Dendrimers in Tissue Engineering

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Abstract

Dendrimers are highly branched and multivalent, and monodispersive making them perfect candidates for a myriad of controlled drug delivery applications. Dendrimers possess many other advantages, such as the possibility of modulating surface chemistry and charge, and biodegradation rate and to be processed as scaffolds that can emulate natural extracellular matrices thus opening up unique applications in tissue engineering. The combination of dendrimers and other macromolecules (proteins and carbohydrates), as well as other traditional scaffold polymers, has led to the creation of hybrid scaffolds with new physical, mechanical, and biochemical properties. However, despite the wide-spread use of dendrimers in biomedical applications, their use in the fabrication of tissue engineering scaffolds remains some-how narrow. The most promising applications of dendritic macromolecules in TE area such as drug delivery strategies, cell differentiation and/or tissue regeneration, 3D/Dynamic platforms and *ex vivo/in vivo* testing are overviewed and discussed herein.

Keywords: Dendrimers; Nanoparticles; Tissue engineering; Drug delivery; Microfluidics.

1. Introduction

Tissue Engineering (TE) principles are based on interdisciplinary research arising from combination of different fields including cell biology, materials science, chemistry, and medicine [1]. The objective of TE is the regeneration of native tissues by supplementing the natural healing process of the body or the creation of entire organs [1]. Based on its original concept, TE consists on synthesizing and using biocompatible materials (scaffolds), which are used to sustain or direct differentiation of cells, forming constructs, after which they are implanted *in vivo* [2].

In the last few decades, we have witnessed incomparable innovations in polymer synthesis and advances in the design of biodegradable chemistries [3]. Regarding polymeric scaffold, these can be divided into two types: natural and synthetic. Natural scaffolds are made of proteins, carbohydrates, or glycoproteins. These biopolymers already play important roles as ECM components within the body, such as collagen or fibrin [4]. On the other hand, synthetic linear polymers, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(caprolactone) (PCL), and poly(ethyleneglycol) (PEG) have also proven extensively useful as scaffold materials in TE. However useful, one of the main limitations of linear polymers is that they have only two functionable groups in each molecule, making it difficult to tune their mechanical and biochemical properties if needed [5].

The non-linear polymers comprise a more recently, nature-inspired type of branched polymers: dendrimers. These are highly branched and nanospherical macromolecules [6]. Dendrimers consist of three-dimensional nano-sized and branched structure, with an overall spherical geometry. The word “dendrimer” comes from the Greek word

"Dendron", which means tree, as it presents a similar branching in his structure like a tree [7].

The structure of the dendrimer is divided into three parts: the core, the interior, and the shell. The core affects the 3D shape of the dendrimer (*i.e.*, spherical scaffolds). The interior affects the host–guest properties of the dendrimer, namely for drug loading capacity and hydrophobic/hydrophilic interactions [8]. Finally, the dendrimer's surface can be further engineering, polymerized or modified with functional groups, allowing for a great variety of applications, from theranostic to targeting applications [8].

Exploratory research in nanotechnology, namely using dendrimer nanoparticles is still considered to be emerging, disruptive and multidisciplinary field. The field promises are exciting, from new diagnoses to new treatment routes. Consequently, there have been tremendous investments in this area. The relevance of the dendrimers and dendritic polymers, and the convergence and usefulness of these nanotools in TE applications are overviewed herein.

2. Fundamentals

Dendrimers are characterized as branched polymers whose value has continued to mature since their discovery more than thirty years ago [6], [9]. Despite their immense popularity in biologically-related fields like drug delivery [10] and molecular imaging [11], dendrimers have been under-employed in the field of tissue engineering and wound repair, with some exceptions in ocular applications such as cornea wound healing [12].

