Branched and dendritic polymer architectures: functional nanomaterials for therapeutic delivery

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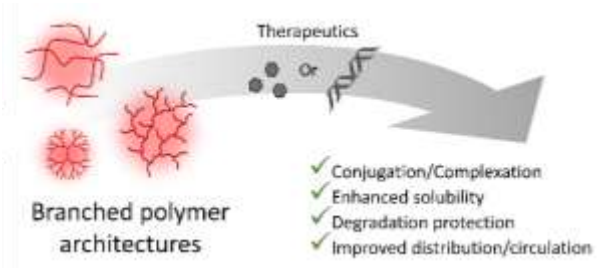

Keywords: Bionanotechnology, Polymeric materials, Dendrimers, Drug delivery, Hierarchical structures

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Abstract

Barriers to therapeutic transport in biological systems can prevent accumulation of drugs at the intended site, thus limiting the therapeutic effect against various diseases. Advances in synthetic chemistry techniques have recently increased the accessibility of complex polymer architectures for drug delivery systems, including branched polymer architectures. In this contribution, we first outline drug delivery concepts, and then define and illustrate all forms of branched polymers including highly branched polymers, hyperbranched polymers, dendrimers, and branched-linear hybrid polymers. Many new types of branched and dendritic polymers

continue to be reported, however there is often confusion about how to accurately describe these complex polymer architectures, particularly in the interdisciplinary field of nanomedicine where not all researchers have in-depth polymer chemistry backgrounds. In this context, the present review describes and compares different branched polymer architectures and their application in therapeutic delivery in a simple and easy to understand way, with the aim of appealing to a multidisciplinary audience.

1. Introduction

Drug discovery and development is the process of finding new pharmaceutical candidates and bringing them to market, and includes identifying new drug molecules, pre-clinical research, clinical trials, and the task of obtaining relevant regulatory approvals.[1,2] Recent estimates put the average cost of this research and development in the region of 10's of millions to billions USD.[3] Historically this R&D has focused on small organic molecule new chemical entities, but has now expanded to recent biotech drugs, such as, peptides, proteins, antibodies, and nucleic acids. [4,5]

However, these pharmacologically active agents are not necessarily effective in their most simple forms due to a number of biological barriers which can limit the therapeutic efficiency.[6-10] Therapeutics are typically administered in formulations to increase their efficacy by controlling solubility, absorption, hydrolytic or enzymatic degradation, pharmacokinetics, biodistribution, excretion, and off-target toxicity.[11-13] A wide variety of materials have been developed as formulations and drug delivery systems, with the choice of material selected depending on the type of drug, conjugation/release strategies, and route of administration.[14-16] Materials chemists can elegantly engineer materials of various sizes, shapes, compositions and physicochemical properties.[17-20] Inorganic materials such as iron oxide nanoparticles, carbon nanotubes, metal organic frameworks, and mesoporous silica constructs, have been applied with good effect.[21] Biological materials are also being investigated including viral vectors for the delivery of gene-based therapeutics, and more recently extracellular vesicles have been receiving interest for drug delivery.[22-24] Among the materials developed for drug delivery systems, polymers have possibly been studied the most. Advances in synthetic polymer chemistry and coupling techniques, have led to the ability to precisely control both nanomaterial composition and function.

As well as looking at different compositions of drug and gene delivery polymers, varying architecture has also been investigated.[25-28] Research on differing polymer-based nucleic acid transfection systems has led to a variety of insights into possible architectural and design parameters that could lead to the optimal non-viral delivery agents.[29,30] In particular, graft, star, and branched polymer systems appear very promising, due to reports of low toxicity and high transfection efficiencies.[31] Branched polymers also offer benefits related to multifunctionality, which can offer synthetic routes to multivalent ligand display and also increased possibilities for covalent drug attachment. The multifunctionality of branched and dendritic architectures also opens the door to new opportunities for theranostic applications (combined diagnostic as well as therapeutic).[32]

Many new and creative examples of branched and dendritic polymers continue to be reported, but there is often confusion about how to accurately class these complex polymer architectures. In this review, we first outline therapeutic delivery concepts and guiding principles in overcoming certain biological barriers. We then define, illustrate, and outline synthetic strategies for all possible forms of branched polymers including highly branched polymers, hyperbranched polymers, dendrimers, and branched-linear hybrid polymers. A selection of the most important recent examples of branched polymers in drug delivery applications will be highlighted and discussed, with a particular focus on the ability to control polymer composition, degradability, shape, and external functionality. Overall, the present review describes and compares different branched polymer architectures and their recent (primarily post 2015) application in therapeutic delivery in a simple and easy to understand way, with the aim of appealing to a multidisciplinary audience. A number of excellent reviews cover more specific details of dendrimer and hyperbranched polymer synthesis,[33,34] or biological applications of these polymers in great depth,[32,35-39] should the readership be interested in further information.

2. Biological barriers to therapeutic delivery

In order to achieve therapeutic effect, pharmacologically active molecules need to reach their sites of action, typically on a cellular level, and with a high enough dose or concentration. However there are a number of hurdles to successful therapeutic delivery, including both extracellular and intracellular barriers.[40-42] Many recent efforts have involved designing particles and delivery systems with certain properties or stimuli response to specifically

overcome some of these barriers,[43] while others have focused on increasing fundamental understanding of these clearance mechanisms.[10]

2.1. Extracellular barriers

In case of oral, inhalation, and some local administration routes, a major barrier to drug delivery are mucus layer barriers. Mucus coat regions include the gastrointestinal tract, lung airways, vaginal mucus membranes, and nasal cavities, and is composed of a viscoelastic mucin fibre hydrogel.[44] These mucus gels are typically negatively charged due to sialic acids and sulfate groups in the sugar fibre chains, and contain many different salts, proteins, bacteria, lipids, and other species which form a complex network which protects the underlying cells from external species including nanoparticle systems.[45,46] Oral delivery routes also have the added barrier of harsh stomach and intestine pH conditions which can hinder the delivery and stability of therapeutics.[13]

In the case of systemic routes of administration directly into the circulatory system, these potential hurdles can be avoided. However, there are further barriers to be navigated, including avoidance of polymer aggregation and destabilisation in blood which could occur with non-specific protein adsorption and also red blood cell association and aggregation.[47] Enzymatic drug degradation is recognized as a growing problem with regard to antibiotic resistance, as one of the mechanisms by which this resistance occurs is through bacteria production of enzymes that selectively target and destroy the activity of antibiotics.[48] In addition, enzymatic degradation of nucleic acids has been reported to occur within the order of minutes *in vivo*, which is a major challenge for gene therapy treatments.[49,50]

During circulation drug delivery systems are subject to physical clearance by lung, slpeen hepatic, and renal pathways.[41] Elimination of larger injected material occurs *via* fenestrations in the pulmonary capillaries and sinusoids of the lungs and spleen respectively. In the liver, polymers, biopharmaceuticals, and nanoparticles with sizes <200 nm are primarily cleared by liver sinusoidal endothelial cells, and particles of larger sizes (>200 nm) or higher rigidity are generally taken up by Kupffer cells.[51] The kidneys are responsible for removal of the smallest sized particles, proteins, or foreign bodies from blood (<5 nm).

The inevitable interaction of nanoparticles with the immune system poses a number of problems, including opsonization and clearance by the mononuclear phagocyte system (MPS), and also pro-inflammatory response to certain nanomedicines.[52,53] Opsonization involves the binding of an antibodies or proteins to the drug delivery vehicle surface, and subsequent

recognition and sequestration by phagocytes - either resident macrophages in the spleen or liver, or circulating macrophages. The formation of this protein corona is dependent on nanoparticle size, surface charge, and exterior chemical composition.[54,55] A common strategy is to functionalise the nanocarrier exterior with a stealth non-fouling polymer such as poly(ethylene glycol) (PEG), poly(poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), poly(2-oxazoline) (Pox), or poly(poly(ethyl ethylene phosphate) (PEEP).[56,57] These hydrophilic polymers form a hydration layer which can hinder protein absorption and thus reduce MPS clearance.

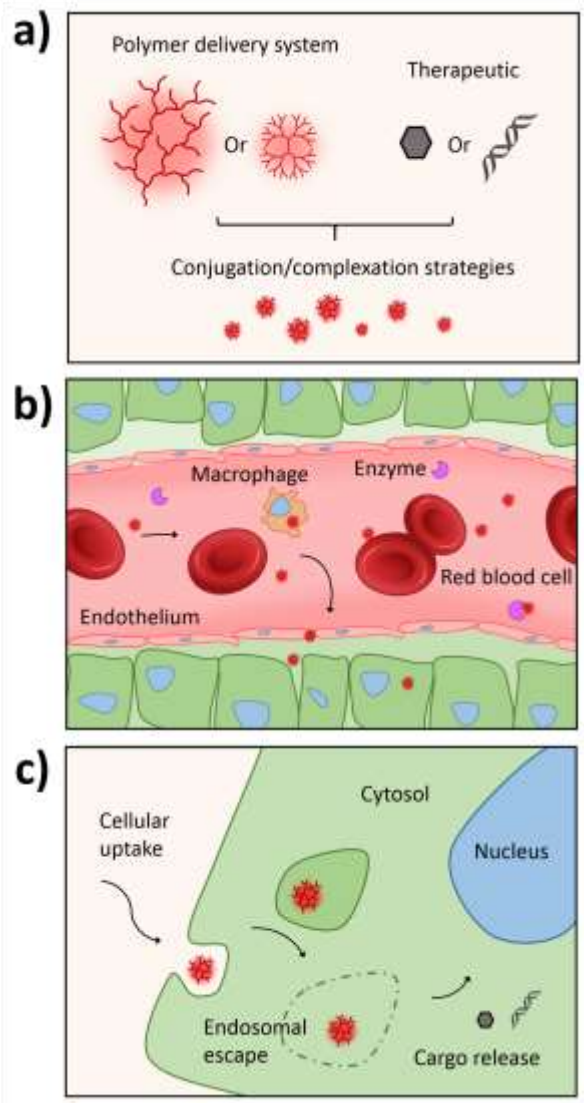


Figure 1. *Barriers in the delivery pathway of polymer based nanomedicines,* ***a)*** *formation of therapeutic conjugate/complex with polymer* ***b)*** *avoidance of rapid clearance and unspecific interactions with blood components,* ***c)*** *cellular uptake, therapeutic release, and intracellular trafficking.*

Polymer and other nanoparticle based therapeutics need to extravasate from the circulatory system and cross the vasculature endothelium in order to reach their intended site of action. This can occur by passive endothelial transcytosis, but also occurs via the enhanced permeation and retention (EPR) effect described by Maeda and colleagues.[58,59] The EPR effect is due to blood vessel dysfunction and fenestrations at sites of tumours, and has been utilized to passively target cancerous tissue and enhance nanoparticle accumulation.[60]

