Chapter 9
Pluronic Nanotechnology for Overcoming Drug Resistance

Pallabita Chowdhury, Prashanth K.B. Nagesh, Santosh Kumar, Meena Jaggi, Subhash C. Chauhan and Murali M. Yallapu

Abstract Chemotherapy is one of the most conventionally used therapeutic interventions for treating various diseases. Chances of acquiring multidrug resistance in response to chemotherapeutic agents are exceedingly common among patients. Drug resistance arises mainly due to overexpression of efflux transporters such as P-glycoprotein and multidrug resistance-associated protein of the ATP-binding cassette superfamily of proteins, which significantly limits intracellular drug accumulation and drug activity. Although many approaches exist to overcome drug resistance, their uses are significantly limited in clinical practice. In this chapter, we demonstrate the superior functions of Pluronic-based technologies to overcome drug resistance. The present chapter highlights various aspects of Pluronic polymers, Pluronic conjugates, Pluronic nanotechnology, as well as their therapeutic implications for effective treatment strategies. We include the role of Pluronic polymers as a pharmacuetic excipient and drug delivery vehicle in this review. In addition, we highlight examples of Pluronic nanosystems that are currently in preclinical development, clinical trials, and clinically translatable formulations. Furthermore, a number of innovative Pluronic nano-designs of advanced therapeutics for future medicinal applications are presented. Collectively, the use of Pluronic-based nanoformulations discussed in this chapter suggests sensitization and prevention of drug resistance. Such an approach not only minimizes the dose required for treatment, but also minimizes the number of treatment cycles, which is useful in a clinical scenario.

Keywords Pluronics • Nanoparticles • Nanotechnology • Drug resistance
9.1 Introduction

According to the National Institute of Allergy and Infectious Diseases (NIH), drug resistance (DR) or multidrug resistance (MDR) is defined as the ability of microbes, such as bacteria, viruses, parasites, or fungi to grow even in the presence of chemical(s) (therapeutic drug molecules) that would usually inhibit their growth. Paul Ehrlich, the father of modern chemotherapy, proposed that drug resistance was acquired by the biological system when there was “reduced avidity of the chemoreceptors so that they are no longer able to take up” the drugs [1]. Antimicrobial resistance has resulted in increased morbidity, mortality, and a waste of healthcare resources. Centers for Disease Control and Prevention (in April 2011) estimated that antibiotic resistance in the United States alone costs approximately $20 billion a year in excess healthcare costs [2].

Drug resistance or multidrug resistance in cancer therapy is frequently experienced. In fact, this is considered as one of the major impediments for the success of many forms of chemotherapy. In general, tumors consist of drug-sensitive and drug-resistant malignant cells. Conventional chemotherapy inhibits the drug-sensitive cells, leaving behind a considerable amount of drug-resistant cells. These cells have already acquired resistance and are not affected by chemotherapy. Additionally, cancer cells adopt and develop drug resistance to frequently administered drug molecules.

In chemotherapy, DR/MDR is governed by several factors. First, resistance occurs due to drug elimination from cells by the efflux ATP-binding cassette (ABC) transporters like P-glycoprotein (P-gp), ABCB1, MRP1, ABCC1, MCRP, ABCG2, BCRP, and ABCG2. Resistance inhibits the diffusion of drugs to the cells due to loss of receptors on the cell surface, loss of drug transporters, or the alteration in membrane lipid composition. Resistance can occur when the drug is compartmentalized in cellular vesicles. A change or alteration in drug targets can cause resistance. Extensive drug metabolism, a change in the cell cycle and the inhibition of apoptosis can cause resistance, as well. Finally, resistance can occur when active damage is repaired [3]. At times, cells undergo mutations, which change the cell’s structure or biochemical pathway in a harmful way. These groups of growing cells are no longer affected by the presence or absence of drugs. Some mutations might even change the part of cells that are affected by drugs, creating a thriving resistance of the cell to the drug. Moreover, when drugs are no longer administered or not properly administered, the body’s natural defenses fail to abolish the resistant survivors, allowing the ability to reproduce and pass the resistance to their descendants.

P-glycoprotein and multidrug resistance-associated protein (MDRP) are responsible for the efflux of drug molecules, and causing the development of resistance to drug action(s) [4]. Evidence of P-gp overexpression and mediated drug resistance was confirmed back in 1982 when deoxyribonucleic acid (DNA) from resistant cells transferred to nonresistant cell lines, which conferred resistance to the latter [3]. On the other hand, MRP was cloned in 1992 and found responsible for
drug resistance [4]. Subsequently, both ATP-binding cassette transporters (ABC transporters) have been extensively studied and are considered significant targets for anticancer drugs. Similarly, HIV is another disease that frequently develops resistance to known therapeutic drug molecules.