Regarding its synthesis, dendrimers are conventionally synthesized by two major routes: the divergent method, introduced by Tomalia *et al.* [13], and the convergent method, developed by Hawker and Fréchet [14], developed to overcome the limitation of the divergent synthesis [15]. In the first method (divergent), the final molecule grows radially

from a core by the sequential addition of layers of monomers, each layer constituting a new generation (G). The number of surface groups multiplies according to the functionalities in each monomer ramification. Dendrimers benefit from straightforward, iterative syntheses that produce highly monodisperse, globular macromolecules. Their high degree of branching results in an exponential increase in end groups with each generation size [16]. In the convergent methods, the dendrimer is produced stepwise, starting from the end groups and growing inside. When dendrons, (growing branched polymeric arms) are large enough, they are attached to a multifunctional core molecule. The convergent growth method has several advantages. They are quite easy to purify and the appearance of defects in the structure are minimal. Furthermore, it is possible to add subtle engineering into the dendritic structure through accurate placement of functional groups at the surface of the macromolecule. However, convergent method does not allow the formation of high generations due to steric problems that happen between reactions of the dendrons and the core molecule [17].

Dendrimers are recognized as nanosized, nonlinear, hyperbranched polymers. Whose overall 3D shape is key for their biological activity. The number and type of surface charges on each dendrimer can be controlled since different generations of dendrimers can be prepared and the termini can be modified to bear any desirable synthetic group [16].

The cytotoxicity of dendrimers depends strongly on the number and nature of functional surface groups. Anionic and neutral dendrimers present slight or no toxic effects while cationic dendrimers frequently show high toxicity. Poly(propylene imine) (PPI) and poly(amido amine) (PAMAM) dendrimers obtaining terminal primary amines are defined by concentration- and generation-dependent toxicity [18], [19], whereas grafted carbosilane – poly (ethylene oxide) (CSi – PEO) dendrimers and other dendrimers

terminated with anionic or neutral groups demonstrate to be much less toxic [20]. Furthermore, modification of the surface of the cationic dendrimer with negatively charged or neutral molecules declines its cytotoxicity. Surface functionalization with pyrrolidone, polyethylene glycol (PEG) or another biocompatible compound can decrease cytotoxicity to levels better than those of currently available products [21].

This unique characteristics of dendrimer become possible their use in several applications such as drug carries of drugs delivery, anti-tumor systems, immunology, blood anticoagulant, and microbicidal activities, among others [3].

3. Different approaches in TE

3.1 Drug Delivery Strategies

Dendrimers have received great attention in biological application due to their adjustable surface functionalization, well-defined structure, high water solubility, compact globular shape and monodispersed size. These features make them versatile carrier for drug delivery applications. Dendrimers are being considered as additive in various routes of administration such, oral, transdermal, ocular and intravenous administration [22], [9]. Dendrimers for drug delivery are employed using two different approaches. Drugs, enzymes, antibodies and other bioactive agents can be physically encapsulated in a dendrimer using non-covalent interactions [23], [24] or be bounded on the surface of the dendrimers via hydrogen bonding, electrostatic interaction, van der Walls force or covalently attached [25], [26]. Small drugs are frequently encapsulated into the dendrimers while larger (bio)molecules preferably bind to the surface [27].

Drugs encapsulation depends on the structure of both the dendrimer and the drug. It is crucial understand the chemistry behind dendrimer structure. Selecting a suitable dendrimer is the principal step for drug for the encapsulation of drugs [10]. The internal

structure of a dendrimer is normally hydrophobic due to hydrophobic interactions and hydrogen bound formations and it is appropriate for encapsulating hydrophobic drugs/bio-actives [28]. The drugs molecules sterically encapsulated in the interior are protected from the external environment. Furthermore, this characteristic offers promising advantages of extending the residence time of the drug in the blood circulation and enhancing the stability of active and tissue targeting [29].

The physical interaction between specific groups within dendrimer and the drug it is a simple process, just mixing dendrimers and drugs in the either aqueous solutions or a mixture of solvents [30]. The chemical structure of the drugs to be loaded remains unchanged after the connection with dendrimer, which assures the therapeutic efficacy of the drug [31]. The effectiveness of encapsulation is highly dependent surface functional groups of dendrimers, chemical structure, and molecular weight. It is often complicated to control the encapsulation capacity and the rate of drug release from the dendrimer because the entrapment of the drug is based on physical interactions [32].

