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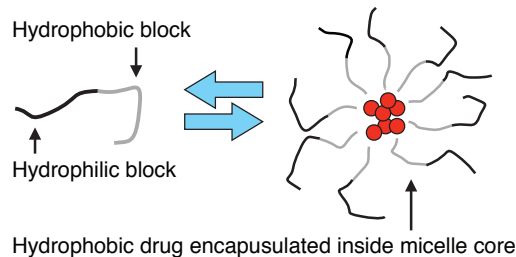
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Drugs may enter cells by endocytosis when the drugs are entrapped inside the polymeric micelles (Fig. 1) [9,10].

The polymeric micelles' core-shell structure can also mimic naturally occurring transport systems such as plasma lipoproteins and viruses [11]. The micelle protects the drug from chemical degradation or being metabolised during its journey to the desired target area, making delivery more efficient [11]. The nanoscopic size of micelles enables quick and easy sterilisation by simple filtration [11]. The major advantage of polymeric micelles is that the required polymer/drug ratio can be as little as 5:1, which significantly increases the efficiency and decreases the toxicity of the delivery system [12].

APs having diverse architectures can be formed from various polymeric systems, including block copolymers [1,13-16], graft polymers [2], star shaped polymers [17,18] and dendrimers [19,20]. Block copolymers are fabricated from the polymerisation of two or more types of monomer unit (Fig. 2) [21]. Typically one hydrophobic moiety and one hydrophilic moiety is polymerised together giving an amphiphilic diblock copolymer e.g. poly(ethylene oxide)-block-poly(lactic acid) (PEO-b-PLA) [5]. Triblocks, tetrablocks, pentablocks etc. can also be synthesised using the same reaction. In aqueous solution amphiphilic block copolymers form polymeric micelles (Fig. 2).

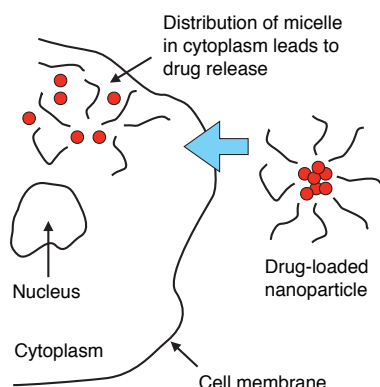


**Figure 2:** Schematic representation of dynamic micellation of diblock copolymers in aqueous environment.

As drug delivery vehicles, the hydrophobic moiety of block copolymers is usually composed of biodegradable and biocompatible monomers such as polyesters e.g. poly(caprolactone) (PCL) and poly(lactic acid) (PLA) or poly(amino acids) e.g. poly(aspartic acid) [13,21,22]. Although the most common hydrophilic segment for block copolymers is poly(ethylene glycol) (PEG), other hydrophilic monomers have also been reported e.g. poly(ethylene oxide) (PEO), and poly(N-isopropylacrylamide) (PNIPAAm) [1,23,24]. PEG is an FDA approved polymer for intravenous administration [13]. It is highly biocompatible and resistant to protein adsorption and cellular adhesion due to its steric repulsion effect [1,13]. PEG is commonly used due to its highly soluble, non toxic nature [15,22], and its ability to be excreted via renal filtration [15,22].

Block copolymers have been investigated extensively as potential delivery vehicles for many hydrophobic drugs. Formulation of hydrophobic drugs range from simple drugs such as propofol [25] through to anticancer drugs such as doxorubicin, etoposide, paclitaxel and camptothecin [26-33]. Polymer chains have a much wider range of physicochemical properties than low molecular weight surfactants [34]. Varying the MW or hydrophobicity of the polymer allows control of the properties of the subsequent nano aggregates [34]. Polymeric micelles formed from block copolymers possess greater kinetic stability upon dilution, the length of hydrophobic segment of the block copolymer influences the stability of the nano aggregates formed whereby