Although great advances have been made using polymeric drug delivery systems for therapeutic delivery to tumours and other non-brain organs, the central nervous system (CNS) is a particular challenge for drug delivery.[61] The blood brain barrier significantly restricts the passage of systemically delivered therapeutic drug molecules to the brain; the benefits of polymeric nanoparticles represent a promising solution to these problems but have yet to be fully exploited.[62,63] The role of this barrier is to regulate the homeostasis of the brain, and thus maintain the unique extracellular environment of the CNS. Structurally, the BBB consists of endothelial cells which form the walls of the capillaries of the brain and spinal cord.[64] There are tight continuous junctions between these particular endothelial cells, which restricts any aqueous paracellular passages from the blood. Small molecules and also larger biomolecules and nanoparticles can only access the CNS by a transcellular mechanism through the vasculature endothelium. This primarily requires harnessing of various endogenous transport systems and receptors in the capillary endothelium. The different mechanisms for endogenous transport across the BBB can be categorised generally as: the transcellular lipophilic pathway; use of transport proteins (utilized by glucose and amino acids); receptor mediated transcytosis (utilized by transferrin and insulin);[65] and absorptive transcytosis.[64]

2.2. Intracellular barriers

After a period of circulation and extravasation, nanocarriers need to be uptaken by target cells after which release of therapeutic payload can occur in order for the active agent to achieve therapeutic effect on the relevant cytosol, nuclear, or intracellular organelle objective. Cellular internalization can occur via the mechanisms of passive diffusion or various endocytic pathways, such as macropinocytosis, receptor mediated endocytosis, or phagocytosis.[66] The initial step involves particle interaction with components of the outer surface of cells, and then formation of internalized vesicle structures of various sizes and internal environments. This process of crossing cellular membranes can depend to a large extent on size, charge, and surface

morphology of the species being internalised, but also the nature of the cell-type in question. Naked nucleic acids and other biomacromolecules can often be too negatively charged to enter cells efficiently *via* endocytosis.[67] Attempts to increase internalisation include moderation of particle surface charge and hydrophobicity, with cationic species reportedly being uptaken significantly more, but also have higher toxicity due to non-specific membrane lysis.[68] Attachment of ligands to drug delivery systems is also a popular method of enhancing uptake in certain cells expressing the appropriate cell surface receptors.

Once inside the cell, the delivery vehicle then needs to escape from the endosome.[69] Endosome escape is essential for the avoidance of lysosomal degradation of therapeutic molecules from lysozyme enzymes and acidic pH environment. Endosomal escape of nanomedicines is also required to avoid the eventual exocytosis of internalized material. Other approaches include the incorporation of pH responsive functionalities designed to become cationic at the reduced endosomal pH values, thus triggering membrane interaction and rupture, and escape of the drug delivery system to the cytosol.[70-72] Following progression of the drug delivery system to the cytosol, the final stages for optimal therapeutic delivery and efficiency involve release of therapeutic from the nanocarrier, either for the mechanism of action to occur in the cytosol, or for diffusion to the appropriate cellular organelle. A number of elegant conjugation chemistries have been developed for precise therapeutic release in response to certain cellular environments or stimuli, including redox responsive disulfide bonds which are known to cleave in the presence of intracellular levels of glutathione.[73,74] For successful DNA transfection the DNA needs to pass the double membrane nuclear envelope and enter the nucleus to be transcribed. Entry to the nucleus occurs through the nuclear pore complex through either passive or active transport mechanisms, again, with size being a determining factor.[75-77]

3. Polymer architectures for therapeutic delivery

3.1. Increasing complexity

Polymers have a key role in drug delivery systems and have the potential to provide solutions by simplifying administration, reducing toxicities, and improving efficiencies through additional functionality.[78] The progression of polymer architectures from linear to more complex branched topologies by use of easily accessible chemistries while maintaining reasonably large scale production, offers further opportunities to improve therapeutic

delivery.[25,79] Recent advances in polymer chemistry, including new step-growth polymerisation routes, continued advancement of controlled radical and ring-opening polymerisation methods, and further development of simple, high yielding, and orthogonal coupling chemistries, has brought unprecedented access to complex polymer architectures.[80,81] Branched polymers are a special class of polymer architecture characterised by their high branching densities.[33] The branched polymer topology imparts a number of favourable properties compared to their linear polymer equivalents including: high surface functionality, globular conformation, low intrinsic viscosities, high solubilities, and interesting rheological modifying properties.[33,34,82] This has led to branched polymers being increasingly important for biomedical applications over the past 20 – 30 years.[35,83]

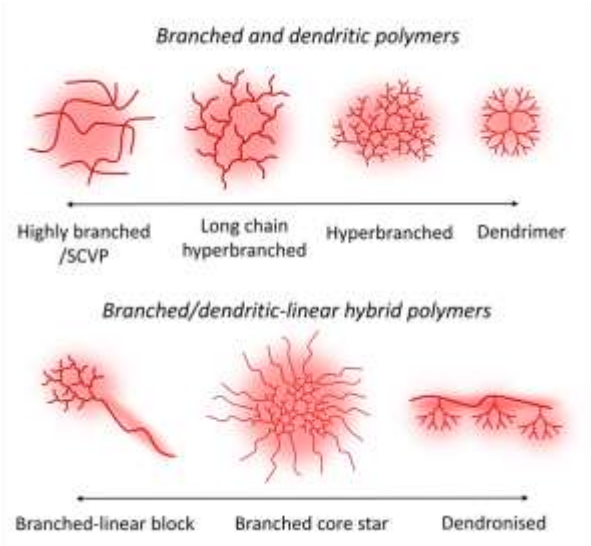


Figure 2. *Cartoon representation of various branched polymer architectures able to be synthesised with modern polymerisation and coupling synthetic strategies.*

In certain polymers such as thermosets and rubbers, branching is typically on a macrocscopic/crosslinked scale, leading to interesting physical properties of these materials. This review focuses of branching in soluble nanoscale forms, and will refer to branched polymers of the following definitions (illustrated in **Figure 2**). The term highly branched polymer refers to high frequency main chain branching of linear polymers, with the branch points in a highly branched polymer being distributed randomly throughout the polymer. The major advantage of highly branched polymers is their simple synthetic methodologies. In general, the term dendritic polymer is used to refer to a class of branched polymers including dendrimers, dendrons, hyperbranched polymers, and hybrid variants containing dendrons and hyperbranched polymers. The term originates from the Greek word dendron, δένδρον, which

translates to tree. The various subclasses of dendritic polymers can be further defined. Dendrimers were first synthesised in laborious multi-step procedures, in the late 1970's and early 1980's, and are defined by their perfectly symmetrical and layered branching patterns (with no irregular or non-branching points, DB), and therefore single molecular weight with a dispersity of 1.[84-86]

$$DB = \frac{D + T}{D + T + L} \qquad (\boldsymbol{Equation\ 1})$$

Degree of branching (DB) was defined by Fréchet and coworkers, **Equation 1**, where D, T and L are the fractions of dendritic, terminal or linear monomer segments in the resulting dendritic polymers (obtained from NMR spectroscopy).[87] Dendrimers have DB's of 1. Hyperbranched polymers are synthesized by step-growth polymerization via condensation or addition of AB_n monomers in one-pot reactions. Here, A and B are the two functionalities that can react with each other but not with themselves. In an AB_2 monomer system, the degree of branching is controlled by statistics and only reaches around 0.5, far from the value of 1 usually achieved with dendrimers.[88] Further functionality and control over branching distributions can be introduced by the AB_2 polymerisation of macromonomers leading to long chain hyperbranched polymers.[89] Recently, evolution of these branched topologies has progressed towards dendritic-linear hybrid polymers from combination of well controlled linear polymers with dendritic polymers, *via* creative coupling and polymerisation chemistries.[90] These structures include linear hybrids of dendrimers and hyperbranched polymers, and can be in the form of branched-linear block copolymers, branched-core star polymers, and dendronised polymers.

From a synthetic chemistry perspective, these structures can by produced from an ever expanding toolbox of polymer chemistry and coupling chemistry techniques. A summary of these procedures can be found in **Figure 3**. Dendrimer formation has typically proceeded using a series of iterative growth and activation steps.[91] Dendrimers can be synthesized following the divergent approach which can lead to branching irregularities at higher generations, and also the convergent approach, which was introduced in pioneering work by Fréchet and Hawker and can lead to higher purity.[92] Due to their structural precision this has been achieved with robust organic reactions, including amidification and esterification reactions (**Figure 3)**. Recently improvements to dendrimer synthesis, in terms of reaction times and purity, have been achieved using accelerated techniques based on efficient and orthogonal chemistries.[93]

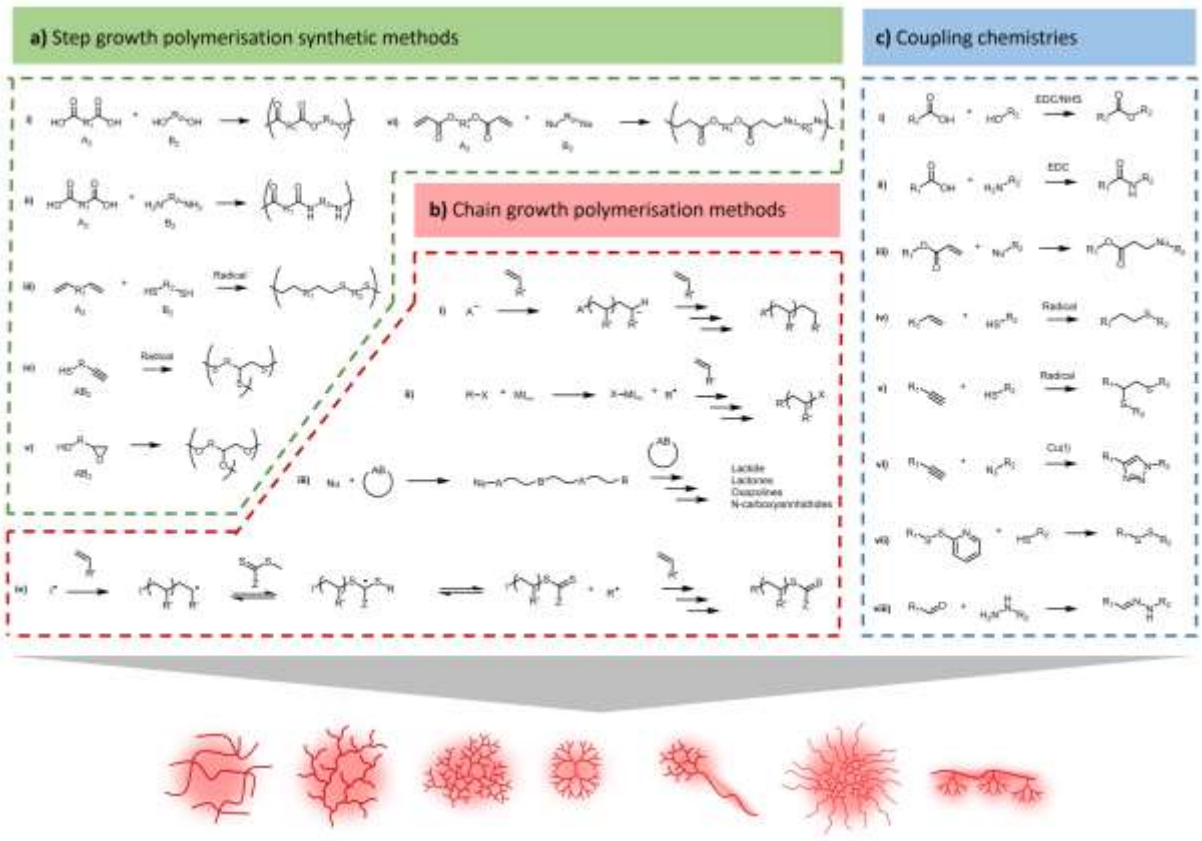


Figure 3. *Schematic representations of some of the synthetic strategies to achieve branched polymer architectures with modern polymerisation and coupling synthetic strategies:* ***a)*** *branched and linear polymers via step growth polymerisations, i) esterification condensation, ii) amidification condensation, iii) thiol-ene addition, iv) thiol-yne addition, v) asymmetric epoxide ring opening, vi) michael addition type,* ***b)*** *branched and linear polymers via controlled chain growth polymerisations, i) living anionic, ii) Cu(0) radical polymerisations, iii) ring opening polymerisations, iv) RAFT polymerisation,* ***c)*** *various coupling strategies for formation of branched-linear hybrid materials, and also therapeutic conjugation, i) ester, ii) amide, iii) michael addition, iv) thiol-ene, v) thiol-yne, vi) azide-alkyne cycloaddition, vii) disulfide formation, viii) hydrazone.*