There are several studies that show the dendrimers were able to effectively encapsulate anticancer agents as doxorubicin (DOX), drug-2-methoxyestradiol (2-ME) [33], and methotrexate (MTX) [34].

Despite several drugs are encapsulated in dendrimers, it is usually difficult to control the retention a consequent release of drug in the dendrimer so sometimes it is necessary different conditions [35]. The reason is that the stability of drug-encapsulated dendrimer are powerfully affected by the strength of their interaction and the water solubility of drug [36]. Therefore, attaching the drugs to surface functionalities is attractive approach. This is a great approach for precise drug delivery because a single dendrimer molecule can stably transport many drug molecules using many functional groups (amine and carboxyl groups) on the surface and release drug molecules precisely on the target [35], [29]. The

drug molecules can be linked to the dendrimer through electrostatic interaction, non-covalently or covalently. When the molecular groups present on the surface of the dendrimer are charged, the surface can electrostatically attract oppositely charged. It is possible to attach a variety of ionizable drugs or molecules electrostatically to dendrimers duo to numerous ionizable groups present on the dendrimer surface allowing this complex show adequate solubility in water [37], [38]. Recent studies have shown that nonsteroidal anti-inflammatory drug with carboxyl groups, including ibuprofen [39], piroxicam [40], and indomethacin [41] can interact electrostatically with different dendrimers [22]. The covalent conjugation of drug onto the dendrimer surface offers different advantages due to hydrolysable or biodegradable bound between dendrimer and drug allow a better control over drug release when compare drug-encapsulated dendrimer [32]. Dendrimer/drug conjugation are frequently stable *in vivo* due to covalent bound between dendrimer and drug and release kinetics is controlled by the type of the linker systems. The normal linkers comprise ester, amide, hydrazine, imine, carbamate disulfide and enzyme-cleavable peptide sequences [42], [43], [44]. Each linkage system has his mechanism of cleavage modality that splits the drug molecule from dendrimer. To improve the effectiveness of drug delivery, it is crucial to control the drug release from the dendrimer transporter in the target site with the minimal contact to normal tissues [32]. Several studies showed that anticancer drugs such as cisplatin [45], doxorubicin (DOX)[46], epirubicin [47], methotrexate [48], and paclitaxel [49] were effectively conjugated with PAMAM dendrimers. Furthermore, some drugs such as penicillin V [50], venlafaxine [51], 5-aminosalicylic acid [52], naproxen [53], and propranolol [54] were also successfully conjugated with PAMAM dendrimers. The several therapeutic moieties that have been used with dendrimers carriers are summarized in **Table 1**.

Table 1. Different drug delivery strategies making use of dendrimers.

Application	Dendrimer type	Drug	Strategy	Reference
Cancer	PAMAM dendrimer (G5)	2-methoxyestradiol (2-ME)	Drug encapsulation	[33]
Cancer	Poly(ethylene glycol) monomethyl ether (M-PEG)-PAMAM dendrimers	Methotrexate Adriamycin	Drug encapsulation	[34]
Inflammation	Perstrop Polyo (G5) PAMAM dendrimer (G3),(G4)	ibuprofen	Electrostatic interactions Drug encapsulation	[39]
Inflammatory disorders	PAMAM dendrimer (G3),(G4)	piroxicam	Electrostatic interactions Drug encapsulation	[40]
Inflammatory disorders	PAMAM dendrimers (G 4), (4.5)	Indomethacin	Electrostatic interactions Drug encapsulation	[41]
Cancer	sodium carboxylate-PAMAM dendrimers (G 3.5)	Cisplatin	Drug conjugation	[45]
Cancer	PEGylated dendrimer	Doxorubicin	Drug conjugation	[46]
Cancer	PEG-dendrimer	Epirubicin	Drug conjugation	[47]
Cancer	tris(hydroxymethyl) aminomethane (TRIS)-PAMAM dendrimer	Methotrexate	Drug conjugation	[48]
Infection	PEG-PAMAM dendrimer	Penicillin V	Drug conjugation	[50]
Antidepressant	PEG- PAMAM dendrimer (G2.5)	venlafaxine	Drug conjugation	[51]
Inflammatory bowel disease	PAMAM dendrimer (G3)	5-aminosalicylic acid	Drug conjugation	[52]
Inflammation	PAMAM dendrimer (G0)	Naproxen	Drug conjugation	[53]
Colon cancer	PAMAM dendrimer (G3	propranolol	Drug conjugation	[54]