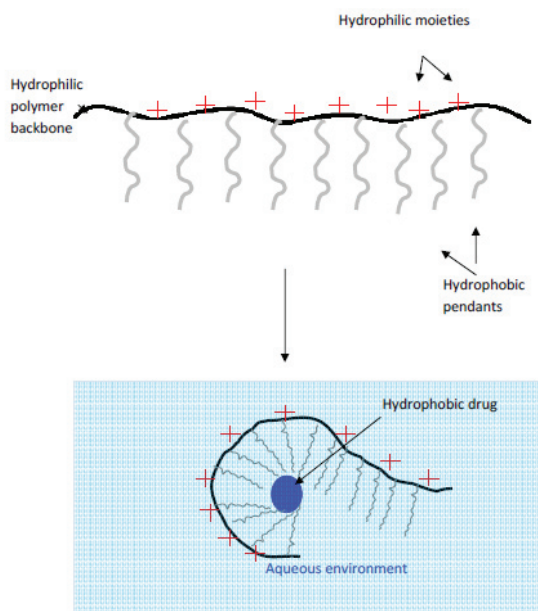
Amphiphilic polymers (AP), comprising hydrophobic and hydrophilic domains, have been widely investigated as hydrophobic drug solubilising agents. In aqueous environments, they associate through weak non-covalent hydrophobic-hydrophobic interactions whereby the hydrophilic moiety remains in contact with the aqueous phase whilst the hydrophobic moieties shield themselves. APs form a wide range of structures in aqueous environments including polymeric micelles, vesicles and nano-particles [1,2,3,4]. Their ability to form spontaneous self-assembled polymeric micelles and nano-particles in aqueous environments demonstrated great potential for drug solubilisation [1]. Like traditional micelles, the hydrophobic core of the micelles can encapsulate hydrophobic drugs and thus increase the water solubility [1,5]. The lowest concentration required for a polymeric micelle to form in an aqueous environment is called the critical aggregation concentration (CAC). The CAC is affected by a number of factors including the structure and nature of the hydrophobic group and the addition of electrolytes [2].



**Figure 1:** Schematic representation of the cellular internalisation of drug loaded micelles by endocytosis.

Polymeric micelles have several advantages compared to traditional surfactant micelles. They possess lower CAC values (approximately 1000 times lower) than traditional micelles [1,6,7], making their aggregates more stable in aqueous solution and hence reducing the risk of disruption upon dilution in vivo [8]. The size of the self-assemblies formed in aqueous environments are usually between 30-100 nm, although this can be modified by drug loading depending on the molecular architecture of the polymer and of the drug [5].

# Amphiphilic polymers



**Figure 3:** Schematic representation of self-assembly formation of comb shaped polymers in aqueous environment.

increased hydrophobic content results in increased stability [35]. The length of hydrophobic chain also effects drug loading capacity of the nano aggregates [35,36]. Lee investigated polymeric micelles of poly(2-ethyl-2-oxazoline)-block-poly( $\epsilon$ -caprolactone) as potential drug delivery systems for paclitaxel. The studies showed that the higher the content of the hydrophobic block in the copolymer, the higher the loading efficiency for the drug [31].

Higher hydrophilic content in a block copolymer results in a higher degree of solubility in aqueous environments. The hydrophilic monomer forms weak hydrogen interactions with the water molecules. Gaucher reported that increasing the hydrophilic chain of the block copolymer resulted in longer circulation times and greater 'stealth' effect whereby the blood protein adsorption of the polymeric micelles was hindered [35]. However, increasing the hydrophilic content excessively will result in unstable micellation or even no micellation occurring. This is due to the hydrophobic interaction driving forces becoming too weak for self-assemblies to occur. The weaker hydrophobic forces present in higher hydrophilic content amphiphiles also result in lower levels of drug loading efficiency and greater instability.

The level of compatibility between hydrophobic drug and amphiphilic block copolymer influences self-assembly stability, drug loading capacity and release rate [35-37]. Shuai loaded monomethoxy poly(ethylene glycol)-b- poly( $\epsilon$ -caprolactone) (MPEG-B-PCL) micelles with doxorubicin and concluded the drug loading capacity was dependant on both hydrophobic associations and hydrogen bonding between the drug and block copolymer [26].