There have been many approaches to overcoming drug resistance. However, very few of these approaches could shift from research to bedside practice. A few of such investigations include co-delivery of gene therapy, such as adding siRNA, shRNA, DNA, or dsRNA with the drugs. One such combination was causing downregulation of cyclin B1 mRNA, thus creating a delay in growth of the tumor. Another approach was to develop a multifunctional carrier system to deliver both the drug and siRNA [5]. MicroRNA (miRNA) also plays an integral role in developing resistance and is shown to have various mechanisms involved in resistance development [6]. Synthetic analogue of dsRNA was exploited for combination therapy to develop a strategy against drug resistance [7]. On a similar attempt, various groups have investigated using mitochondria targeting for drug delivery [8].

Although many of these approaches help to some extent in overcoming drug resistance, their use is still unknown in clinical practice. An extensive literature search revealed that Pluronic-based technologies are highly successful when used to overcome drug resistance. Thus, we aim to present a review which highlights the novel role of Pluronics in chemotherapies. Poloxamer (Pluronics®) [9] is a nonionic triblock copolymer with surfactant properties that is amphiphilic in nature. These copolymers are also marketed as Synpersonic® or Tetronic®. It is believed that Pluronics consist of hydrophobic poly(propylene oxide) (PPO) chains, which has a tendency to be immersed in the hydrophobic core of the biomembrane, resulting in an alternation of the membrane and thus, sensitization of the MDR tumor cells to the anticancer drugs [10]. This function further aids in active drug transport across both blood–brain barriers and intestinal barriers, which can cause transcriptional activation of gene expression both in vitro and in vivo [11, 12].

### 9.2 Pluronic Polymers

Poloxamers were introduced in 1950 and are classified as nonionic copolymers. These polymers are odorless, tasteless, white, waxy granules with free flowing properties. Amphiphilic in nature, they are soluble in both polar and nonpolar solvents. Pluronics are composed in a triblock fashion, consisting of a hydrophobic [poly(propylene oxide) (PPO)] unit in between two basic hydrophilic units [poly (ethylene oxide) (PEO)] with the basic sequence of A–B–A structure (PEOₐ–PPOₜ–PEOₐ) (Fig. 9.1) [11]. The number and average size of PEO and PPO blocks are shown in Table 9.1. These polymers have the same chemical structure but differ in molecular weight. The hydrophilic–lipophilic balance (HLB) determines the amphiphilic property of Pluronic polymers, which is dependent on the number of PEO or PPO units. Pluronics are a major pharmaceutical excipient due to their
superior stability in aqueous solutions in the presence of acid, alkali, or metals ions over other molecules. Pluronics are synthesized by sequential anionic polymerization by adding ethylene oxide (EO) or propylene oxide (PO) monomers in the presence of an alkaline catalyst, such as sodium or potassium hydroxide [13]. They exhibit a reversible thermodynamic property, which helps to convert the solution into a solid gel form by varying their chemical composition.

Pluronics are unstructured molecular solutions at low temperature. However, as the temperature rises and reaches the critical micelle temperature (CMT—also termed as Krafft temperature or Krafft point), the copolymers aggregate, resulting in the formation of spherical micelles. As the temperature increases above CMT, the micelles align in a uniform fashion, laying the hydrated and swollen PEO units at the outer lining, while the dehydrated PPO units occupy the inner core of the micelles. This self-assemble process is called micellization [11]. This leads to an increase in the hydrophobicity and a decrease in the degree of hydration. This critical micellar concentration (CMC), which influences the micellization process, is of prime importance. The possible dilution of the micelles by body fluids determines the stability of the solution, and is dependent on the CMC [16, 17]. CMC also determines the biological effect that the Pluronic® micelles have on the exposed cells [18].

The interactions of the PPO blocks are the primary driving force for the micellization. Depending on the type of Pluronic used, the micelles are about 20–80 nm in size [19]. In general, block copolymers have approximately ≥ 30% PEO content,
especially in diluted solutions at body temperature [11]. The benefit of the hydrophobic core in the Pluronic micelles makes them an efficient carrier tool for delivering hydrophobic drugs and other therapeutic agents. The process of transferring lipophilic molecules to the core of the micelles is referred to as solubilization. Solubilization can be used to improve the metabolic stability, solubility, and pharmacokinetics of encapsulated therapeutic molecules at the physiological condition. The hydrophilic outer layer maintains the dispersed state of micelles and prevents undesirable interactions with cells and proteins [11]. Pluronics are capable of forming pores on cellular membranes [20], which gives them the added advantage of the ability to interact with the MDR cells and to develop sensitization of the cells causing apoptosis to anticancer drugs [11, 21]. Pluronics are much favored in drug delivery because of their ability to embody into the membrane, allowing translocation in cells, and thus changing cellular functions, such as mitochondrial respiration, ATP synthesis, activity of drug efflux transporters, apoptotic signal transduction, and gene expression [22]. Additionally, they have the ability to enhance the drug transport of various drugs through impervious barriers, such as blood–brain barrier and intestinal barriers, hence improving bioavailability [11, 12].

More importantly, Pluronic polymers have either a prevention or a reversal effect on MDR due to several associated mechanisms, which include but are not limited to:

(a) **Inhibition of P-gp drug efflux transport system.** As per literature review, Pluronics specifically inhibit the P-gp-dependent transport route in MDR cells and not in cells that do not express P-gp [18, 23].