3.2 Dendrimers for tuning stem cells functions

Regenerative medicine reveals fundamental biological processes, replacing or regenerating human cells, tissues, or organs to restore or establish normal function [55]. The use of stem cells as therapeutic agent in an attractive approach for targeting tissues or organs of interest. Combined delivery of cells with several information molecules as therapeutic agents has the potential to increase, modulate and initiate local or systemic repair processes, enhancing stem cell efficacy for regenerative medicine applications [56].

Several end groups of dendrimers can potentially present more control over cell proliferation rates and biodegradation profiles by systematic variation of concentration, generation size and end groups chemistry [57]. The clinical treatment rises to better approaches to repair diseased or damage tissues, so do design requirements for the material to be used [58].

The biodendrimer must be offer a temporary matrix for supporting cells functions (e.g. adhesion, proliferation and differentiation), before the regeneration of tissues occurs and orientate the arrangements of some cells type to specific location within a matrix. Furthermore, it is necessary modulates the biological activity in tissue repairs and it must have mechanical and physical properties similar to the host-damaged tissue [59]. For tissue repair the dendrimer can be crosslinked to form hydrogels.

A study by Bi *et al*., developed PAMAM/thiolated Hyaluronic acid (HS-HA) hydrogel with rat bone marrow stromal stem cells (RBMSCs) and Arginylglycylaspartic acid (RGD) peptide encapsulated. The results showed that PAMAM/HS-HA hydrogels significantly improved the cell viability, proliferation and attachment. So, they concluded PAMAM/HS-HA hydrogel system could be a promising platform for various applications in biofabrication [60]. Another study made by Bi *et al*., produced PAMAM-polyethylene glycol (PEG) hydrogels to differentiate mesenchymal stem cells (MSCs) for 21 days. The hydrogels system showed to influence the differentiation of MSCs and can be important in developing of therapeutic strategies [61].

Oliveira *et al*., investigated the effect of combining dexamethasone (Dex) loaded carboxymethlychitosan (CMCht)/PAMAM dendrimer, macroporous hydroxyapatite (HA) and SPLC scaffolds on the proliferation and osteogenic differentiation of RBMSCs *in vitro* [62]. The results demonstrated that Dex-loaded CMCht/PAMAM dendrimer nanoparticles with the HA increase osteogenesis and mineralization of the extra-cellular

matrix. Another study made by Oliveira *et al*., developed Dex loaded CMCht/PAMAM dendrimer, exposed them to RBMSCs onto starch-polycaprolactone *ex vivo*, followed by subcutaneous implantation in the back of Fischer 344 rats for 4 weeks [63]. The results showed that stem cell "tune-up" approach can be a new regenerative strategy for new bone tissue. Santos *et al*., evaluated the use of different generation of PAMAM dendrimer for the *in vitro* transfection of MSCs [64]. Transfection results showed transfection efficient very low however, it was sufficient to promote the *in vitro* differentiation of MSCs towards the osteogenic lineage. A study reported by Hu *et al*., evaluated the use of PEGylated PAMAM dendrimer as the nanocarrier for the cytoplasmic delivery of kartogenin (KGN) to induce chondrogenic differentiation of MSCs [65]. This approach demonstrated to induce higher expression of chondrogenic markers and the fluorescein labelled PEG-PAMAM was able to persist in the joint cavity for a prolonged time of healthy and osteoarthritis (OA) rats. The strategies mentioned above are summarized in **Table 2**.

Table 2. Different applications of dendrimers for tuning stem cells functions.