The solubility of APs is determined using the same considerations that apply to low molecular weight surfactants e.g. chain length, nature of solubilise, effect of temperature etc. [34]. However, due to the extensive size of polymers, their interactions with water are a lot more complex than for the smaller molecules, as a result some polymers do not possess a saturation point [34]. Water soluble polymers increase the intrinsic viscosities of their

solvents when they are present in low concentrations [38].

Cross-linking of block copolymers is a technique frequently used to enhance structural integrity of their nanostructures in aqueous environments [39,40]. Crosslinking is achieved at either the core or the corona of the micelle, and results in a decrease in hydrodynamic radius [37,39]. Crosslinking efficiency of polymeric micelles is dependent on molecular weight of the polymer [37]. By crosslinking the core of the micellar structure, the outer shell structure is unaffected, giving rise to functional group attachment for the design of smart polymers [39].

The other main class of APs are graft polymers which consist of a homo polymer as the backbone to which hydrophobic pendant groups are 'grafted' to form a comb like structure. The hydrophobic pendant groups of a comb shaped polymer may consist of homopolymers, copolymers or small molecular weight hydrophobic molecules. In aqueous environment, self-assemblies are formed with a hydrophobic core stabilised by a hydrophilic corona (Fig. 3) [2,41,42]. In 1998 Chiu developed amphiphilic graft copolymers consisting of stearyl methacrylate, methyl acrylate, acrylic acid and PEG acrylate [43]. They reported the amphiphilic graft copolymers formed polymeric micelles in aqueous environments, these aggregates showed a bimodal size distribution whereby two size populations of 50 and 389 nm were observed [43]. The rationale behind such aggregation was proposed due to loose aggregates and large association complexes being formed which were in dynamic equilibrium with smaller more compact micelles [43]. The aggregate formed showed potential for encapsulation of hydrophobic drugs. However, they concluded more work was required to further investigate the properties of these systems i.e. biocompatibility, bioavailability, cytotoxicity etc [43]. Sugimoto fabricated poly(N-hydroxyethyl L-glutamine)-graft-poly(L-tryptophan) and successfully incorporated hydrophobic azobenzene into hydrophobic region of the polymeric micelles showing their potential for drug solubilisation [44].

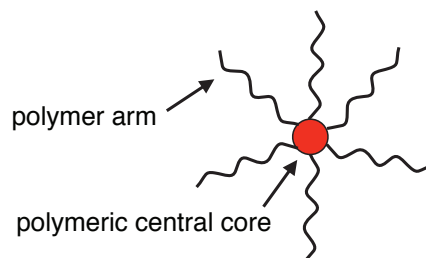
The type of aggregates formed in aqueous environments include polymeric micelles, nano-particles and vesicles [2-4]. The physical properties of the amphiphilic comb shaped polymers can be controlled by adjustment of structural components, e.g. type of hydrophobic pendant group and level of grafting [2,45]. Hydrophilic moieties such as quaternary ammonium ions or poly(ethylene glycol) (PEG) can also be attached to the homopolymer backbone to improve the safety profile and determine the in vivo fate of the self assemblies [46,47]. Zhu and colleagues attached PEG monomers onto a chitosan-N-trimethylaminoethylmethacrylate (CS-TM-PEG) graft polymer [47]. They investigated the haemolytic activity when red blood cells were exposed to 250–2000  $\mu\text{g mL}^{-1}$  polymer solutions [47]. The CS-TM-PEG polymer showed a reduction in haemolytic activity when compared to its unpegylated counterpart, the percentage haemolysis was reduced from 10–13 % (CS-TM) to 5-7 % (CS-TM-PEG) [47], resulting in higher biocompatibility of the polymer. Recently Thompson reported that addition of hydrophilic quaternary ammonium ions onto the polymer backbone of modified poly(allylamine) (PAA) polymers not only increased the aqueous solubility of the polymers but also made the polymers less cytotoxic against human epithelial colorectal adenocarcinoma (Caco-2) cells [2,48]. Lin investigated the aggregation of poly( $\beta$ -benzyl-L-aspartate)-graft-poly(ethylene glycol) (PBLG-g-PEG) [45]. They concluded that lower CMC values were observed at higher grafting levels of hydrophobic PBLG [45]. This perhaps due to the increased hydrophobicity driving aggregation into the core – shell formation [45]. The polymeric micelles formed were spherical at lower concentrations, however they found that as

the concentration was increased the conformation of the aggregates changed to rhombic and further increase of the polypeptide content and backbone length resulted in aggregates with a spindle-like morphology [45].