One-pot synthetic strategies for formation of highly branched and hyperbranched polymers arose as an alternative and simpler route to polymers with similar favourable properties to dendrimers, but without demanding multi-step syntheses and purifications. Hyperbranched polymers are synthesised by the step growth polymerisation of AB_n monomers (where $n \geq 2$), and also the step growth copolymerisation of combinations of monomers, as in the $A_2 + B_m$ approach (where $m \geq 3$).[33] These reactions were theorised by Flory decades ago and require monomers different A and B functionalities that can react with each other but not with themselves.[94] Step growth polymerisations can also be performed with telechelic AB_2 macromonomers leading to long chain hyperbranched polymers.[95]

The design and synthesis of highly branched polymers by chain growth polymerisations is a more recent development in polymer chemistry. In the self-condensing vinyl polymerisation (SCVP) route, which was introduced by Fréchet and co-workers, a vinyl monomer bearing an initiating group can propagate through the vinyl bond and also form branching points through the initiating group.[96] This SCVP has been extended to RAFT, ATRP, NMP, and SCROP. Another popular chain growth strategy for highly branched polymers is the Strathclyde route, which involves the copolymerisation of vinyl monomers with small amounts of divinyl monomers and in the presence of a chain transfer agent.[97] Similarly to SCVP, this method has also been extended to the controlled radical polymerisations RAFT and ATRP. Linear polymers with pendant vinyl moieties are formed, which then have the opportunity to polymerise into other linear chains to form highly branched polymers.

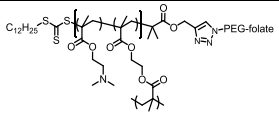
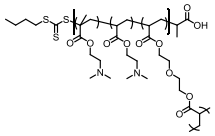
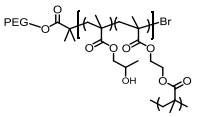
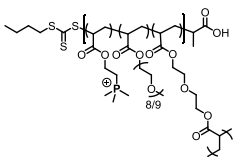
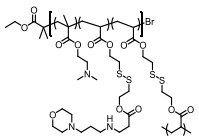
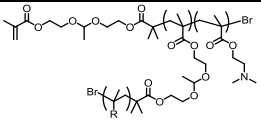
Since the development of living anionic polymerisations by Szwarc in 1956, there have been many chain growth polymerisations developed for synthesis of linear polymers with controlled molecular weights, narrow molecular weight dispersities, and precise functionality.[98,99] RAFT polymerisation in particular is becoming increasingly popular for biomedical applications, in part due to its ease of use and compatibility with a wide range of monomers.[100] In addition to synthesis of highly branched polymers by chain growth methods as mentioned previously, by combining these chain growth systems with efficient coupling chemistries, researchers can now easily synthesise new types of branched-linear hybrid architectures including, branched-linear block copolymers, branched-core star polymers, and dendronised polymers. The expansion of highly efficient coupling chemistries, after the seminal work of Sharpless *et al*., has led to further options for synthesis of these types of architectures.[101] Available click-type reactions include, the Huisgen alkyne-azide cycloaddition, thiol-ene/yne radical additions, various thiol-ene Michael additions, and tertiary isocyanate amine coupling among others.[80,102]

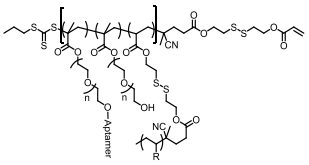
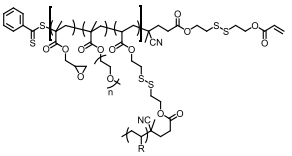
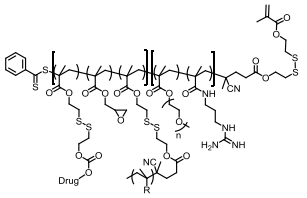
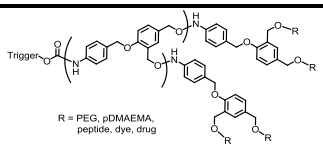
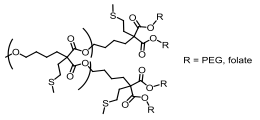
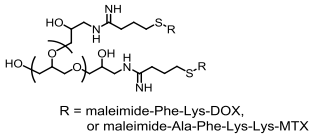
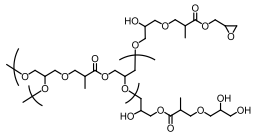
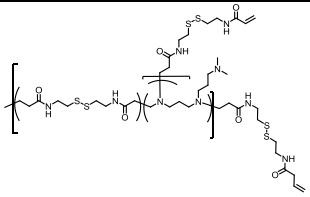
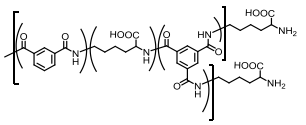
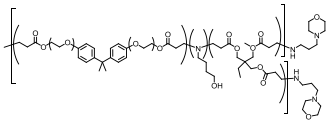
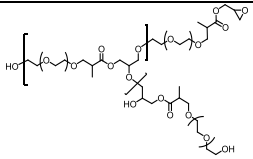
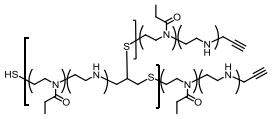
Therapeutic molecules can be carried by these polymer systems by two main methods: encapsulation with either hydrophobic or electrostatic interactions, and also covalent attachment to the polymeric carrier. Encapsulation methods have been widely investigated, and branched polymer architectures offer the benefits of having globular three-dimensional topologies capable of encapsulating high loadings of cargo. A benefit of this approach is the ability to obtain unimolecular micelle type of structures without concentration dependent disassembly at low concentrations.[36] However it can be difficult to control the release of molecules from the polymer. Many interesting chemistries (**Figure 3**) have been developed for covalent attachment guest molecules, including stimuli responsive linkers able to release

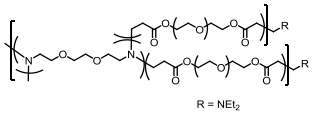
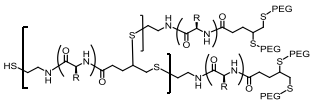
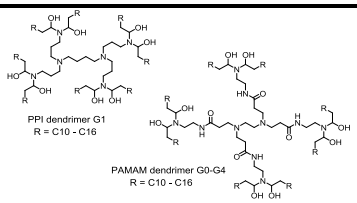
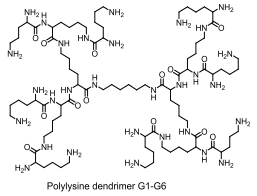
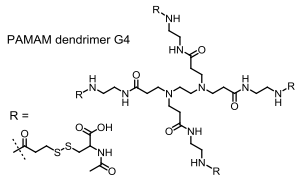
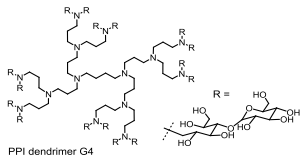
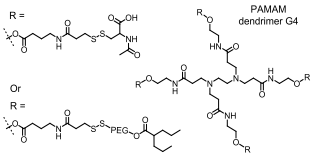
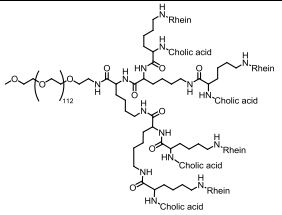
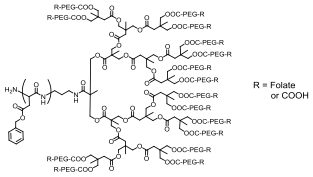
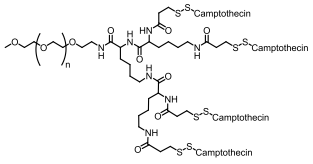
therapeutic molecules on certain specific triggers, including pH, redox environments, glucose, and enzymatic cleavage.[43] Branched polymers also offer advantages for this drug attachment method, due to their high number of functionalisable groups on the periphery of the constructs.

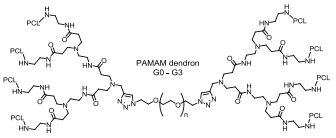
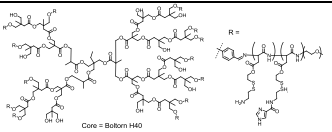
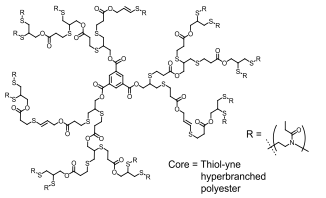
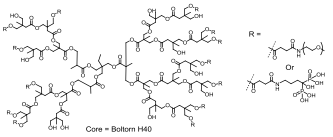
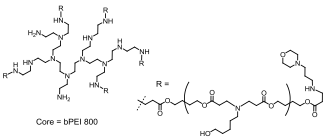
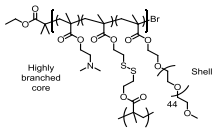
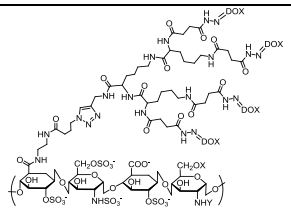
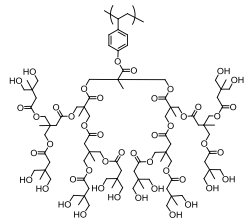
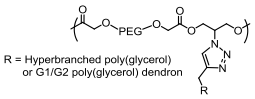
It is worth noting that branched and dendritic polymers have also gained use as active ingredients themselves, without the need for additional therapeutic molecules. For example, in the treatment of inflammatory and autoimmune diseases such as rheumatoid arthritis and multiple sclerosis, dendrimers and hyperbranched polymers have been used as anti-inflammatory agents.[103-105] Dendrimers are also emerging as treatments of infections, based on antimicrobial activity of the polymer itself.[106] Currently Starpharma dendrimer product Vivagel® has completed phase III trials, and been launched in multiple countries, for topical treatment and rapid relief of bacterial vaginosis. In addition carbosilane dendrimers with sulfonate end groups,[107] and phosphorous based dendrimers with cinnamic acid terminating groups,[108] have been investigated as HIV-1 retrovirucides.