Application	Dendrimer type	Drug / Cells	Strategy	Reference
Proliferation	PAMAM/HS-HA hydrogels	RBMSCs	Biofabrication	[60]
Differentiation	PAMAM- PEG hydrogels	MSCs	3D model	[61]
Osteogenic differentiation	Dex-loaded CMCht)/PAMAM	RBMSCs	3D model	[62]
Bone regeneration	Dex-loaded CMCht)/PAMAM	RBMSCs	*Ex vivo* / *In vivo*	[63]
Osteogenic differentiation	PAMAM dendrimer	MSCs	Transfection	[64]
Chondrogenic differentiation	PEGylated PAMAM dendrimer	cytoplasmic delivery of kartogenin/ MSCs	*N/A*	[65]

N/A – Not applicable.

3.3. *In vitro* validation of dendrimers NPs in 3D/Dynamic platforms

Dynamic mechanical, biochemical and physicochemical cues present in the ECM delineate cell-cell and cell-tissue behaviors in native healthy and diseased tissues [66]. To get closer to the *in vivo* scenario, there are now new 3D *in vitro* tools that best emulate some important features. The use of 3D models or dynamic models such as microfluidics in various fields of such as drug discovery, diagnostic tools, and therapeutic approaches in regenerative medicine and tissue engineering holds undisclosed potential [67].

Microfluidics is both the science that studies the behavior of fluids in micro-channels, as well as the technology of fabricating miniaturized devices with 3D tunnels and chambers, in which fluids flow [68]. They can be valuable investigation tools due unique advantages: high sensitivity, high throughput, less material-consumption, low cost and enhanced spatiotemporal control [69]. The physical laws on microscale offer an advantage enabling the control of physics, biology, chemistry and physiology at cellular level. These benefits have furthermore driven the integration of biomaterials (naturally 3D in its matrix) with microfluidic structures and cell culture techniques, traditionally used in TE triad. For example, Carvalho *et al*. [70] developed a 3D microfluidic model to emulate colorectal cancer, in which its validation was performed by viability studies integrated with live imaging to confirm the dose-response effect of cells exposed to the Gemcitabine (GEM) released from carboxymethylchitosan/poly(amidoamine) dendrimer nanoparticles (CMCht/PAMAM) gradient. The CMCht/PAMAM dendrimer (G3) nanoparticles were synthetized and characterized previously, showing an average size of 50 nm [71]. Colorectal cancer cell line HCT-116 was embedded within Matrigel, and subjected to delivery-induced gradient of GEM was generated from a maximum concentration of dendrimer nanoparticles sourced from the lateral microchannels in a

dynamic fashion [70]. That work highlights the importance of the range of concentrations in a single experiment in an *in vivo* like scenario of colorectal cancer.

Agarwal *et al.* [72] described the fabrication and characterization of PAMAM (G4) conjugated cellulose filter paper-based 3D liver model. Since paper itself cannot be used for cell culture uses, it was functionalized with PAMAM dendrimer using glutaraldehyde. [72]. Results show PAMAM-paper promoted greater cell adhesion, proliferation, and viability of the HepG2 cells when compared to non-functionalized paper. Moreover, mature hepatocyte markers (albumin, ATP7b, CK19, and SULT2 A1) improved expression profile when in comparison to monolayer cultures. Additionally, the team showed the application of the PAMAM-paper 3D liver model for drug toxicity evaluations against hepatotoxins. The results showed a significantly higher sensitivity of the HepG2 cells upon repeated drug treatment when compared to a traditional 2D monolayer culture [72].

Bugno *et al*. evaluated the penetration of ultra-small nanoparticles (<10 nm) in terms of physical properties' influence penetration through solid tumors [73]. Sub-10 nm PAMAM dendrimers and gold NPs were evaluated in terms of penetration in multicellular 3D tumor spheroids (MCTS). Despite increased accumulation within the 3D spheroids, electrostatic cell interactions and ligand (folic acid, FA)-mediated targeting had low impact on penetration. Also, nanoparticles' rigidity played a minor role in penetration, with smaller rigid AuNP (2 nm) penetrating significantly more than larger AuNP (4 nm) (3-fold, $P = 0.014$; G2-NH2 vs. G4-NH2, 2.8-fold, $P = 0.033$) [73]. Results findings suggest that NP size is the key factor of tumor penetration. Remarkably, factors such as cellular uptake, surface charge, and even the conjugation of targeting ligands didn't play as significant of a role in the penetration behaviors as initially theorized [73].