Polymeric micelles formed by comb shaped polymers are generally smaller in size than those formed by block copolymers. This is attributed to the phenomenon known as intramolecular aggregation whereby, a single polymeric chain has the ability to aggregate with itself forming the self-assembly [49,50]. However, a number of groups have reported larger particles formation, possibly due to the hydrophobic pendant groups in the self-assemblies being in close proximity to the water, hence multiple micelle aggregation occurs [49,51].

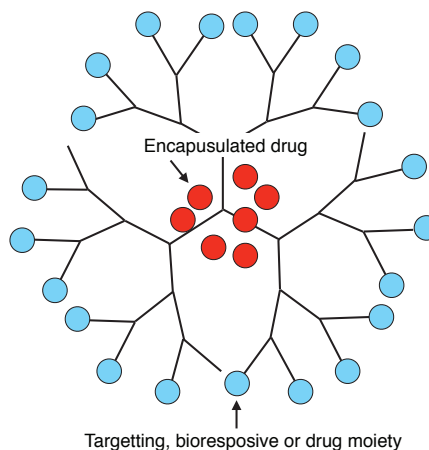
Amphiphilic graft polymers are commonly synthesised from soluble polyamine backbones such as poly(allylamine) (PAA) [2,52,53], poly(ethylenimine) (PEI) [54-56], poly(L-lysine) (PL) [4,57]. Carbohydrate polymers have also been reported using glycol chitosan [58,59] and dextran [60]. The hydrophobic pendant groups grafted onto the homopolymer backbone are usually alkyl chains [2,54,56], acyl groups [61,62] or cholesterol moieties [2,53,63]. The hydrophobic core of the amphiphilic comb shaped polymer is capable of encapsulating hydrophobic drug through hydrophobic or electrostatic interactions [35]. When drugs are encapsulated inside the core, their fate is dependent on micellar properties rather than intrinsic properties [64]. Cheng showed that varying concentrations of cyclosporine A were absorbed orally in vivo into the bloodstream of male Wistar rats when encapsulated inside a range of poly(ethylenimine) amphiphilics [54]. Graft polymers have previously been reported to achieve solubilisation of hydrophobic drugs such as cyclosporine A [3], doxorubicin [64] and paclitaxel [65]. They have also been reported to be good solubilising agents for other therapeutic agents such as proteins and genes [52,66]. McPhail and colleagues studied the combination of liposomes and graft polymers for drug delivery. They developed a vesicle in vesicle system whereby polymeric vesicles were encapsulated inside liposomes to yield a more advanced excipient for enhanced controlled release of drugs, enzymes, vaccines or proteins from the polymeric nano-carrier [67]. They fabricated the vesicle in vesicle system using palmitoyl glycol chitosan polymers and cholesterol polymers and egg phosphatidylcholine (egg PC) and cholesterol liposomes [67]. The structural formation was confirmed using freeze fracture electron microscopy. The polymeric vesicle was loaded with 5(6)-carboxyfluorescein (CF) [67]. Drug release studies concluded that the vesicle in vesicle structure slowed down the release of the drug compared to a normal polymeric micelle [67]. This system has huge implications not only for controlled release of therapeutic agents but also for the possibility of administering more than one active ingredient in combination therapy etc.