Table 1. *Summary of current state-of-the-art in branched polymer therapeutic delivery systems, including design strategies for various branched polymer architectures, and specific application details in drug/ gene delivery.*

Architecture	Structure	Polymer synthetic method	Therapeutic conjugation method	Application	Ref
Highly branched Divinyl copolymerisation		RAFT copolymerisation with divinyl monomer	Electrostatic nucleic acid complexation	Plasmid DNA delivery	109
		RAFT copolymerisation with divinyl monomer	Electrostatic nucleic acid complexation	Controlled release of dsRNA	110
		ATRP copolymerisation with divinyl monomer	Encapsulation of SPIONs	SPION delivery for therapy/diagnosis	111
		RAFT copolymerisation with divinyl monomer	Electrostatic nucleic acid complexation	Plasmid DNA delivery	112
		ATRP copolymerisation with divinyl monomer	Electrostatic nucleic acid complexation	Plasmid DNA delivery	113
SCVP		ATRP SCVP of degradable inimer and DMAEMA	Drug encapsulation	Niclosamide and amonafide drug delivery	114

		RAFT SCVP of disulfide inimer and PEGMA/aptamer monomer	Hydrophobic drug encapsulation	Delivery of doxorubicin to breast cancer cell line	115
		RAFT SCVP of disulfide inimer and PEGMA/GMA	Covalent attachment, with acid and redox cleavable groups	Camptothecin intracellular delivery	116
		RAFT SCVP of disulfide inimer and functional monomers	Disulfide linked drug monomer (Gd MRI imaging via epoxide)	Camptothecin intracellular delivery	117
Hyperbranched AB_n		AB_2 polycondensation (isocyanate-hydroxy)	Covalent attachment	Doxorubicin delivery and DNA delivery	118
		AB_2 polycondensation	Hydrophobic drug encapsulation	Taxol anticancer therapy	119
		AB_2 ROP of epoxide containing glycidol	Enzyme cleavable covalent attachment	Doxorubicin and methotrexate anticancer drugs	120
		AB_3 ROP of epoxide containing monomer	Ester linked covalent attachment	Methotrexate anticancer drug	121
A_2+B_m		A_2 +B_3 Michael addition	Electrostatic nucleic acid complexation	Tumour siRNA delivery in vivo	122
		A_2 +B_2 +B_3 polycondensation	Cell penetrating peptide mimic	Intracellular delivery via endosome disruption	123
		A_2 +B_2 +B_3 Michael addition	Electrostatic nucleic acid complexation	Plasmid DNA delivery for skin gene therapy	124
Longchain hyperbranched		Proton transfer AB_2 polymerisation	Ester linked covalent attachemnt	Methotrexate anticancer drug	125
		CROP and thiol-yne AB_2 photoaddition polymerisation	Electrostatic nucleic acid complexation	Plasmid DNA delivery	126

		A_2 +B_4 Michael addition polymerisation	Electrostatic nucleic acid complexation	Plasmid DNA delivery	127
		CROP and thiol-yne AB_2 photoaddition polymerisation	Hydrophobic drug encapsulation	Doxorubicin drug release in vitro	128
Dendrimer		Divergent dendrimer synthesis (commercial) then Michael addition modification	Electrostatic nucleic acid complexation	siRNA gene delivery to lung vasculature	129
		Divergent strategy using standard peptide coupling chemistry	Electrostatic nucleic acid complexation	Plasmid DNA delivery	130
		Divergent dendrimer synthesis (commercial) then activated ester coupling	Disulfide linked covalent attachment	NAC anti-inflamatory agent delivery to B2-V microglial cells	131
		G4 PPI dendrimer Reductive amination coupling of maltose shell	Electrostatic drug complexation	Fludarabine triphosphate delivery	132
		Divergent dendrimer synthesis (commercial) then amide coupling	Disulfide linked covalent attachment	NAC and valproic acid anti-inflammatory	133
Branched-linear hybrid Branched-linear block		Divergent lysine dendron synthesis and amidification with PEG and functionality	Hydrophobic drug encapsulation	Delivery of doxorubicin to lymphoma tumour	134
		NCA ROP, divergent dendrimer synthesis, activated ester coupling	Hydrophobic drug encapsulation	Paclitaxel drug delivery	135
		Commercial PEG, divergent dendrimer synthesis, activated ester coupling	Disulfide linked covalent attachment	Camptothecin delivery in vivo	136

		Alkyne azide click coupling of PAMAM dendrons and PEG	Hydrophobic drug encapsulation	Doxorubicin encapsulation and release	137
Branched-linear star		Polycondensation (commercial H40) ph sensitive polypeptide shell coupling	Electrostatic nucleic acid complexation	siRNA gene delivery	138
		CROP and thiol-yne photoaddition polymerisation	Hydrophobic drug encapsulation	Hydrophobic dye as drug model	139
		Polycondensation (commercial H40) activated ester PEG arm coupling	Hydrophobic drug encapsulation	Doxorubicin anticancer drug delivery	140
		Azirine ROP (commercial PEI) Michael addition arm coupling	Electrostatic nucleic acid complexation	Plasmid DNA delivery	141
		ATRP with divinyl comonomer	Electrostatic nucleic acid complexation	siRNA gene delivery to lung vasculature	142
Dendronised		Divergent lysine dendron synthesis and azide alkyne click coupling to heparin	Covalent attachment, through acid cleavable hydrazone	Doxorubicin delivery in 4T1 breast tumor model	143
		Polystyrene based backbone grafted with polyester dendrons	In vivo biodistribution studies with radiolabel	Radiolabel as proof-of-concept for drug delivery	144
		PEG azido polymer backbone grafted with polyglycerol dendrons	Hydrophobic drug encapsulation	Hydrophobic dye as drug model	145

3.2. Highly branched polymers

Synthesis of highly branched polymers by use of chain growth polymerisations, is a versatile and scalable approach for the synthesis of functional polymers.[146] Radical chain growth polymerisation methods have always produced branching in some cases through the radical

polymerisation side reactions of intramolecular backbiting, intermolecular transfer to polymer, and polymerisation of vinyl terminated disproportionation products.[147] However, introduction of branching in radical polymerisations through design was more recently established.

3.2.1. Divinyl monomer copolymerisation

Network formation through the radical polymerisation of vinyl monomers with difunctional comonomers is analogous to step growth polymerisations of multifunctional monomers. These polymerisations have been considered theoretically by Flory and Stockmayer, among others.[148-151] Whilst considerable experimental work has also been carried out with various monomer pairs, including: MMA and EGDMA, styrene and divinyl benzene, vinyl acetate and divinyl adipate. Theory predicts that macroscopic crosslinking will occur when the number of difunctional branching monomers per polymer chain is greater than one. In practise there is often a discrepancy between the theory and the observed polymerisation gel points. Theoretical gel point values are generally underestimated due to pendant vinyl group of the multifunctional commoner species causing intramolecular cyclisation during the polymerisation or becoming less reactive as one group is polymerised and remaining as an unreacted pendant vinyl group throughout the polymerisation.[152] These free radical polymerisation systems are often difficult to predict and can require considerable optimisation in order to achieve high conversions without macroscopic gelation.[153,154]

However, in 2000 Sherrington *et al.*, introduced an improvement to free radical polymerisations of divinyl monomers to form highly branched but soluble polymer architectures, by inclusion of thiol chain transfer agent (CTA).[97,155,156] This method, known as the 'Strathclyde route', reduces the primary chain length, can delay gelation, and can allow inclusion of additional functionality through functional CTAs. Atom transfer radical polymerisation (ATRP) was then employed to synthesise similar branched polymers with a more controlled polymerisation.[155] The difunctional copolymer method to branched polymers was first employed with reversible addition fragmentation chain transfer (RAFT) polymerization by our group in 2005,[157,158] and further investigated by Armes and coworkers.[152]

Early work on application of these branched polymer systems to DNA delivery was established by the Davis group, who synthesised highly branched PDMAEMA-b-PEG using RAFT polymerisation.[159,160] The structures were formed with a redox sensitive divinyl comonomer,

to yield high molecular weight polymers able to be cleaved into lower molecular weight polymer chains. Efficient binding of DNA was shown to occur through electrostatic interactions. This method has also been adopted by the Thurecht group who have used the RAFT copolymerisation of DMAEMA and EGDMA to synthesise highly branched structures for a variety of biomedical applications including gene delivery.[109,161] Polymer ability to deliver DNA was investigated using in vitro cell uptake assays in HeLa cells with flow cytometry. Branched pDMAEMA conjugated with the targeting ligand Folate (overexpressed on HeLa cells) showed improved cell uptake compared to oligofectamine and non-folate branched pDMAEMA, however polymer toxicity was observed above N/P ratios of 10.

More recently, Rannard and Owen, have shown that ATRP copolymerisations with small amounts of EGDMA leads to branched polymers which can form stable nanoparticles with tuneable sizes and functionalities.[162] It was shown, with a gut epithelium model, that these nanoparticles are able to cross mucus barriers and have potential of use as orally-administered nanocarrier systems. Amphiphilic highly branched polymers were used to stabilise oil-based emulsions and deliver different dissolved antiretroviral drugs for HIV/AIDS therapy. Using an epithelium monolayer transwell membrane the permeation of emulsion formulated HIV protease inhibitor Lopinavir was around 10 times higher than the aqueous application of the drug.[163] In a viral activity assay the antiviral effect (against HIV-1 IIIB) of drug-loaded nanoemulsions was similar to the aqueous based control. Additionally, it has been demonstrated that these highly branched polymers, synthesised by ATRP, can form stable nanoparticle composites with super-paramagnetic iron oxide nanoparticles (SPIONs) with future uses for delivery of drugs, imaging agents, or hyperthermia agents within cancer therapies.[111]

Wang and coworkers have investigated the effect of branching on transfection of plasmid DNA coding for G-luciferase and also green fluorescent protein (GFP).[113] Variation of primary chain length and density of branching between individual polymer chains was achieved using ATRP with redox responsive divinyl comonomer. The authors found that the most highly branched polymer had the least adverse cytotoxic effects, whilst having higher transfection efficiencies than the linear pDMAEMA control.