In an innovative study, Holden *et al*. developed a dendrimer hydrogel made from ultraviolet-cured polyamidoamine dendrimer G3 linked to three polyethylene glycol molecules, was studied for the delivery of brimonidine (0.1% w/v) and timolol maleate (0.5% w/v), two antiglaucoma drugs [74]. The strategies mentioned above are summarized in **Table 3.**

Table 3. Different strategies for *in vitro* validation of dendrimers NPs in 3D/Dynamic platforms

Application	Dendrimer type	Drug	Strategy	Reference
Colorectal cancer	Carboxymethylchitosan/ PAMAM dendrimer nanoparticles	Gemcitabine	Microfluidics/3D	[70]
Liver cancer	PAMAM (G4) conjugated cellulose filter paper	Diclofenac Acetominofen	3D liver model	[72]
Tumor penetration	PAMAM dendrimers G2-NH_2, G4-NH_2 and G7-NH_2	N/A	3D multicellular tumor spheroids	[73]
Glaucoma	PAMAM dendrimer G3.0 + 3 PEG (12,000 Da)–acrylate chains	timolol maleate; brimonidine	3D Dendrimer Hydrogel	[75]

N/A – Not applicable.

3.4. *In vivo* and *ex vivo* applications of Dendrimers

Hedge *et al.* evaluated skin delivery of ketoprofen when covalently tethered to mildly cationic (2^+ or 4^+) peptide dendrimers [76]. Passive diffusion such as sonophoresis- and iontophoresis-assisted permeation of each peptide dendrimer-drug conjugate (D1–D4) was studied across mouse skin, both *in vitro* and *in vivo*. The study effectively demonstrated that peptide dendrimer conjugates of ketoprofen, when combined with non-invasive modalities, such as iontophoresis can enhance skin permeation with clinically relevant concentrations achieved transdermally.

In another approach, Patel *et al*. explored the use of thiamine-poly(propylene imine) (PPI) dendrimers for increased delivery of paclitaxel (PTX) across the blood brain barrier BBB [77]. The approaches making use of dendrimers have shown a great promise for diagnosis

and treatment of brain tumor due to its targeting capabilities of molecular cargoes to the tumor sites, as well as the efficiency of crossing the blood brain barrier and penetration to brain after systemic administration [78].

In vitro, PTX loaded thiamine conjugated PPI dendrimers (PTX-Tm-PPI) presented improved drug loading and reduced hemolytic toxicity with suitability for prolonged delivery of PTX. *Ex vivo* cytotoxicity studies of free PTX, PTX-PPI and PTX-Tm-PPI dendrimers over IMR-32 human neuroblastoma cell line revealed higher potential of PTX-Tm-PPI nanoconjugate to retard tumor cell viability as compared to plain PTX or PTX-PPI. Pharmacokinetics studies revealed significant slow clearance of PTX from the body via Tm-PPI nanoconjugate [77].

In another approach to tackle brain cancer, Sharma *et al*. aimed at establishing a platform for the possibility of effective and safe delivery of Temozolomide (TMZ) to the brain using surface engineered chitosan-PAMAM (G4.0) dendrimer for the treatment of glioblastoma (**Figure 1-i**) [79]. The *in vivo* pharmacokinetic parameters proved sustained release fashion such as half-life of 22.74 h of dendrimer constructs rather than 15.35 h of TMZ alone. When analyzing the biodistribution, higher concentration was found in heart when compared to the brain. This study exhibits the potential applicability of dendrimers in Central Nervous System in improving the anticancer activity and delivery of TMZ to brain. The attractive *ex vivo* cytotoxicity against two glioma cell lines; U-251 and T-98G and phase solubility studies of TMZ revealed remarkable results. *In vivo* studies of the prepared nano-formulation revealed to be promising and achieved double the concentration of TMZ in brain due to surface functionality of dendrimer [79].