(b) **Inhibiting the MDRPs** [24] and breast cancer resistance protein (BCRP) [25]. Significant accumulation and cytotoxic effect of MDRP substrate and MDRP-dependent drugs were observed in MDRP cells when compared to non-MDRP cells using Pluronic-85, probably due to its ability to sensitize selected MDRP overexpressing cells. As demonstrated by Yamagata et al. [25], uptake of mitoxantrone was enhanced in BCRP-expressing cells and was less effective in green fluorescent protein (GFP)-expressing cells, suggesting that Pluronics could be a potent BCRP inhibitor in the small intestine. Although role of Pluronics on BCRP and P-gp inhibitory effects still remains unclear.

(c) **Eradicating drug sequestration inside the cytoplasmic vesicles.** [26, 27]. The MDR cells deter drug delivery by sequestering the drugs within vesicles following drug release, to the cytoplasm, and accumulation in the nucleus [27–31]. This limits the potency of the drug before it can even implement any therapeutic action. The presence of H+-ATPase, an ATP-dependent pump on the membranes, increases the pH gradients aiding drug sequestration in the resistant cells [32].

(d) **Significant ATP level depletion.** Mitochondria, where metabolic activities of the cell occur, may be a prospective site of action for the copolymer. As per Kabanov et al. [33], metabolic activities in the MDR cells are more responsive to Pluronics than the non-MDR cells, thus resulting in significant ATP inhibition by Pluronics in MDR expressed cells. Another hypothesis is that
Pluronics are supplements of K ionophores (lipid-soluble entities that transport ions across a cell membrane) [34–36] and are capable of uncoupling oxidative phosphorylation [37, 38], which may contribute to inhibit the metabolic activities on the mitochondria, reducing ATP level(s). It was reported that these copolymers directly target the hydrophobic site of NADH “nicotinamide adenine dinucleotide (NAD) + hydrogen (H)” dehydrogenase complex that are located in the mitochondrial membrane, which in turn inhibits the metabolic activity and the ATP depletion [37, 38].

(e) **Induction of membrane fluidization**. Pluronics can alter the structure of the lipid bilayer of the membrane leading to microviscosity. It is also important to note that both membrane fluidization and the ATP depletion are of critical importance for inhibiting the P-gp drug efflux transport system [10]. It is referred to as “double punch” effect, as the synergistic effect of both is important [11].

(f) **Inhibition of the Glutathione (GSH)/Glutathione S-transferase (GST) detoxification system**. Elimination of drug occurs due to the presence of GSH/GST detoxification system. It is believed that there is a complex and interrelated mechanism for drug elimination through the MRP and GSH/GST detoxification system in MDR cells when exposed to Pluronic polymers. Thus, it may be attributed that Pluronic® copolymers are responsible for the inhibition of the drug efflux transporter, causing accumulation of drugs in the resistant cells [11, 40].

(g) **Promoting release of cytochrome C**. Pluronics promote production of reactive oxygen species (ROS) in the cytoplasm due to the decreased potential of the mitochondrial membrane. This potential causes respiration deficiency in the mitochondria of the MDR cells [41].

(h) **Enhances drug-induced apoptosis**. Alterations in drug-induced apoptosis trigger pro-apoptotic signaling and prevent/minimize the activation of the anti-apoptotic defense in MDR cells [42].

A graphical representation of the working mechanism of Pluronic block copolymer is shown in Fig. 9.2.

To the best of our knowledge, Pluronic SP1049C (L61 and F127) is the first anticancer (doxorubicin) micellar formulation to reach clinical evaluation. Results of Phase I clinical trials in 26 advanced stage IV cancer patients reveal that the maximum tolerated dose (MTD) was 70 mg/m² and dose-limiting toxicities (DLT) was 90 mg/m² (neutropenia). It was considered as an acceptable safety profile and was efficacious against highly resistant oesophageal cancer [44]. In Phase II clinical trials on 19 patients evaluate SP1049C treatment, which showed a partial response (PR) in 9 patients and a minor response or stable disease in 8 patients. The median overall survival and Progression-free survival (PFS) were observed as 10 and 6.6 months [45]. In addition, various other Pluronic-based drug formulations are under pipeline for therapeutic evaluations.
9.3 Pluronic Conjugates

Pluronic copolymers owing it to their versatile nature for the hydrophilic and lipophilic block of PPO and PEO exhibited several advantages. The formed micelles (particles size, <100 nm) allowing significant accumulation in pathological tissues via the enhanced permeability and retention (EPR) effect, hence, they are capable of delivering drugs to tumors. Chemical or biomacromolecular conjugation with functional groups or drug(s) helps to enhance the property of the core-forming micelles and for drug delivery.