Our group has recently focussed on expanding the application of highly branched polymers by RAFT polymerisation as nucleic acid delivery systems, by incorporation of alternative functionalities for nucleic acid complexation, and also controlled payload release

strategies.[110,112] Highly branched polymers containing phosphonium cationic moieties, and also the equivalent polymer with ammonium cationic groups, were synthesised by a post polymerisation modification route. Bromoethyl acrylate (BEA) and polyethyleneglycol acrylate (PEGA) were copolymerised with diacrylate comonomer DEGDA by RAFT polymerisation, this precursor was functionalised via the bromine with trimethylphosphine and trimethylammonium. The phosphonium polymers showed good ability to complex nucleic acids, ~~good biocompatibility,~~ lower cytotoxicity to a NIH-3T3 celline over a polymer concentration range from 0.5 µg/mL to 2 mg/mL, and higher GFP transfection efficiencies compared to the ammonium equivalent. In another study, we investigated the ability of highly branched polymers to complex and release dsRNA in a sustained manner, *in vitro*, by complexation of nucleic acids with highly branched p(DMAEMA-*co*-DMAEA) (**Figure 4**). By copolymerising we were able to tune the hydrolysis rate of the acrylate side chains, which transform from cationic amine containing groups, into anionic acrylic acid groups, therefore tuning the release of dsRNA. This release mechanism is also beneficial in terms of cytotoxicity compared to cationic pDMAEMA or bPEI, as the polymers hydrolyse over time to ~~biocompatible~~ less cytotoxic polyacrylic acid.

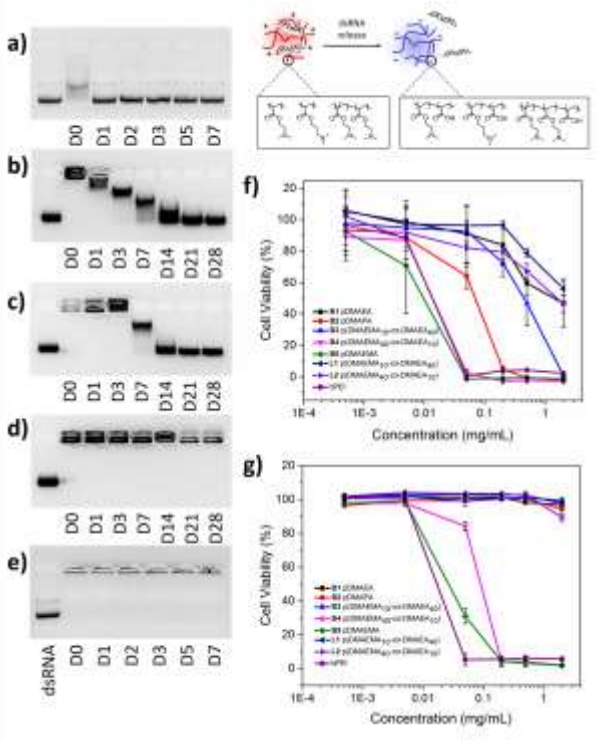


Figure 4. *Highly branched and hydrolysable copolymers by RAFT for dsRNA release, including agarose gel electrophoresis assay showing dsRNA release (polyplexes all at N/P ratio 5) over time periods up to 28 days* ***a)*** *branched pDMAEA;* ***b)*** *branched p(DMAEMA$_{10}$-co-DMAEA$_{40}$);* ***c)*** *branched pDMAPA;* ***d)*** *branched p(DMAEMA$_{40}$-co-DMAEA$_{10}$);* ***e)*** *branched pDMAEMA; and polymer toxicity*

*to NIH-3T3 cells following 24 hr incubation of **f)** initial polymers; **g)** polymers pre-incubated for 2 weeks in D_2O at pH ~ 7.4. Figure adapted with permission [110]. Copyright 2018 Royal Society of Chemistry.*

3.2.2. Self-condensing vinyl polymerisation

In 1995, Fréchet *et al*. showed that polymerisation of a vinyl monomer bearing an initiating group allowed polymerisation through the vinyl group and also through the initiating site, leading to the formation of highly branched polymers.[96] The authors termed this self-condensing vinyl polymerisation (SCVP). The SCVP process has been extended to various chain growth polymerisation methods, such as, nitroxide mediated polymerisation (NMP),[164] reversible addition chain transfer polymerisation (RAFT),[165] atom transfer radical polymerisation (ATRP),[166] and ring opening polymerisation (ROP).[167,168] Chain growth methods to highly branched polymers, such as divinyl commoner method, and SCVP, allow for facile incorporation of stimuli responsive groups, prodrug monomers, and imaging moieties for theranostic applications.[169]

The group of Gao has investigated branched polymers by SCVP for breast cancer therapies. The researchers used ATRP of inimers in microemulsion, and were able to load a drug combination of DNA damage repair agents and also STAT-3 inhibitors (amonafide and niclosamide respectively).[114] Selective and synergistic growth inhibition of triple negative breast cancer cells (IC_{50} of 2-4 times lower than the individual drugs) was seen with the combination of drugs encapsulated in the branched polymer delivery system. Luo *et al*., synthesised branched hydroxypropyl methacrylamide copolymers by a RAFT SCVP method, incorporating a DOX prodrug monomer and cathepsin-B enzyme cleavable branching units.[170] The high molecular weight and large (102 nm diameter) branched polymers could be degraded into lower molecular weight and smaller (8.2 nm diameter) species. These branched drug conjugates were investigated for breast cancer therapy both in vitro and with a mouse model. Enhanced antitumour efficacies in a 4T1 tumour model was observed by TG1, immunohistochemical results, and the in vivo toxicity assays, highlighting potential benefits of designing polymer drug delivery systems with stimuli responsive and degradable properties compared to the non formulated free drug. Recently RAFT SCVP was also employed by Wei and colleagues, to form highly branched polymer prodrug conjugates with both redox sensitive disulphide groups and acid sensitive groups carbonate groups.[116] The presence of acidic pH conditions or glutathione environments enhances the release of camptothecin, with the polymer system having IC50 values of 365.1 μg/ml, to HeLa cell-line with an MTS cell viablilty assay.

3.3. Hyperbranched polymers

Hyperbranched polymers have a more defined branching pattern than highly branched polymers, as branch points are introduced at high proportion of monomer units, compared to randomly along a chain growth polymer chain with highly branched polymers.[82] This feature makes hyperbranched polymers interesting for therapeutic delivery applications as the degree of branching in terms of branching units, linear units, and terminal units is much more easily defined and characterised.[34]

3.3.1. Step growth polymerisation of AB_n monomers

Much of the theory of branched and hyperbranched polymers was outlined by Flory in the mid-20th century, based on polycondensation reactions.[94] In order for AB_n hyperbranched polymers to be formed, a number of requirements were outlined by Flory: the A moiety must react selectively with B groups, B groups must have equal reactivity, and no cyclisation reactions should occur. These reactions proceed in a manner similar to most step growth polymerisations with rapid loss of monomer early in the reaction, high conversions required for high molecular weights, and the case of AB_n polymerisations there is no possibility of crosslinking (in theory). The resulting polymers contain dendritic units (fully reacted B moieties), linear units (singly reacted B moieties), terminal units (unreacted B moieties), and one focal A group. One of the most well-known examples of a hyperbranched polymer formed from AB_n polycondensation is the commercial polymer Boltron, synthesised from the monomer bis(methylol)propionic acid (bis-MPA).[171] Boltron hyperbranched polymers with multiple surface hydroxyl functional groups have been synthesised, which have been used in a large number of applications, both as the native polymer and also post-polymerisation modified *via* the hydroxyl groups to impart further functionality or different solubility properties.[172] In this case control over the reaction (resulting molecular weight, molecular weight distribution, and degree of branching) can be achieved by addition of monomer in discrete portions, later developed into the 'slow monomer addition' method.[173,174]

Klok *et al*. have investigated the effect of degree of branching of polylysine on DNA complexation and delivery.[130] Transfection efficiency was affected by both polymer architecture and molecular weight. At similar molecular weights the hyperbranched polylysines showed greater transfection and gene knockdown compared to their linear and

dendrimer analogues. In the 1990's, Mulhaupt and Frey developed the chemistry of hyperbranched polyglycerols which are formed from the step growth polymerisation of glycidol, a latent AB_2 monomer.[175] ~~The polymers have very high biocompatibility~~ Over many studies the polymers have displayed low cytotoxicity both *in vitro* and *in vivo,*[176] similarly to the established linear polyethylene glycol, however great control over the branching and architecture can be achieved, opening up the application of these materials as nanocarriers for therapeutic purposes. In 2014, the Frey group showed that conjugating the MUC1 glycopeptide B-cell epitope and the tetanus toxoid T-cell epitope to the surface of hyperbranched polygycerol, enabled optimal presentation of antibodies due to the 3d topology of the branched structure.[177] This synthetic vaccine led to significant immune responses in a mouse study, highlighting the potential of these systems to be used in anticancer immunotherapy. Haag and colleagues have further expanded these hyperbranched polyglycerols as drug delivery systems, by attaching enzymatically cleavable therapeutics to the surface of these nanocarriers.[120] Further work by the group has shown that conjugating doxorubicin via an acid cleavable hydrazine linker had high drug loadings (5-10 molecules per hyperbranched polymer of 2 – 5 kg/mol) and an improved antitumour efficiency compared to free doxorubicin in a mouse tumour model.[178]

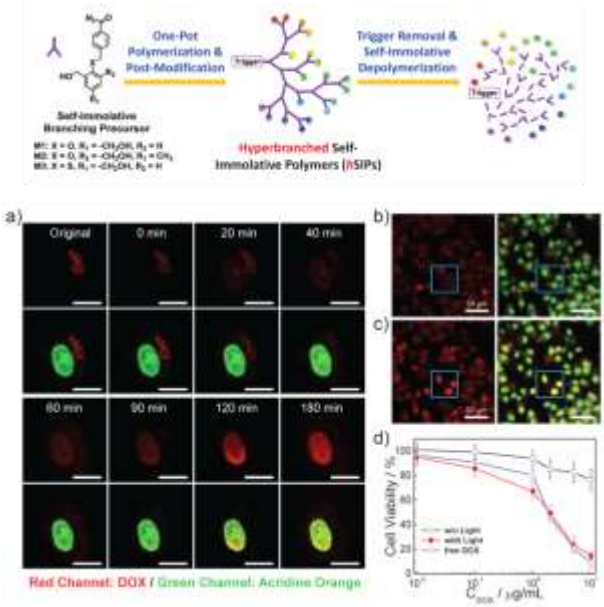


Figure 5. *Synthetic scheme and confocal laser scanning microscopy images of HeLa cells incubated with hyperbranched and self-immolative polymers conjugated with doxorubicin and cRGD peptide. Figure adapted with permission [118]. Copyright 2015 American Chemical Society.*

Hyperbranched and self-immolative polymers undergo a cascade depolymerisation process after stimuli responsive removal of a trigger at the focal point of the hyperbranched polymer. Liu and coworkers synthesisd hyperbranched self immolative polymers in a onepot AB_2 polycondensation method, after which, the polymer were funtionalised with various imaging, targeting, and therapeutic groups including, cRGD peptides, Doxorubicin, coumarin, choline, and DMAEMA for nucleic acid complexation.[118] Depolymerisation triggered by blue light was investigated, and the polymer was determined to be completely degraded after 6 hours. Additionally intracellular release (HeLa cell-line) of doxorubicin conjugated to the exterior of the hyperbranched polymer was followed with confocal microscopy (**Figure 5**). Colocalisation studies indicated polymer cellular uptake via endocytosis and release of doxorubicin into the cytosol, which overtime was seen to enter the nucleus with use of acridine orange stain.