Other more diverse architectures have also been fabricated including star shaped polymers and dendrimers. Star shaped APs are made up of 3 or more linear polymer chains linked to a central polymeric core (Fig. 4) [17,68]. The arms are of comparable lengths and consist of homo-, co- or terpolymers [17]. The nano aggregates formed in aqueous solutions possess a lower hydrodynamic radius and viscosity in solution when compared to linear polymers of the same molecular weight and composition [69-71]. The star shaped polymers form unimolecular micelles with increased stability compared to block copolymers [69,70]. The physical properties of the polymer can be altered by changing the chemical structure and composition of the arm chains [17,70]. Star shaped polymers have been reported to enhance aqueous solubility of hydrophobic drugs [18,71,72, 73]. The use of star polymer as thermo responsive delivery



**Figure 4:** Schematic representation of star shaped polymer. vehicles is at the forefront of today's research [69,73].

Dendrimers are highly branched three dimensional macromolecules [74-76], consisting of a central root core from which regular layered branching occurs. Their surface comprises an abundance of terminal groups to which functional groups or drug molecules can be attached (Fig. 5) [19,70,74]. Repeated layers of branching can be well defined due to sophisticated layer by layer chemical synthesis, each layer is called a generation [70,78]. Amphiphilic dendrimers form intramolecular aggregates (unimolecular micelles) in aqueous environments. Aggregation can involve hydrophobic or electrostatic interactions or hydrogen bonding [79]. The aggregates formed possess increased stability at lower concentrations in comparison with block copolymers as the micelles do not disrupt upon dilution [70]. The aggregates are more uniform in size than the formation of micelles from block copolymers, due to the nature of the chemistry used in the addition of generations [70,74,80]. The intramolecular hydrophobic core of the dendrimer is capable of encapsulation and controlled release of hydrophobic moieties such as drugs, genes and imaging agents [76,78]. Entrapment is also achieved within the multivalent branching network or through adsorption onto the outer shell [78,81]. When a drug is covalently attached to the outer shell of a dendrimer, it exhibits a decreased release rate when compared to drug encapsulation by hydrophobic or electrostatic interactions [82]. Dendrimers with high drug conjugation can swiftly enter the cell and localise in the nucleus [82]. Dendrimers hold great potential as carriers of hydrophobic compounds as they can be tailor-made by adapting their surface groups and branches [19,75,76]. Generation and nature of packing of the dendrimer effects aggregation size, physicochemical properties, stability and drug loading capacity of the intramolecular aggregates formed [70,75,80].



**Figure 5:** Schematic representation of dendrimer structure.

Stimuli responsive or 'smart polymers' have emerged as new front runners in the race for advanced delivery systems. Stimuli responsive polymers undergo active responses to environmental signals or external change, making them ideal candidates for active targeting [23,83]. The stimuli include physical (temperature, ultrasound, light), chemical (pH, ionic strength) and biological (biomolecules) [23,84,85]. The major application for this technology is in the delivery of anti cancer therapeutics. Temperature responsive polymeric micelles are the most extensively studied to date [23,85,86]. Cancerous tissues possess increased metabolic rate compared to normal healthy tissues [83,87]. As a result they exist at higher temperatures of 40 – 44 °C [83,87]. This condition makes thermoresponsive polymeric micelles ideal carrier vehicles as once inside the cell, the temperature increase will cause the polymeric micelle to release its payload into the surrounding cytoplasm.

Thermoresponsive poly(2-(N,N-dimethylamino) ethyl methacrylate)-b-poly (caprolactone)-b-poly(2-N,N-dimethylamino) ethyl acrylate (PDMAEMA-b-PCL-b- PDMAEMA) polymers were synthesised by San Miguel and colleagues [88]. The polymer showed a reversible dispersion/aggregation pattern in response to temperature change [88]. The lower critical solution temperature (LCST) was 54°C. Slight changes in the temperature below or above the LCST can activate disruption of micelle structure and result in drug release [86]. They reported that the polymer possessed lower CMCs and better drug loading capacity for anti cancer drug chlorambucil compared to PDMAEMA non-responsive polymers [88]. The in vitro release results suggest that the thermoresponsive polymers would be suitable for transport of anticancer therapeutics, however no in vivo data has been reported to date [88].