Kabanov et al. [43] developed a solution of haloperidol using P-85 in water, which was further conjugated with insulin or antibody. The resultant micelles could enhance the haloperidol delivery to the brain of mice almost 500-fold. This is due to the insulin moiety on the micelles surface, which could interact well with the insulin

Fig. 9.2 Mechanism of action of Pluronics in MDR cells: (a) inhibition of drug efflux transporters (P-gp and MRP), (b) inhibition of other MDR protein (BCRP), (c) eradication of drug sequestration inside cytoplasmic vesicle, (d) ATP depletion, (e) decrease in the membrane microviscosity, (f) inhibition of the GSH/GST detoxification system, (g) increase in ROS level, (h) enhancement of pro-apoptotic and p53 signaling and decrease in anti-apoptotic signaling
receptors on the cell membrane. Coupling of micelles with the brain specific antibody could be target specific to the brain antigen. In contrast to this, haloperidol, when delivered using Pluronic solution without antibody conjugation, did not cross the hematoencephalic barrier (HEB) well, resulting in lower haloperidol concentration. In another study, a conjugate system using poloxamer 188 and grafting hydrophilic heparin leads to shell cross-linked micelles, which offers not only lowered CMC to the micelle system, but stabilized formulation [44, 45]. Additionally, such a heparin cross-linked poloxamer conjugate system could be a potential system to defy dilution in the body fluids and could enhance the stability of the micelles (in vivo), making it a potential temperature sensitive system for protein drug delivery [46].

Pluronic copolymer has shown promising results for delivering chemotherapeutic drugs, mainly because of their ability to reverse MDR effects in tumor cells. Song et al. [47] demonstrated that anti-human hypoxia inducible factor-1a (HIF-1a) antibody functionalized Pluronic P123 micelles for selective targetability and inhibition of cancer cells by releasing paclitaxel, and lowered the incidence of paclitaxel associated systemic toxicity. Similarly, synergistic action of doxorubicin was achieved when employing a combination of mixed/conjugated Pluronic micelle formulation [Pluronic P-105, PEG2000-diacylphospholipid, and poly(ethylene glycol)-co-poly(beta-benzyl-L-aspartate)] [44]. Li and Tan [48] demonstrated that Pluronic mixed micelles of P105 and PEG resulting in phosphatidyl ethanolamine conjugate (PEG-PE) could be much more efficacious when compared to P105 micelles. P105 and PE, when mixed in molar ratio less than 7:3, demonstrated higher stability and less adverse effects due to their low CMC value. This conjugate could also maintain the integrity, even when diluted in the blood. When tested on human breast carcinoma MCF-7 cells, this nanocomposite conjugate showed a higher cytotoxicity in contrast to the micelles with P105. This suggested that Pluronic, in conjugation with another polymer relative to one Pluronic, demonstrated better dilution stability (preventing dissociation of micelles into monomers) and efficacy.

Pluronics have gained considerable popularity in enhancing stability, solubility, and other added advantages. However, there are a few drawbacks associated with the use of Pluronic polymers, especially with reverse Pluronics (such as 10R5): high CMC, fast release/dissociation rates, and poor drug loading capacity. To overcome these innate shortcomings of Pluronics, F127 and 10R5 were conjugated with Folic acid (FA) and Quercitin (Q), respectively. Based on their structural characteristics, these two conjugated systems of F127-FA and 10R5-Q were mixed to form the final micelles. This final formulation was able to stabilize the doxorubicin in micelles, lower the CMC, and enhance loading capacity in contrast to mixed micelles of F127 and 10R5 [49]. Further, it was proposed previously that co-delivery of quercetin with doxorubicin enhances cytotoxicity to tumorous cells and minimized effects on healthy cells, which was also confirmed from this study.

Pluronics, being amphiphilic in nature, can co-deliver two anticancer drugs simultaneously to the tumor cells, one of which being hydrophobic (paclitaxel) and hydrophilic (doxorubicin). Both drugs being substrates of MRP, P-gp, and BCRP
have been used before to demonstrate efficient tumor regression [50, 51]. However, due to solubility differences of the two drugs, nanomicelles prepared from the amphiphilic Pluronic polymers F127 and P105 have been of significant help, aiding synergistic effects of both the drugs. As a result, enhanced cellular uptake, stronger growth inhibition, and better apoptosis in MDR cells were achieved. Results indicate that this dual drug loaded Pluronic micelles enhance drug accumulation in the tumor cells and in the plasma, thus achieving a higher incidence of antitumor efficiency with respect to single drug loaded Pluronic micelles or combined drug administration. This suggests that a combination of dual drugs with Pluronic micelles offers advantages of synergistic effects, passive tumor targeting, and reversed MDR effect, and therefore, could be a viable option for reversing MDR effect of cancer chemotherapy [52].

Apart from offering several advantages for cancer chemotherapy line of action, Pluronics have also been successful in offering anti-adhesive property when conjugated with protein lysozyme. The antibacterial lysozyme conjugate enables the lysozyme to stretch out more into the solution. This suggests that attaching the lysozyme to the PEO chain of the Pluronic could be better adsorbed to the attaching surface, as the free PPO chain adheres to the hydrophobic surface and the PEO lysozyme conjugate faces towards the hydrophilic solution. Therefore it is inferred that the PPO attached to the surface is an indication of the high stability that the Pluronic is capable of [53, 54], and the attachment of the antimicrobial lysozyme to the Pluronic provides resistance to particle deposition and selective lethal interaction with microorganisms, thus providing anti-adhesive activity [55].

