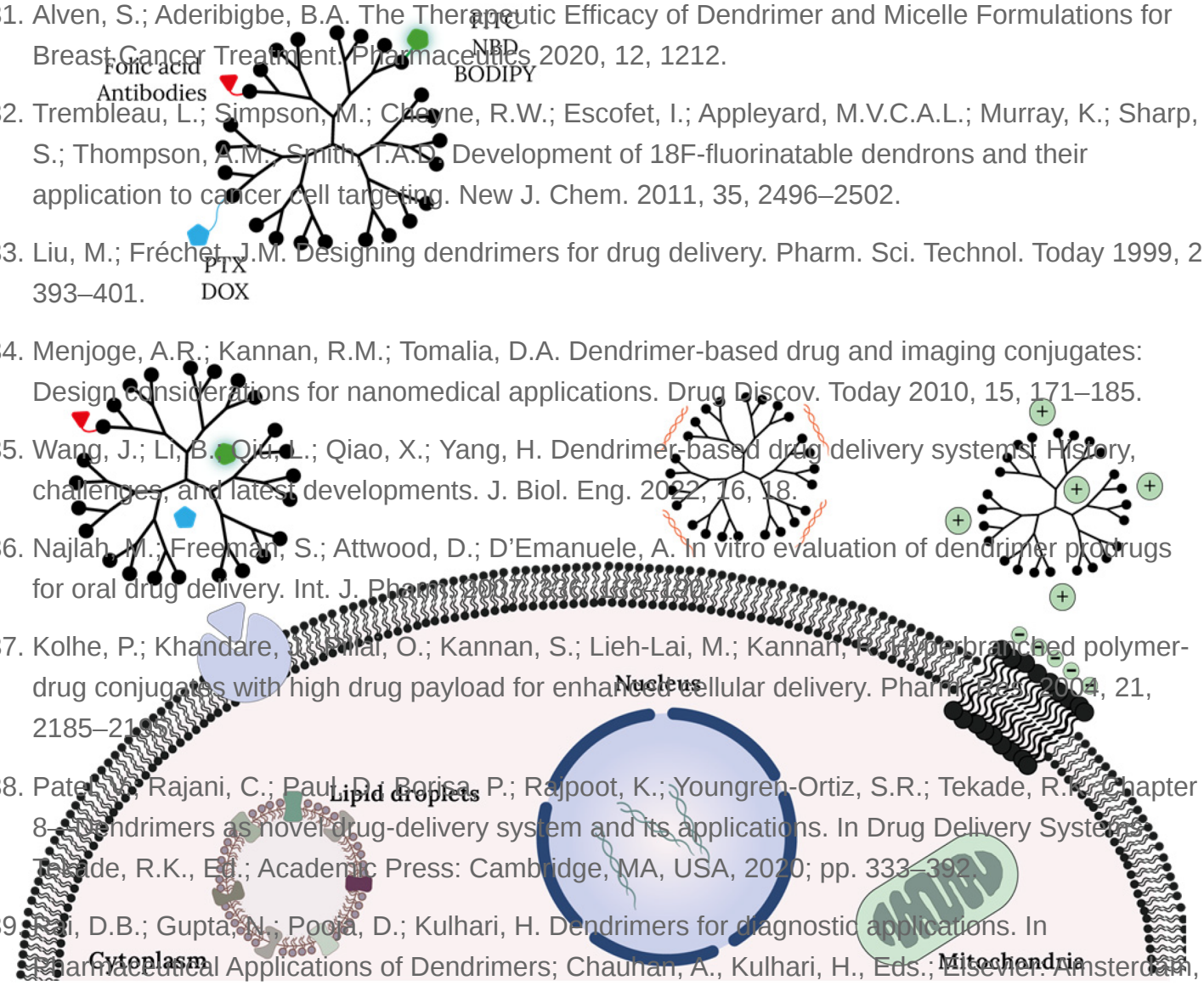

Figure 3. Schematic representation of potential intracellular cellular organelles (including plasma membrane, lipid droplets, cell nucleus, and mitochondria) targeted by dendrimers used in anticancer strategies. Cationic surface of cationic-modified dendrimers is known to strongly interact with negatively charged lipids present in cancer cell membranes. Cationic dendrimers are also shown to specifically target mitochondria.

In another study, the impact and target of PAMAM dendrimer generation (G4, G5, G6) on mitochondria and other cellular organelles such as lysosomes was evaluated in HaCaT (human epidermal keratinocyte cells) and SW480 (primary adenocarcinoma) cell lines [63]. In general, SW4810 cells were more sensitive to these cationic dendrimers than HaCaT cells. After treatment, the production of ROS was higher in SW480 and reached a maximum after 4 h of exposure. These results suggest the specificity of PAMAM dendrimers towards cancer cell mitochondria in comparison to normal cells [64]. The increase in ROS and the expression of apoptotic markers such as BAX and PARP are well reported for the activity of dendrimers used in anticancer strategies [65].

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