However many thermoresponsive polymers created possessed poor biocompatibility and were not approved for in vivo use [89]. Recently mixed micellar systems consisting of simple block copolymer methoxy poly(ethylene glycol)-b-poly(D,L-lactide) (mPEG-b-PLA) and temperature sensitive polymer methoxypoly(ethylene glycol)-b-poly(N-n-propylacrylamide-co-vinylimidazole) (mPEG-b-P(NnPAAm-co-VIm)) have been synthesised [89]. It was reported that the mixed micellar systems successfully encapsulated the anticancer agent doxorubicin. In vitro cytotoxicity was reduced when exposing human cervical epithelial carcinoma (HeLa) cells to the formulation, this indicated increased biocompatibility [89]. Confocal microscopy was used for cellular visualisation of the mixed micelle fate in vitro. The mixed micellar system showed increased drug in the cytoplasm after 1 h in comparison with the free drug [89]. Lo and colleagues concluded that this system showed great potential for intravenous delivery of hydrophobic drugs [89].

pH responsive micelles are also useful in cancer treatment. The external pH of tumour tissue cells have been reported to have a lower pH (6.75) than normal healthy tissue cells (pH 7.4) [83,84]. pH sensitive micelles are formed by conjugation of drugs into the monomer blocks of polymeric micelles [22]. The drug is attached via an acid cleavable bond [90]. These micelles disrupt upon acidic pH ranges hence releasing their payload [90]. Furthermore, if the polymeric nanostructures enter the cell by endocytosis, the micelles will become localised within the endosomes or lysosomes which have pH 5 - 5.5 [84,90]. The release of drugs from the endosomes and lysosomes into the cytosol is important to achieve high therapeutic effect and thus killing the cancer cells [83].

pH sensitive micelles of poly(ethylene glycol)-pol(aspartate hydrazide) (PEG-

p(Asp-Hyd) were synthesised [22,91]. It was reported that 75 – 80 % hydrazide substitution onto the polymer gave the optimal drug release when loaded with anticancer agent Adriamycin [91]. The micelles were shown to undergo disruption at lower pH's [91]. Confocal microscopy was used to determine cellular localisation, the micelles were trapped inside the lysosomes where they were 'programmed' to disrupt through hydrolysis of the hydrazone linker [91]. The results indicated that PEG-p(Asp-Hyd) had great potential for the transport and targeting of cancer therapies. Gu and colleagues fabricated comb shaped polymers with hydrophobic cholic acid groups grafted onto a poly(L-lysine) backbone [92]. They then grafted hydrophilic poly(ethylene glycol) groups onto the comb shaped polymer via benzoic imine bonds to form poly(ethylene glycol)-g-poly(L-lysine)-g-cholic acid [92]. Benzoic imine bonds are known for their pH responsive nature [92]. The membrane disruptive ability of the nano self-assemblies was investigated using a haemolysis assay at the extracellular pH of solid tumours (pH 6.0) and physiological pH (pH 7.4) [92]. Gu found that at pH 6 the self-assemblies exhibited a large increase in haemolytic activity towards the red blood cells in comparison with pH 7.4, from 37.5 % to 90 % haemolysis at 0.75 mgmL<sup>-1</sup> respectively [92]. Gu concluded that the micelles showed a pH responsive nature with a higher degree of membrane disruption occurring at extracellular pH's hence showing potential for drug delivery to tumours with intracellular transport [92].

To date, all the amphiphilic polymers mentioned have been used to passively target cancerous tumours through the phenomenon known as the enhanced permeability and retention effect (EPR) (Fig. 6). Tumour tissue multiplies at an increased metabolic rate compared with normal tissues. As a result of the rapid growth rate the blood capillaries in the tumours are under developed forming defective, dilated or 'leaky capillaries' [93]. The tumour tissues also experience poor lymphatic drainage [93]. Polymeric nano-particles from 10 – 500 nm are capable of entering the highly permeable blood capillaries which supply the rapidly growing cancerous site [84,93,94]. Once inside the capillaries they accumulate and are retained in the tumour as a result of the poor lymphatic drainage [94]. The accumulation of drug loaded nano particles in tumour tissue allows for successful passive targeting of anticancer drugs [93]. This however does not occur in normal tissue as the blood vessels are well formed and non porous.