9.4 Pluronic Nanotechnology

Nanotechnology in drug delivery research has gained significant attention for developing novel techniques and/or delivery of classic medicine to the body. The benefits of nanotechnology [56–58] include:

- The delivery of poorly soluble drugs, proteins, and peptides
- The delivery of macromolecules to the intracellular site of action
- The delivery of two or more drugs simultaneously to achieve synergistic effect
- Enhancing visibility at the site of drug action by combining therapeutic agents with imaging modalities
- Targeted delivery to cell or specific tissue
- Transcytosis of drugs across tight epithelial and endothelial barriers
- Enhancing therapeutic in vivo efficacy
- Controlled release of the therapeutic agent.

Pluronics, as mentioned earlier, are triblock copolymers with PEO and PPO units that exhibit surfactant properties, enabling them to interact with hydrophobic surfaces of biological membranes. Thus, Pluronics are an interesting candidate for
drug delivery across biological membrane, increasing drug solubility and drug stability, enhancing pharmacokinetics and biodistribution of drugs. These copolymers at concentrations above critical micelle concentration (CMC) can self-assemble into micelles, forming Pluronic micelles with diameters between 10 and 100 nm [16]. For example, Pluronic P85 was utilized in enhancing the cytotoxicity of daunorubicin in MDR cells [59]. This was achieved due to enhanced transport of daunorubicin into the cells, enhancing drug influx into the cytoplasm to enable a better binding with the DNA of the MDR cells. It is evident that with P85, an alternative delivery system can be developed to enhance the activity of the antineoplastic agents against MDR tumors [59]. The advantage of Pluronics is the ability to reverse MDR effects and to cross intestinal and blood–brain barriers, as well as gene expression in vitro and in vivo models, all of which have drawn interest of researchers globally.

Pluronic-based nanotechnology is making progressive advancements and is one of the fast flourishing fields in pharmaceutical research. Various nano-carriers, such as polymeric nanoparticles, metal nanoparticles, nano-suspensions, liposomes, dendrimers, nanogels, polymeric micelles, and solid lipid nanoparticles have been studied extensively over the past two to three decades. Recent trend follows modification/functionalization of nano-carriers with Pluronics in order to investigate their ability to reverse multidrug resistance in a precise manner, and is considered as a safe and more efficient delivery system for drugs, genes, and imaging molecules [60–62].

9.4.1 Pluronic Nanoparticles

Nanoparticles are colloidal systems with size ranging between 10 and 1000 nm. In nanoparticles, the drug can be dispersed, entrapped, encapsulated, or attached to a nanoparticle matrix. The unique structure of polymeric chain allows to achieve a specific shape, size, physical state, and surface. Due to their small size and uniform dispersion of drug molecules in polymer matrix, they provide a sustained drug release that avoids frequent administration. It can also help in attaining target specific delivery and intracellular penetration, and thereby a better absorption. Polymeric nanoparticles have the ability to target particular organs/tissues as carriers of drug, DNA, proteins, peptides, and genes. To prevent rapid elimination from human body system, they are often coated/conjugated with Pluronic polymers, polyethylene glycol, chitosan, and hyaluronic acid or even thermosensitive gels, to impart mucoadhesive property and thus improve the drug bioavailability [63]. Evidence of using polymeric nanoparticles in enhancing drug delivery often utilizes PLGA [64, 65], PEG [66, 67], PLGA–PEG [68–70], PCL [71, 72], and PLA [73] copolymers due to their biocompatibility and US-Food and Drug Administration (FDA) approved polymers for human use. To demonstrate the ability of Pluronics to cause immense sensitization of MDR tumors to several anticancer agents, Mei et al. [74] developed and characterized nanoparticles with PCL and Pluronic F68.
These nanoparticles increased the uptake in resistant breast cancer cells by 1.67-fold at all tested concentrations up to 500 µg/ml. Further, the cytotoxicity achieved on resistant breast cancer cell lines with PCL-F68 nanoparticles was significantly more ($p < 0.05$) than the PCL nanoparticles and a marketed formulation Taxotere. These findings confirm the potential of Pluronics to reverse MDR and achieve better therapeutic effects [74]. Similarly, nanoparticles prepared from Polyethyleneimine (PEI) and Pluronic P85 resulted in overcoming resistance in lung cancer cells. A conjugated nanoparticle of PEI-P85 was mixed with D-α-tocopheryl and polyethylene glycol 1000 succinate (TPGS) and survivin shRNA (ShSur) (which is used to down regulate the expression of survivin that is upregulated in resistant cancer cell lines). This complex nanoparticle of P85-PEI/TPGS/PTX/shSur was further loaded with paclitaxel. The complex nanoparticle demonstrated 87-fold higher cytotoxicity than free paclitaxel and enhanced cellular uptake by 36-fold, in contrast to blank nanoparticles. In addition, better antitumor efficacy was achieved on nude mice models with this complex nanoparticle conjugate due to the co-delivery of paclitaxel and shSur, both of which helped to overcome resistance in human lung cancer cell lines [75].