Step growth polymerisation of AB_n monomers, where $n \geq 3$, has also been used to synthesise hyperbranched polymers for therapeutic delivery. For example, Zhu et al. synthesised biodegradable hyperbranched polyglycerol by *in situ* formation of an AB_3 monomer, to which they then conjugated the anticancer drug methotrexate (MTX) and fluorescent dye rhodamine.[121] The polymers showed ~~good biocompatibility~~ low cytotoxicity with over 90% NIH-3T3 cell viability after 48 hours over a polymer concentration range from 1 µg/mL to 10 mg/mL, and biodegradability through the polymer ester bonds. MTT assay against a cancerous cell line suggested high anticancer efficiency of the hyperbranched polymer drug delivery system.

3.3.2. Step growth polymerisation of A_2+B_m monomers

Synthesis of branched polymers *via* a double monomer methodology, $A_2 + B_m$, is attractive due to the range of much more readily available monomers, however the approach can lead to gelation at high conversions and critical concentrations.[34] These syntheses also require careful optimisation of the ratio of functional groups, monomer concentrations, purity of reagents, reaction time and temperature, in order to achieve controlled and reproducible reactions of high molecular weights without purification methods.[168,179] The growth and final structure profile of $A_2 + B_m$ systems is also not fully comparable to AB_n systems with their cascade type of branching patterns, leading to some in the community not considering them true hyperbranched polymers.[33]

A particularly simple but elegant step growth polymerisation method was developed by Lynn, Anderson, and Langer in the early 2000's, involving Michael additions of amines to multifunctional acrylate groups to form poly(β-aminoesters).[180,181] This was further developed to hyperbranched poly(β-aminoesters) by $A_2 + B_m$ routes more recently by a number of research groups. In 2016, Wang *et al*, investigated highly branched poly(β-aminoesters) for gene therapy, synthesised by the Michael addition polymerisation of an A_2 amine monomer with B_3 and B_2 triacrylates and diacrylates.[124] The authors found that the branched polymer topology imparts favourable properties of improved transfection efficiencies and reduced toxicities *in vitro*. Additionally, the highly branched poly(β-aminoesters) effectively delivered genetic material *in vivo*, and resulted in the expression of significant functional proteins in the skin. A similar strategy was employed by Oupicky and colleagues, who prepared hyperbranched poly(β-amido amines) through the Michael addition polymerisation of and A_2 diacrylamide monomer and a B_3 amine monomer (**Figure 6**).[122] The polymers were degradable with a glutathione redox stimuli through use of a disulphide containing diacrylamide, and were also functionalised with fluorine moieties. Good ability to bind siRNA by the polymers was confirmed, and gene silencing was successfully demonstrated with an *in vivo* luciferase expressing tumor model. Against B16F10 cells and 4T1 cells, the fluorinated bioreducable hyperbranched polymer was able to knockdown luciferease expression the most (20-50% expression of luciferase relative to PBS control). While for the animal study, the same polymer siRNA formulation gave 10-40% expression of luciferase relative to PBS control, depending on whether lucerferase activity was determined *in/ex vivo*.

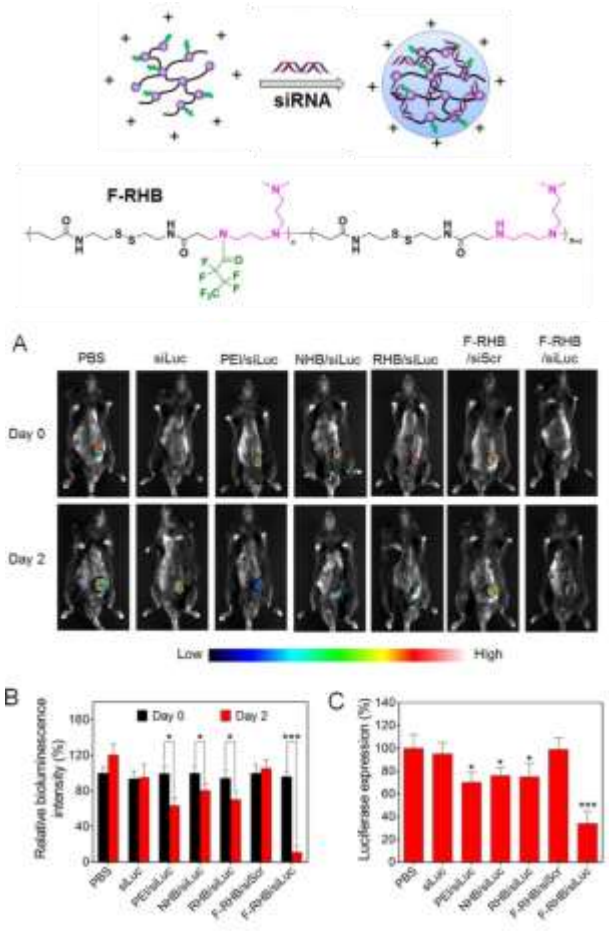


Figure 6. *Hyperbranched poly(amido amines) and in vivo luc gene silencing (FRHB: fluorinated reducible hyperbranched polymer, RHB: reducible hyperbranched polymer, NHB: non-reducible hyperbranched polymer),* ***a)*** *bioluminescence images of mice with B16F10-luc tumours,* ***b)*** *quantification of whole-body images,* ***c)*** *ex vivo anaylsis of luc activity in isolated tumour samples. Figure adapted with permission* [122]*. Copyright 2017 American Chemical Society.*

In a different example, the $A_2 + B_m$ system has also been recently employed to synthesise hyperbranched lysine based polymers that mimic cell-penetrating peptides. Chen *et al*. used a polycondensation reaction involving $A_2 + B_3 + B_2$ monomers, and showed that the resulting hyperbranched polymers had high cellular internalisation rates which were dependent on pH.[123] The branched architectures enhanced the membrane lytic properties of the polymers compared to the linear version, and thus showed potential for cytoplasmic delivery of therapeutic molecules.

3.3.3. Long chain hyperbranched polymers

Hyperbranched polymers from macromolecular units are a particularly interesting class of dendritic polymer architecture due to the ability to introduce additional functionality along the

macromonomer chain, and the control over distance between branch points by tuning the degree of polymerisation of the linear macromonomer.[89] Synthetic strategies involve combination of chain growth polymerisation methods to gain well-defined AB_2 macromonomers which can be further polymerised in an AB_2 step growth method.

Long chain hyperbranched PEG materials have been synthesised in this manner by Zhu and coworkers, and used for anticancer drug molecule delivery and plasmid DNA delivery.[125] The researchers were able to combine the advantages of a long chain hyperbranched architecture with the favourable biological properties of PEG to produce promising branched materials for use as drug delivery systems. An alternative route to hyperbranched polymers has been developed by our group, utilising thiol-yne radical chemistry.[95,139,182-184] This reaction involves the addition of a thiol to a reactive alkyne followed by the addition of another thiol to the resulting vinylthioether at a faster rate. This leads to hyperbranched polymers with very high degrees of branching. This approach can be used for both small molecules and polymers with thiol and alkyne end groups.[185,186] In a recent study, we reported the synthesis of hyperbranched poly(ethyleneimine-*co*-oxazoline) by combination of thiol-yne photoaddition chemistry with well-defined linear ethyleneimine-*co*-oxazoline copolymers (**Figure 7**).[126] This new PEI hyperbranched architecture with only secondary amines (compared to bPEI with primary, secondary, and tertiary amines) was used to complex and deliver plasmid DNA coding for GFP. *In vitro* toxicity assays and gene transfection experiments with HEK293T cell-line, highlighted the impact of polymer architecture, as the hyperbranched structure showed lower toxicity but similar transfection efficiencies compared to the equivalent linear p(ethyleneimine-*co*-oxazoline) copolymers. The AB_2 thiol-yne step growth approach to long chain hyperbranched polymers has also been employed by Dong *et al.*, who synthesised hyperbranched polypeptide with a PEG shell for encapsulation and delivery of doxorubicin.[128] The researchers produced poly(e-benzyloxycarbonyl-L-lysine) with thiol and alkyne end groups which formed hyperbranched polylysine under UV irradiation, to which a linear PEG shell was attached. The hyperbranched polymer gave a higher drug loading than the linear counterpart block copolymer, and a slower drug release rate.

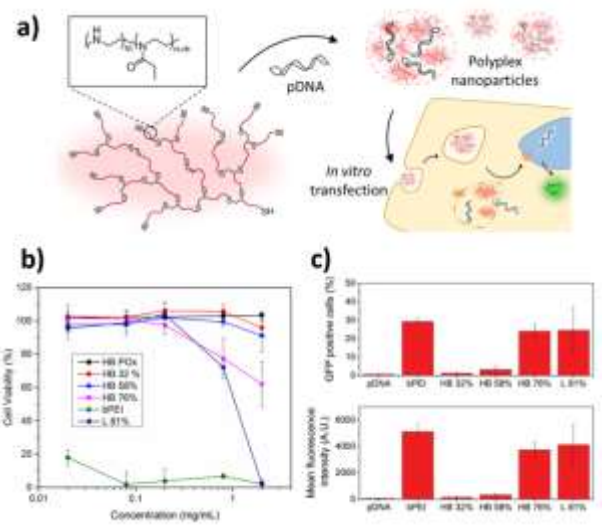


Figure 7. a) *Hyperbranched p(ethyleneimine-co-oxazoline), with varying ethyleneimine contents from 32% to 78%, by thiol-yne chemistry for use in the delivery of plasmid DNA with a GFP reporter gene,* ***b)*** *polymer toxicity as determined by XTT assay in HEK293T cells,* ***c)*** *proportion GFP positive cells and mean fluorescent intensities of transfected HEK293T cells compared to commercial branched PEI. Figure adapted with permission* [126]*. Copyright 2019 Royal Society of Chemistry.*

3.4. Dendrimers

Dendrimers are possibly the most studied of branched polymers for therapeutic delivery applications. This is due, in part, to their structurally perfect branching patterns and also to being very well defined unimolecular species.[35] Poly(amido amine) (PAMAM) dendrimers were the first dendrimers to be widely studied and are now commercially available, in addition to the large variety in backbone structures and coupling chemistries that have since been developed.[93] The dendrimer architecture offers the attractive property of multivalent surface functionality for increased interaction with biointerfaces, while also allowing efficient drug conjugation to the surface or encapsulation in the unimolecular micelle-like core.