Gupta *et al.* study attempted to deliver berberine (potential anticancer activity) through G4 PAMAM dendrimers by conjugation as well as encapsulation approach, to tackle breast cancer (**Figure 1-ii**) [80]. The developed encapsulated and conjugated berberine

formulations were found to have size in the approximate range of 100-200 nm. The *in vivo* hematological parameters were analyzed through auto-analyzer and the formulations were found to be safer and biocompatible with very least but insignificant ($p>0.05$) effects. The in vivo pharmacokinetic parameters were found to be impressively improved in albino rat model. The pharmacokinetic parameters such as half-life (t1/2) and AUC of berberine were impressively improved in the plasma level time *in vivo* studies in albino rat model. The obtained t1/2 was 14.33 h for encapsulation method compared to 6.7 h for berberine alone.

Wei *et al.* performed a comprehensive study where a novel drug delivery system based on a self-assembling amphiphilic dendrimer can generate supramolecular nanomicelles (with a larger space in their core for drug encapsulation) [81].

A small hydrophilic poly(amidoamine) (PAMAM) dendron and two hydrophobic C18 alkyl chains were bridged via click chemistry (**Figure 1-iii**). Doxorubicin was used as a model drug, and studied in 2D using Doxorubicin-sensitive and -resistant breast cancer cell lines (MCF-7S and MCF-7R); in 3D using tumor spheroids of MCF-7R cells, and also *in vivo,* where biodistribution and tumor penetration were assessed [81]. *In vitro* results of 3D tumor spheroids revealed that the AmDM/DOX nanomicelles could effectively penetrate deeper into the interior of the tumor spheroids when compared to free DOX. These results were corroborated in vivo, where dendrimer nanomicelles can gather efficiently at the tumor site via the EPR effect, as well as penetrating deeper within the tumor, also exhibiting better antitumor activity compared with free drug.

Heyder *et al*. evaluated the generation effect as well as the and surface PEGylation of degradable polyester-based dendrimers nanocarriers on their interactions with an *in vitro* model of the pulmonary epithelium as well as to assess the ability to formulate such carriers in propellant-based, portable oral-inhalation devices (**Figure 1-iv**) [82].