9.4.2 Dendrimers

Dendrimers are made up of polymeric chains with a highly branched star-shaped structure. Dendrimers are nanoconstructs with unique physical and chemical properties, such as high water solubility, encapsulation ability, monodispersity, and a large number of surface functionalizable groups. The ability to functionalize surface groups makes them suitable candidates for delivery of both hydrophilic and lipophilic drugs [76, 77]. They have been an immense help in delivering anticancer [78, 79], antibacterial, antiviral [80–83] drugs, as well as high molecular weight compounds [84, 85]. Dendrimer modification with Pluronic polymers, especially polyamidoamine (PAMAM) dendrimers, has attracted considerable interest, yielding a better product with improved reversion of MDR phenomenon. Highly lipophilic Pluronics (P123, F68, F127 and F108) that were conjugated on 4th generation of PAMAM dendrimer, i.e., PAMAMG4.0-Pluronics resulted in nanoformulations with a diameter of 60–180 nm [86]. PMMAMG4.0-P123 not only exhibited 76.25% of 5-fluorouracil (5FU) loading efficiency, but also promoted a highly anti-proliferative activity of 5-FU against MCF-7 breast cancer cells. Similarly, a F127 conjugated to the 5th generation PAMAM dendrimer showed a reduced hemolytic toxicity [87]. This formulation is efficient to encapsulate doxorubicin for a sustained release. While another PAMAM modified Pluronic (PAMAM:F127, 1:35.37 mole ratio) exhibited DOX complexation, which resulted in a pH-sensitive and sustained release behavior [88]. Additionally, this DOX-PAMAM-F127 complex showed stronger anticancer effects in MCF-7/ADR cells with a 33.15% resistance reversion index. Another PAMAM-F127 demonstrated formation of unimolecular micelles, and can be loaded with DOX, promoted
100% uptake in MCF-7/ADR cells and decreased cell viability even at 2 µg/ml concentration [89]. This implies a further superior role of dendrimer-Pluronic conjugates in the treatment of drug resistance cells.

9.4.3 Liposomes

Liposomes are lipid vesicles composed of one or more phospholipid bilayers with a central aqueous compartment [90]. They are capable of forming vesicles 25 nm to 10 µm in diameter and offers better encapsulation of both hydrophilic and lipophilic drugs due to the presence of a central aqueous compartment and lipid layer(s). Liposomes have a higher degree of biocompatibility than the polymer-based systems [91]. Commonly used polymers for liposome preparation are PEG, poly(vinyl pyrrolidone) (PVP), and poly(acrylic acid) (PAA). Pluronic P123 was conjugated with galactosyl (Gal), which is a specific ligand to target hepatocellular carcinoma (HCC) cells. The resultant Gal-P123 was used to develop liposomes (LPG) in order to enhance the ability of this molecule to reverse MDR effects by specifically targeting the HCC receptors. Mitoxantrone (MX) is the model drug for this study, which is a BCRP substrate that can overexpress BCRP in MDR cells. The MX loaded LPG had a good nanosize diameter of 100 nm. The in vitro capability of MX-LPG was evaluated using HCC Huh-7 cells, and then demonstrated a 2.3-fold increase in cytotoxicity by the MX-LPG over MX. Further, a 14.9-fold enhanced uptake of MX-LPG was reported in BCRP overexpressing MDCKII/BCRP cells over plain MX. Both in vitro data and in vivo study conducted on BALBc mice demonstrated improved uptake, target ability, and bioavailability of MX-LPG over LPG. The superiority of this formulation in reversing BCRP mediated MDR effects was clearly demonstrated [92]. Poloxamer P85 and F68 were modified into liposomes to overcome the effect of MDR. In this context, the group formulated modified poloxamers and tested it against normal cancer cells and MDR cells. Results demonstrate that Pluronic P85 at 50 µM enhanced the PML accumulation in MDR cells by 2-fold compared to control and 10-fold when compared to plain liposomes and Pluronic P68. This suggests that liposome modified Pluronic P85 is a potential carrier for anticancer drugs to reverse MDR cells [93].