Anderson *et al*., employed a combinatorial approach to obtain a library of modified dendrimers of varying generation PAMAM and p(propylenimine) (PPI), with different alkyl chain substituents (C10 – C16).[187] SiRNA formulated dendrimers were found to preferentially accumulate in Tie2-positive endothelial cells in the lung, when studied with an in vivo mouse model. The materials showed promise for the delivery of nucleic acid therapeutics in diseases or injuries involving dysfunctional endothelium, whilst having clinical translation advantages relating to molecularly defined dendrimer cores. Glutathione responsive PAMAM dendrimers have been developed by Kannan *et al*., and recently been investigated in a large animal model

of hypothermic circulatory arrest induced brain injury.[133] Systemically injected dendrimer drug conjugates were able to deliver the antineuroinflamitory therapeutic N-acetyl cysteine, and the antiexcitotoxicity therapeutic valproic acid. The dendrimer delivery system produced 24 hr neurological score improvements of similar values to a 10 fold higher dose of free drug, and with much reduced adverse side effects.

Another application of dendrimers in drug delivery, is the formulation of corticosteroids for the treatment of retinal neuroinflammation, in macular degenerative diseases. An intravitreal injection of hydroxyl-terminated polyamidoamine dendrimer covalently conjugated with fluocinolone acetonide, accumulated in activated microglia, and halted retinal degeneration for one month.[188] Also in the realm of inflammation related diseases, the Hammond group recently showed that cationic and PEGylated PAMAM dendrimers with insulin-like growth factor 1 conjugated to the 4-7 nm macromolecule surface, were able to penetrate cartilage and provide relief from osteoarthritic symptoms in an preclinical rat model. The size and surface charge of the nanocarrier was pivotal in achieving cartilage penetration and drug therapeutic lifetime.[189]

In 2016, the group of Siegwart, reported modular and degradable dendrimers that had low toxicities and high antitumor efficiencies, and gave a significant survival benefit in the *in vivo* cancer model studied (**Figure 8**).[190] The ester based dendrimers were synthesised using sequential thiol or amine Michael additions, which allowed a large library of dendrimers to be produced with differing functionalities and generations. Initial *in vitro* and *in vivo* siRNA luciferase gene silencing screens were performed to evaluate dendrimer candidates to be taken forward to a further aggressive liver cancer model. An optimal degradable dendrimer was identified that was able to inhibit growth in the studied cancerous tumour model, while having low toxicity and biodegradability.

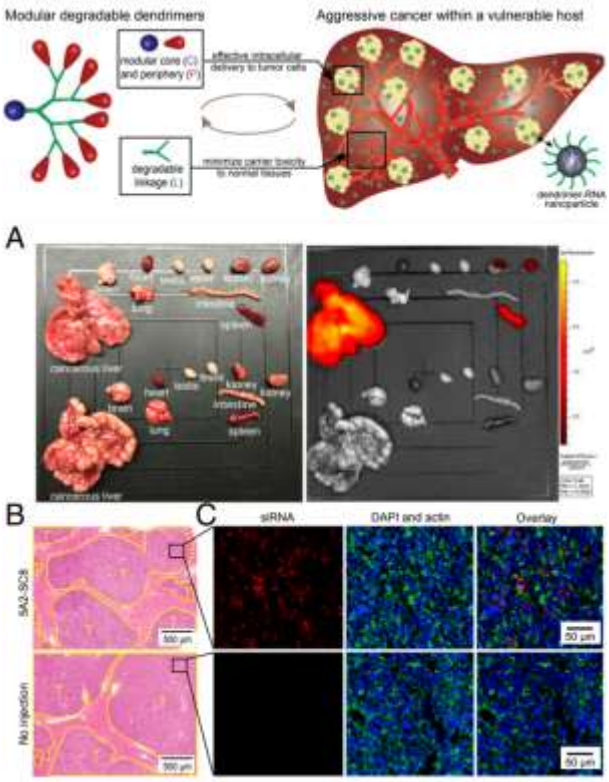


Figure 8. *Modular degradable dendrimers for therapeutic delivery to liver cancer,* ***a)*** *fluorescent imaging showing cancerous liver accumulation of siRNA,* ***b)*** *histology staining comnfirmed livers contained tumours,* ***c)*** *confocal imaging confirmed siRNA intracellular location. Figure adapted with permission [190]. Copyright 2016 National Academy of Sciences.*

3.5. Branched-linear hybrid polymers

As the field of branched and dendritic polymers has rapidly developed, new classes of hybrid polymers have emerged.[90,191] These branched-linear hybrid polymer architectures can contain either dendrimers or hyperbanched polymers and include block copolymers, branched core star polymers, and dendronised polymers.

3.5.1. Branched-linear block copolymers

Hybrid block copolymer structures of branched polymers can be synthesised by either a chain first strategy, dendron/branched polymer first strategy, or a coupling strategy. A particular advantage of hybrid branched-linear block copolymers is the combination of branched topology traits with the self-assembly possibilities of block copolymers, which enables further development of drug delivery systems based on micelle like structures. This is illustrated by Hammond *et al*., who synthesised amphiphilic dendron-linear block copolymers with poly(β-

benzyl-L-aspartate) linear hydrophobic chain and hydrophilic polyester dendron unit functionalised with folate groups.[135,192] The anticancer therapeutic paclitaxel was encapsulated in the micelle core with loading efficiencies of up to 40%, while the exterior of the drug carrier presents a multivalent targeting by the folate groups. Both targeted and non-targeted micelles accumulated in tumour sites by the EPR effect after injection in mice, however the folate system was able to enter tumour cells from the extracellular environment by receptor mediated endocytosis, and had a 4 fold improved anticancer efficiency compared to the non-targeted system.

The Luo group has investigated amphiphilic dendritic-linear copolymers for anticancer drug delivery (**Figure 9**).[134,193] The synthesised polymers form micelles having a hydrophilic linear PEG shell and hydrophobic dendron core functionalised with rhein, or cholic acid, or riboflavin, which are able to bind to the drug doxorubicin thus forming stable nanoparticles. The strong doxorubicin dendron interactions leads to very high drug loading efficiencies of up to 100% immediately after formulation, reducing to between 100% - 40% after 1 day, and further reducing to 100% - 10% after 1 week. The dendritic-linear polymer systems investigated formed particles with high stabilities, long circulation times, reduced toxicities, whilst also showing favourable anticancer efficiencies in the particular subcutaneous Raji lymphoma xenograft mouse model that was employed.

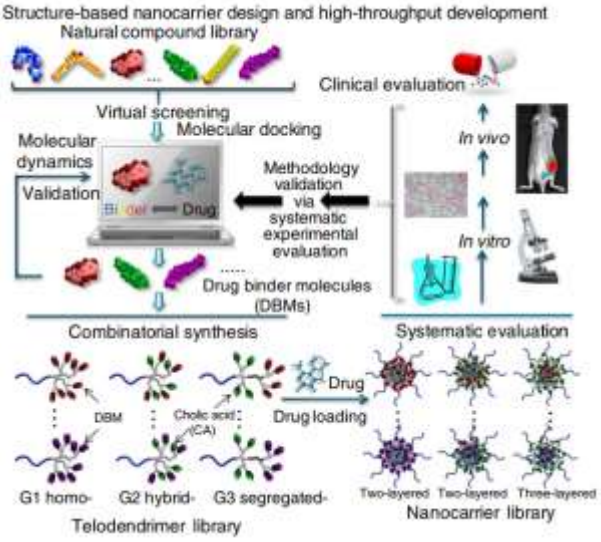


Figure 9. *Dendritic-linear hybrid block copolymers for doxorubicin delivery, synthesised by a rational design and high throughput development process. Figure reproduced with permission [134]. Copyright 2015 Springer Nature.*

Shen *et al*., studied dendritic-linear block copolymers for delivery of camptothecin.[136] The therapeutic agents were conjugated to the hydrophobic dendron segment of the polymer *via* a redox responsive disulphide bridge, while the linear PEG segment provided solubility ~~and biocompatibility~~. Due to the active ingredient being covalently attached to the multivalent dendron, high drug loadings were achieved. The polymer drug conjugate self-assembled into micelles of different morphologies depending on the number of conjugated drugs and thus the hydrophobicity of the core forming block. It was found that medium length rod-like micelles of these branched-linear block copolymers had long circulation times, and released camptothecin intracellularly, demonstrating the advantages of branched-linear block copolymers for therapeutic delivery to tumours.

3.5.2. Branched-core star polymers

Star polymers involving linear polymer chains extending radially from a globlular three dimensional branched polymer, are another interesting class of branched-linear hybrid polymers. This polymer architecture can be rationally designed for use as efficient encapsulation devices for various guest molecules, as well as direct conjugation to the star exterior. In cases where a high number of polymer arms can be attached to the branched polymer core, the star polymer can act as a unimolecular nanocarrier.[36,194] When considering amphiphilic core shell systems with high number of arms, the architecture can offer the advantage of not dissembling into individual polymer chains upon dilution, as would be the case for micelle systems. However, there are also studies looking at the self-assembly of star polymers with low number of arms.

Branched core star polymers have been investigated as nucleic acid delivery vehicles by a number of research groups, either utilising cationic branched cores or cationic linear polymer shells for electrostatic complexation of the therapeutic payload. Gong and colleagues utilised a hyperbranched polyester core (Boltron H40) coupled with linear cationic polymer arms through pH sensitive imine bonds, to complex and deliver siRNA to GFP expressing triple negative breast cancer cells *in vitro*.[138] The linear polymer arms consisted of poly(aspartic acid) with disulphide linked 2-aminoethyl groups for nucleic acid complexation and also disulphide linked imidazole groups to promote endosomal escape. This RNA delivery system showed GFP down regulation capabilities comparable to commercial transfection reagents but with lower toxicity, particularly when further functionalised with GE11 targeting peptide and tested on EFGR overexpressing cell-lines. In contrast, Matyjaszewski *et al*. investigated cationic core

star polymers, synthesised in an one pot ATRP approach, for siRNA complexation and cellular uptake.[142,195] While Wang and coworkers synthesied star polymers combining a branched PEI core and linear poly(β-amino ester) arms.[141] This star poly(β-amino ester) showed excellent gene transfection efficiencies of primary rat adipose derived mesenchymal stem cells, of between 200 and 15000 times higher than either the PEI core, or the poly(β-amino ester) arms on their own.

Similar bPEI core star polymers have been synthesised by the Voit group for small molecule encapsulation, who employed an oligosaccharide shell to stabilise the PEI structures.[196] The researchers studied encapsulation efficiencies of the branched core star polymers with various small molecules of different overall charges, including vitamin-B, an estradiol derivative, and pantoprazole. Interestingly, the core shell glycopolymer architecture was found to be necessary for stable electrostatic complexes. They obtained high encapsulation efficiencies of up to 10 drug molecules per macromolecule, and the maltriose polymer in particular showed good potential for use as a drug delivery system. Cationic core star polymers have been used to deliver platinum based anticancer drugs with high efficiencies, by Nie, Wang, and colleagues.[197] PAMAM dendrimers were conjugated with platinum prodrug, and poly(ethylene glycol)-block-(2-azepane ethyl methacrylate) linear polymer arms, which had pH based size switching behaviour for enhanced tumour penetration and drug delivery *in vivo*.