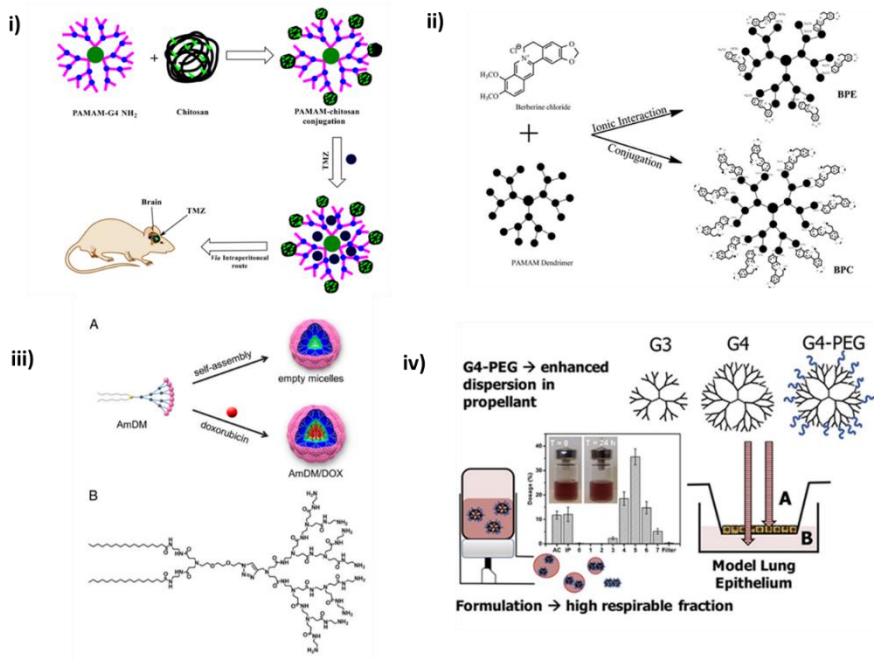

Figure 1. Representation of different strategies for the employment of dendrimer NPs in tissue engineering applications. i) Schematic delivery of TMZ through PAMAM-chitosan conjugate in a rodent model. Reprinted with permission from [79]. Copyright © 2018 Springer. ii) Different approaches for the delivery of berberine: G4 PAMAM dendrimers by conjugation (BPC) as well as encapsulation (BPE) approach. Reprinted with permission from [80]. Copyright © Elsevier 2017. iii) Nanomicelles constructed with AmDM as a drug delivery platform. (A) Formation of empty AmDM nanomicelles and DOX-encapsulated AmDM/DOX nanomicelles. (B) Molecular structure of amphiphilic dendrimer AmDM. Reprinted with permission from [81]. Copyright © 2015 National Academy of Sciences. iv) Research effect of generation and surface PEGylation of degradable, polyester-based dendrimers nanocarriers on their interactions with an *in vitro* model of the pulmonary epithelium. Reprinted with permission from [82]. Copyright © 2017 Elsevier.

That study was performed in trans-well® inserts where Calu-3 cells were seeded on apical compartment. The cells were allowed to grow at air-liquid interface. As a follow up, *in vivo* studies using these formulations were also used [83]. Pulmonary administration of the formulations is performed by means of using the pharyngeal aspiration technique, as well as intravenous (*i.v.*) administration. Both the *in vitro* and *in vivo* results showed that PEGylation can be used to control internalization and transport of dendrimers across the pulmonary epithelium, serving therefore as a tailorable platform to target the lung tissue for treating local diseases, or for systemic delivery, using the lung as pathway to the

bloodstream [83]. In summary, given the advantages of dendrimers and dendritic scaffolds presented here and the successes of these materials in the above applications, it is clear that there is a need for continued innovation through the use of new materials, procedures, and strategies to address challenges in field of tissue engineering.

The strategies mentioned above are summarized in **Table 4.**

Table 4. *In vivo* and *ex vivo* tissue engineering applications of dendrimers.

Application	Dendrimer type	Drug / Cells	Strategy	Reference
Transdermal delivery of anti-inflammatory drugs	Peptide-dendrimers: Gly-Lys(Keto)-Lys-(Arg)2 Gly-Lys(Keto)-Lys-(Lys)2 Gly-Lys-(Arg-Keto)2 Gly-Lys-(Lys-Keto)2	ketoprofen	*Ex vivo*/*in vivo*	[76]
Brain cancer	thiamine-poly(propylene imine) (PPI) dendrimers	Paclitaxel	*Ex vivo*/*in vivo*	[77]
Brain cancer	Chitosan - PAMAM dendrimer G4.0	Temozolomide	*Ex vivo*/*in vivo*	[79]
Breast cancer	G4.0 PAMAM	Berberine	*Ex vivo*/*in vivo*	[79]
Breast cancer	hydrophilic PAMAM dendron +2 hydrophobic C18 alkyl chains	Doxorubicin	*Ex vivo*/*in vivo*	[81]

4. Final remarks and future trends

Conventional therapeutic approaches demonstrate various limitations like low aqueous solubility and short half-life. Dendrimers can provide a platform to modify the basic properties of drug molecules improving their solubility, half-life, biocompatibility and its release characteristics. Furthermore, dendrimers have been shown great potential to be used as intracellular carriers of growth factors aimed at controlling the behavior of stem cells mainly their proliferation and osteogenic and chondrogenic differentiation. These novel strategies based on the use of NPs loaded with drugs offer unprecedented opportunities both at the preclinical and clinical levels. However, some challenges still

remain in the dendrimer research field. For a better application in Tissue Engineering, parameters such as biodistribution, biocompatibility, and mainly the efficacy of these systems *in vivo*, has to be further improved.

Although the number of dendritic polymers that have advanced to the clinic is still remarkably small, the cases of success in both treatment and diagnostic applications indicates that dendrimer research has the potential to make a significant clinical impact. Recent works have demonstrated how dendrimer nanotechnology can be beneficially implemented to foster therapeutic perspectives, namely in the field of Tissue Engineering.

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