9.4.4 Polymeric Micelles

Polymeric nanomicelles are synthesized from a block copolymer, which is generally biodegradable or biocompatible like PEG, PEO that forms amphiphilic monomeric units with distinct hydrophilic shell and hydrophobic core. They contain polymer chains, which are self-assembled due to hydrophobic or ion pair interactions between polymer segments [91]. The polymer blocks are arranged differently as diblock (A–B type), triblock (A–B–A type), or even grafted/branched
type copolymers, where A and B are different polymers used. If the core-forming block structures are efficiently monitored, the nanomicelles may have good thermodynamic and kinetic stability, and that increases the chance of a variety of drugs to be incorporated for drug loading, release, activation, and effective therapy. It is found that polymeric micelles are more stable than nanomicelles that are made from conventional surfactants [33]. Due to their low CMC values, they can retain drug molecules for longer periods of time, even in a diluted environment in systemic fluids. Moreover, polymeric micelles offer advantages like extended circulation time, sustained release, favorable biodistribution, reduced side effects, and lower toxicity [94–96]. Docetaxel was loaded in a polymeric micelle prepared with Pluronic F127 and P105 that was prepared by thin film hydration method. In vitro and in vivo data demonstrate superiority of this formulation over marketed Taxotere due to prolonged release by 1.85-fold. When tested on resistant human lung carcinoma, A549 anticancer efficacy and tumor inhibition were enhanced by 2-fold in contrast to Taxotere [97]. Additionally, when methotrexate was encapsulated into the same micelles of P105 and F127, they exhibited higher antitumor activity and increased cellular uptake over methotrexate injection. Also, higher cytotoxicity and lower systemic toxicity were induced by encapsulated methotrexate than methotrexate alone by injection on MDR cell lines. This confirms the ability of Pluronic as polymeric micelles to reverse MDR effects [98]. Pluronic P105 or P105 and L101 were used to formulate mixed micelles and were further attached to folic acid that was loaded with paclitaxel. This formulation exhibited enhanced targetability and uptake due to the presence of both Pluronic and folic acid, as there is an overexpression of folate receptors on MDR tumors. This caused enhanced internalization of the drug into the resistant tumor sites and prevented systemic toxicity at other bodily sites [99].

9.4.5 Ceramic Nanoparticles

Nanoscaled ceramics, such as Alumina (Al₂O₃) hydroxyapatite (HA), silica (SiO₂), and titanium oxide (TiO₂) [102, 103] are known to be biocompatible with biological environment and are utilized to form the ceramic nanoparticles ranging <100 nm. The ability to release the drug in a controlled fashion is the primary reason for its use in drug delivery. Apart from their porous nature, formation of sol-gel, enhanced stability in biological environment, high loading capacity, and water solubility, they have been advantageous in developing novel drug delivery systems. However, there is evidence of adverse effects by the use of ceramic materials to develop these nanoparticles [104]. They are primarily used to encapsulate proteins, DNA, gene delivery [105, 106], large molecular weight drugs, anticancer agents, as well as in photodynamic therapy [107]. In addition, a variety of organic groups, which may be incorporated on the surface of the outer matrix of ceramics, has shown to have direct effect on these nanoparticles [108], enhancing the ability to deliver hydrophilic drugs at the site of cancer cells specifically [109].
Ceramic particles are widely used for dental and orthopedic applications; however, the reactive nature of these ceramic particles can inhibit the drug release profile and stability of the particles. A well-suitable example of Pluronic modified nanoparticle, reported by Chan et al. [70], in which docetaxel was encapsulated with an amphiphilic hyaluronic acid/ceramide-conjugated Pluronic 85 forms polymeric nanoparticles, which exhibited ability to reverse multidrug resistance of docetaxel. From the in vitro cellular uptake study, it was clearly demonstrated that this formulation, in contrast to Taxotere®, has the ability to reverse MDR effects by reversing drug efflux due to the presence of Pluronic 85. Lastly, by using MTS assay, it was confirmed that their nanoparticle formulation can reverse MDR by lowering the IC₅₀ significantly to Taxotere® (intravenously used concentrated docetaxel clinical formulation), thus suggesting that it was able to reverse MDR effects on resistant tumors. These results were in accordance to the in vivo data where NIR fluorescence imaging showed that the formulation was able to target specifically to the tumor region(s). Similarly, mesoporous silica nanoparticles were coated with a dual polymer lipid material, which was made from pH-sensitive phospholipid DOPE grafted Pluronic P123. The final mesoporous nanoparticle was loaded with irinotecan and its anticancer efficacy was checked against MCF-7/BCRP-resistant cells. It had an efficient intracellular activity due to cellular internalization, antitumor activity, and tumor targetability. Also, it showed the potential of reversing MDR by reducing the tumor growth at a low dose and preventing undesired effects [100].

9.4.6 Nanogels

Nanogels are swollen cross-linked polymeric particles composed of hydrophilic or amphiphilic polymer chains. They offer advantages such as high water content, biocompatibility, high stability, and nanometer size range, which enhance the loading capacity that serves as a carrier for the transport of drugs by multivalent biconjugation. Apart from these, their ability to be responsive to the environmental factors, such as ionic strength, pH, and temperature, makes them a remarkable nanocarrier system for drug delivery. The most common monomers used for the preparation of nanogels that are cross-linked to form the polymeric chain includes polymers such as polyethylenimine (PEI) [101], poly(ethylene glycol) (PEG) [102], poly(propylene glycol) (PPG) [103], poly(methacrylic acid) (PMA) [104], poly(acrylic acid) (PAA) [105]. Pluronic F68 and F127 were used to develop a PEI based nanogel that could sustain the drug stability of nucleoside 5’-triphosphate (NTP) to enzymatic hydrolysis, and were also less cytotoxic in comparison to PEG based nanogels due to 2–2.5 times enhanced interaction with the cellular membrane of the cancer cells. Also, these formulations possess a high loading capacity and thus, a high drug concentration could be achieved at the tumor site, minimizing both adverse toxicities at other sites and chances of developing resistance to the potent drug molecule [103].
9.4.7 Solid Lipid Nanoparticles