Another option for small molecule drug delivery with branched core star polymers, is to employ an amphiphilic system to either conjugate or encapsulate hydrophobic molecules. Amphiphillic star based hyperbranched Boltron H40 has been used by a number of research groups for both encapsulation and drug conjugation.[140,198] A hydrophilic linear polymer such as PEG is typically used as the hydrophilic shell. Our group has recently utilised amphiphilic branched core star polymers for hydrophobic molecule encapsulation and cellular internalisation.[139] A hydrophilic ~~and biocompatible~~ poly(2-ethyl oxazoline) shell was conjugated to a hyperbanched and hydrophobic polyester core polymer that was based on a thiol-yne polymerisation system. The core shell architecture allowed encapsulation of hydrophobic Nile Red as a model drug, and the polymers were readily uptaken by A2780 ovarian cancer cells *via* an energy dependent mechanism, suggesting endocytosis.

3.5.3. Dendronised polymers

Dendronised polymers (linear polymers grafted with dendrons), and hypergrafted polymers (linear polymers grafted with hyperbranched polymers) are hybrid polymer architectures which have only more recently been established for use in therapeutic delivery.[191] The first study evaluating rigid-rod dendronised polymer toxicity, biodistribution, and pharmacokinetics *in vivo*, was undertaken by Fréchet and Szoka.[144] The materials comprising a poly(4-hydroxystyrene) backbone with 4 generation polyester dendrons were evaluated for cytotoxicity against MDA breast cancer cells *in vitro*, which displayed 70% viablilty at a polymer concentration of 3 mg/mL. In vivo biodistribution studies were performed with tumoured and non tumoured mice. The smaller molecular weight dendronised polymers (67 kDa) exhibited urinary excretion, the largest polymer (1740 kDa) was cleared by the reticuloendothelial organs, while the medium molecular weight system (251 kDa) accumulated the most in tumour environments. The long blood circulation times of the dendronised polymers was attributed to their large molecular weights and rigid-rod shapes.

Dendronised amphiphilic polymers have been synthesised using an alkyne azide click reaction to graft polyglycerol dendrons to a PEG based linear backbone.[145] The systems formed supramolecular aggregates able to efficiently encapsulate hydrophobic guest molecules, and then be internalised by cells as followed by flow cytometry and confocal microscopy. In addition, the polyglycerol shell imparted non-cytotoxic properties to the nanocarriers over a range of concentrations, and the dendronised polymers performed better than similar linear-dendron block copolymers which could be destabalised at lower concentrations.

Gu *et al*. investigated *in vivo* doxorubicin delivery using a dendronised heparin based polymer system.[143] The researchers utilised a pH sensitive hydrazine bond to conjugate doxorubicin to a lysine based Dendron, which was then attached to a linear heparin chain with the use of azide alkyne cycloaddition chemistry. Drug loadings of 9 wt% could be achieved and the polymer architecture further self assembled into nanoparticles of around 100 nm. In a mice 4T1 breast tumour model the polymer delivery system produced strong antitumour results, high antiangiogenisis effects, and apoptosis compared to the free drug as observed by a variety of mice and tumour weight analysis, immunohistochemical analysis, and histology.

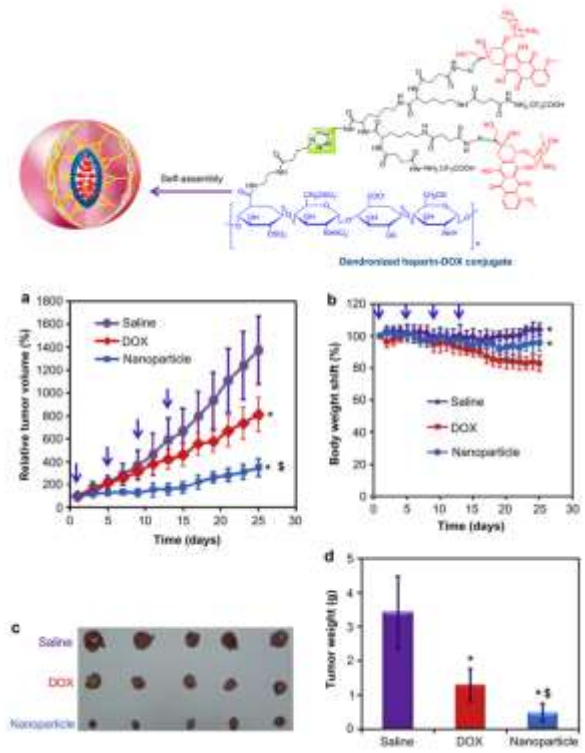


Figure 10. *Dendronised heparin-doxorubicin conjugates as a pH responsive therapeutic delivery system, including in vivo studies showing* ***a)*** *relative tumour volumes,* ***b)*** *mouse body weights,* ***c)*** *tumour size analysis, and* ***d)*** *tumour weight analysis. Figure adapted with permission* [143]*. Copyright 2013 Elsevier.*

Dendronised polymers have also been used for gene delivery applications. Guon *et al.* synthesised a range of biodegradable dendronised polypeptides with variations in structure that were tuned in order to identify superior delivery vectors.[199] A dendronised polymer based on a second generation lysine dendron functionalised with 75% histidine and 25% tryptophan, was found to have the optimal combination of charged and aromatic residues required for successful delivery. The polymers showed good efficiencies when complexed with siRNA to eGFP expressing NIH-3T3 cells *in vitro*, while also exhibiting minimal toxicity.

4. Architecture property relationships

With increased access to complex polymer architectures through new chemical technologies, researchers have been able to start hypothesising relationships between polymer architecture and subsequent properties as therapeutic delivery systems as well as physiochemical properties. The physiochemical properties of linear polymers are largely determined by the monomer repeat unit, however the properties of branched polymers result mainly from the polymer end

groups at the surface of the polymer.[33] The ~~biocompatibility~~ cytotoxicity of polymers is a complex assessment and is mainly affected by non-architecture related factors such as polymer functionality, however branched structures can offer ability to modulate ~~biocompatibility~~ toxicity to cells. Known toxic molecules or functionalities can be embedded within the core of branched topologies, and new polymer architectures could be used to alter the protein corona by recruiting or repelling specific endogenous biomolecules to polymer surfaces by variation branching densities.[200] Polymer toxicity is also known to be affected by polymer flexibility with a number of studies reporting reduced toxicity for branched polymer systems.[29] The globular and approximately spherical conformation of branched polymers in solution is a significant attraction for drug delivery applications. This size and shape of polymers is an important parameter and can alter significantly the final properties of the delivery system, in particular, whether the polymer forms unimolecular and stable objects, or forms larger self-assembled structures, which in turn impacts the circulation times and biodistribution of the polymer systems.[201,202] The tunability of polymer architecture and shape offers opportunities to increase drug loading either through manipulating the polymer core or self-assembled structure, or by the increased number of functionalisable groups on the exterior of a branched polymer.[36] When considering *in vivo* therapeutic delivery barriers, branched polymers offer opportunities for stimuli responsive endosome escape by proton sponge/osomotic pressure changes and subsequent polymer swelling of charge alteration.[203] Cellular uptake has also been reported to depend on polymer architecture, with increased branching potentially resulting in a higher number of multivalent interactions with cell surface receptors, thereby increasing internalisation.

5. Conclusions and future perspectives

New developments in the preparation and application of polymer materials in therapeutic delivery, have been helped by the combined efforts of chemists, biologists, materials scientists, and clinicians. The overall goal of scientists in this field is to improve or maximise the therapeutic effect while minimising unwanted or toxic side effects of the active ingredient. The ability to synthesise increasingly complex architectures has opened up a number of exciting avenues of research involving new classes of polymers. This review outlined and compared different branched polymer architectures and their application in therapeutic delivery in an accessible manner, with the aim of appealing to a multidisciplinary audience. Highly branched

polymers, synthesised by chain growth polymerisation strategies including SCVP or divinyl monomer copolymerisation, are highly functional materials from facile onepot routes. These materials have seen increasing use in therapeutic formulation applications, particularly since the development of the controlled radical polymerisation methods in the late 1990s. Hyperbranched polymers from step growth polymerisations have also seen wide employment in drug delivery systems. New branched-linear hybrid architectures are starting to be explored by researchers. These exciting materials, able to be synthesised with efficient coupling chemistries, enable the combination of favourable aspects of branched polymers with the ablility to create precise amphiphilic polymers for drug encapsulation/conjugation. The capability to synthesise and characterise well-defined polymer architectures could help to further examine structure-function correlations in the field of polymer based therapeutic delivery. The translation of the potential of polymer-based therapeutic delivery systems from successful *in vivo* laboratory studies to efficacy in humans, remains problematic. Current regulations from bodies including the US Food and Drug Administration, and the European Medicines Agency, are a limitation for the translation of branched and dendritic polymer architectures into therapeutic products. Polymers have molecular weight distributions that do not fit regulations for drug products. In this regard, dendrimers, being single molecular weight species, have seen more products enter clinical trials and be commercialised than other branched polymer architectures. The Australian company Starpharma Ltd (which also funded and then acquired the Donald Tomalia founded Dendritic Nanotechnologies Ltd in 2006), has developed an antimicrobial dendrimer based gel product, and has a dendrimer chemotherapeutic delivery system in clinical trials, is leading the way in clinical translation. The scope for combining advances in polymer science and associated developments in dendritic architecture synthesis, with increasing knowledge of disease heterogeneity and limitations of certain animal models, suggests further improvements in translation will be realized over the coming decades.

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Author Biographies

Alexander Cook obtained a BSc in Chemistry from Imperial College in 2013, and completed an MSc in Polymer Chemistry at the University of Warwick in 2014, conducting research involving the design and characterisation of self-assembling peptide-polymer conjugates. Following this, he obtained his PhD from the University of Warwick in the group of Professor Sébastien Perrier, investigating functional polymers for nucleic acid delivery applications. In 2018, Alexander took up a Marie Sklodowska Curie Cofund Fellowship, working with Professor Paolo Decuzzi in the Laboratory of Nanotechnology for Precision Medicine at Istituto Italiano di Tecnologia in Genova.

Sébastien Perrier graduated from the Ecole National Supérieure de Chimie de Montpellier, France, in 1998. He undertook his PhD at the University of Warwick, England, and spent one year as a postdoctoral fellow at the University of New South Wales, Australia. He started his academic career at Leeds in 2002 as a lecturer and then moved to the University of Sydney in 2007, as director of the Key Centre for Polymers & Colloids. In October 2013, Sébastien was appointed as the Monash-Warwick Alliance Chair in Polymer Chemistry, a joint appointment between the Chemistry Department and the Medical School at the University of Warwick, UK, and the Faculty of Pharmacy at Monash University, Australia. Sébastien's team focuses on the use of macromolecular engineering to design functional nanostructured materials, with applications ranging from material science to nanotechnology and nanomedicine. He is a member of the editorial boards of Soft Matter, Macromolecules, European Polymer Journal,

Polymers, Click Chemistry, ACS Macro Letters, Chemical Communications and Chemical Society Reviews and an editor of Polymer Chemistry.

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