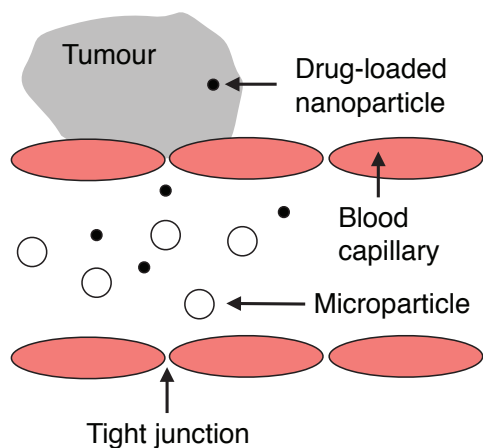
Amphiphilic polymers can be actively targeted to specific sites by conjugating recognition moieties for receptors or components which are upregulated in the cancerous cells [95]. Ligands specific to receptor sites that are over expressed in cancer cells can be attached to amphiphilic polymers e.g. folate, galactose [84,95]. Folate receptors are often used for active targeting of cancerous tumour sites [84,96,97]. Prabakaran *et al.* synthesised a folate conjugated amphiphilic polymer based on Boltorn<sup>®</sup>H40, a hyperbranched aliphatic polyester called Boltorn – poly(L-lactide)-b-methoxy poly(ethylene glycol) / folate conjugated poly(ethylene glycol) (H40-PLA-b-MPEG/PEG-FA) [98]. They incorporated anticancer therapeutic doxorubicin into the polymeric micelle which showed prolonged release of up to 40 h. In vitro analysis was carried out on 4T1 mice mammalian carcinoma cell lines [98]. The results showed that the folate conjugated polymer exhibited a greater cytotoxic effect on the cells than the non-targeted polymer [98]. They suggested that this was perhaps due to folate receptor - mediated endocytosis improving uptake into the cells [98].

Fluorescent polymers have also emerged in recent years as a new class of multifunctional smart delivery systems. Incorporation of fluorophores such as

pyrene or naphthalene into monomer units of amphiphilic polymers results in the nano aggregates formed possessing inherent fluorescence [99,10]. Fluorescent polymers have a range of potential applications in medicine, e.g. fluorescent probes for cell labelling and visualisation of cell localisation, bacteria detection, in vivo tracer for brain research [99,101]. Previously, fluorescent dyes were used to tag well established polymeric delivery systems for in vitro and in vivo visualisation [101]. However, the presence of these dyes could affect the physical and chemical properties of the self assemblies altering tissue interactions and cytotoxicity [101]. To avoid such issues inherently fluorescent amphiphilic polymers have been developed.

Recently, Suchao-in et al. reported the synthesis of a novel amphiphilic thermoresponsive, pH responsive and inherently fluorescent graft polymer [102], comprising thermosensitive poly(N-isopropyl-acrylamide-co-N-vinyl carbazole) monomers conjugated to pH responsive poly(dimethylamino) ethyl acrylate (PNIPAm-co-PNVC)-b-PDMAEA [102]. Carbazole compounds have been investigated and shown to possess intrinsic fluorescent properties [102,103]. Suchao-in concluded that the successful fabrication of the fluorescent, pH thermo responsive nature of the polymer will be of great importance for the successful imaging of therapeutics [102]. This could be an important stepping stone to fully understand the in vivo fate of drug targeted molecules.

Wu developed a fluorescent galactosylated polymer for targeted drug delivery to liver cells. The polymer consisted of galactose-polycaprolactone-g-dextran-fluorescein isothiocyanate (Gal-PCL-g-Dex-FTIC) [104]. Galactose is a common recognition moiety for liver cells. Galactose selectively binds onto the ASGPr on HEPG2 cell surfaces allowing active targeting of cells (Fig. 7) [104]. The polymers were found to form stable self assemblies in aqueous environments both in vitro and in vivo systems [104]. Confocal microscopy enabled cellular localisation through fluorescent imaging of the FTIC moiety. The aggregates were found to accumulate within the liver cells of mice, which was demonstrated using fluorescence imaging of the liver tissue. It was concluded that the polymeric nano aggregates showed great potential for liver targeting drug carriers [104].



**Figure 6.:** Schematic diagram of passive tumour targeting of drug loaded nano-particles through the permeable tumour vasculature.

The major frontrunner in the field of polymeric micelles to date is the discovery of poloxamer also known as Pluronic®. Pluronic is the brand name of a triblock copolymer used as an excipient for the delivery of hydrophobic drugs.

The Pluronic structure comprises of hydrophilic poly(ethylene oxide) (PEO) and hydrophobic poly(propylene oxide) (PPO) monomers, usually in the PEO-PPO-PEO form. The Pluronic polymer forms unimers below the critical aggregation concentration and forms polymeric micelles in the aqueous environment above this concentration [105]. The micellar structure protects the active ingredient from degradation in vivo enabling successful journey to its target destination. Pluronics has attracted a lot of attention because of their low toxicity in the body and the ability to solubilise biologically active lipophilic substances [106,107].

Pluronics are available in many forms, with different ratios of the hydrophobic to hydrophilic monomers and molecular weight. Each variation possesses slightly different characteristic properties. Pluronic polymers have a unique nomenclature whereby the name starts with an F (solid), P (paste) or L (liquid) depending on their physical properties. The letter is followed by three numbers, the first two numbers when multiplied by 100 give the estimated molecular weight of the poly(propylene oxide) polymer, the final digit when multiplied by 10 gives the % poly(ethylene oxide) content for example P105 equates to poly(propylene oxide) mass of 1,000 gmoL<sup>-1</sup> with 50% poly(ethylene oxide) content.

Husseini used an ultrasonic chamber with real time fluorescence detection to measure acoustic release of doxorubicin (DOX) and its paramagnetic analogue ruboxyl (Rb) from Pluronic P-105 in aqueous solution [108]. The ultimate goal was to produce a formulation which could enhance drug release at the tumour sites. The use of ultrasound to enhance the drug permeability of cell membranes had been previously reported [109,110]. Husseini observed that the released drug was re-encapsulated in between the pulses of the ultrasound by the dynamic equilibrium theory [108]. This led to the hypothesis that upon separation of the sonicated volume, the non-extravasated drug would re-encapsulate in the polymeric micelles and circulate in its encapsulated form, preventing drug interaction with healthy tissues and cells [105,106,108]. This work showed Pluronic's ability to control the release of the encapsulated drug.

Pluronics have helped overcome the phenomenon known as multidrug resistance (MDR) in tumour cells. MDR arises when tumour cells develop resistance to the cytotoxic drugs which are designed to destroy them. They also develop cross-resistance to other compounds which are structurally and functionally unrelated [111]. Cells treated with the anticancer agent paclitaxel often develop MDR [112]. Paclitaxel is an anticancer agent which requires site specific delivery using an excipient or vehicle due to its cytotoxic nature causing adverse side effects. Wang and colleagues loaded the paclitaxel drug into Pluronic P-105 and L-101 polymeric micelles, the polymers were modified with folic acid to enable binding to the folate receptors on the tumour cells [112]. From the results it was concluded that the combined mechanisms of folate-mediated active internalization and Pluronic-mediated overcoming MDR may be beneficial in the treatment of MDR solid tumours [112].

The major problem encountered with Pluronic polymers is the significantly high CMC value. This has resulted in the polymeric micelles dissociating at low concentrations and the therapeutic agent which they encapsulated being prematurely released [113]. Cross-linking of the Pluronic structure has led to further advancement within the drug delivery area. Yang *et al.* successfully crosslinked the outer shells, by converting the two terminal alcohols on the Pluronic-L121 to aldehydes. Diamines were then used to form a bridge

between the aldehyde terminals [114]. The micelles formed were spherical, they were found to possess a much lower CMC value ( $5.0 \times 10^{-4}$  % wt) than that of the unmodified L121 polymer ( $5.0 \times 10^{-3}$  % wt) enhancing the stability of the micelles [114,115]. The cross-linked micelles were found to be less cytotoxic than their unmodified counterparts when treated on human foreskin fibroblast (Hs68) cells [114]. The unmodified micelles also appeared to be taken up quicker and in greater abundance than the cross-linked micelles when viewed using a fluorescent microscope, however the CMC for the unmodified micelles was much higher resulting in dissociation upon dilution [114].

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