SLN can be defined as a solid lipid matrix in nanometer range accommodating a drug that is stabilized by one or more surfactants and/or co-surfactants such as Poloxamer 188, steric acid, Tween 80, and many more [106]. They offer advantages like controlling drug release, drug targeting, long-term stability, incorporation of lipophilic and hydrophilic drugs, and endless biotoxicity due to the use of physiological lipids [106]. However, SLN has a limited drug loading capacity (around 25% of lipid matrix) and leads to a burst release of hydrophilic drugs during the initial period [107]. SLN is used for delivering protein and antigens largely as it can be incorporated or absorbed into the lipid molecule of the SLN, which can further be administered into other conventional dosage forms such as oral, nasal, etc. Delivering proteins via SLN confirms protein stability, avoids proteolytic degradation, as well as sustained release of the incorporated molecules. SLN is used as a promising tool to deliver drugs that have a low bioavailability due to its inherent property of colloidal structure employed from both physiological lipids, as well as lipid molecules. To demonstrate the efficiency of SLN against MDR effects, Wong et al. developed and characterized a formulation of SLN using Pluronic F68. Doxorubicin was complexed with this SLN moiety and its cellular efficacy was investigated against MDR human breast cancer cell lines. In contrast to the doxorubicin solution with SLN, in vitro cytotoxicity was increased by 8-fold, cellular uptake was enhanced by 1.2-fold, and cellular retention was increased up to 2-fold, [108]. Further, SLN was used to embed human thymidylate synthase (hTS) inhibitor hydrophilic peptide (LRp) and was found to be effective against cDDP-resistant ovarian cancer cell line, thus extending the lifetime of the nanoparticles at the tumor site by the EPR effect and doubling the percentage of apoptosis [109].

9.4.8 Magnetic Nanoparticles

Magnetic nanoparticles are particles in a nanosized range with magnetic cores covered by a polymer or metal coating such as polyethylene glycol and polysaccharides, which can also be functionalized via cross-linkers [110]. In other words, these possible porous polymers may contain magnetic nanoparticles within its pores. These particles can then be functionalized by attaching carboxyl groups, amines, biotin, streptavidin, antibodies, or other cytotoxic drugs [111]. Magnetic nanoparticles offer the following advantages: it is easy to modulate the travel of the nanoparticles in vivo since they are guided by magnetic field; they can be heated for drug release; and they can be imaged simultaneously [112]. They have shown efficient targeted delivery and therapeutic effects on DNA. Magnetic nanoparticles were effectively used for delivering in vitro gene transfection [113]. Apart from that, magnetic nanoparticles can also be used as an anti-inflammatory agent by
maintaining the local concentrations at the required site and reducing the overall dosage and associated side effects [114]. Daunorubicin and 5-bromotetrandrin were encapsulated into Pluronic F127-iron oxide magnetic nanoparticle (MNP). This formulation was investigated against MDR leukemic cells, and is believed to have a sustained drug release and enhanced drug accumulation in K562/A02 cells after 48 h. Also, use of this formulation is believed to prevent the development of MDR effects in vitro due to the downregulation of MDR gene and P-gp expression [115]. Therapeutic benefit of various Pluronic-based drug nanoformulation with improved activity has been presented in Table 9.2.

**Table 9.2** Therapeutic benefit of Pluronic drug nanoformulations

<table>
<thead>
<tr>
<th>Type of Pluronic-based nanoparticle and composition</th>
<th>Therapeutic molecule</th>
<th>Improvement achieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate and chitosan polymeric nanoparticles coated with F127 polymer</td>
<td>Curcumin</td>
<td>Improved solubility of the lipophilic drug, achieved sustained release of the curcumin, and prolonged retention of curcumin in cancer cells [116]</td>
</tr>
<tr>
<td>PEO-modified poly(ε-caprolactone) nanoparticles and F68/F108 physical adsorption</td>
<td>Tamoxifen</td>
<td>90% drug encapsulation and increased accumulation of tamoxifen in the tumor cells, nonspecific endocytic process, followed by gradual release of the drug [117]</td>
</tr>
<tr>
<td>Poly(β-amino ester) F108 blend nanoparticles</td>
<td>Paclitaxel</td>
<td>Nanoparticles, when blended with Pluronic, showed rapid degradation at tumor cellular (acidic) environment and provided rapid tumorcidal effect in the cytosol when compared to PbAE [118]</td>
</tr>
<tr>
<td>Fe₃O₄ nanoparticles coated with β-cyclodextrin and F127 polymer</td>
<td>Curcumin</td>
<td>F127 coating improves stability in aqueous dispersion, haemo-compatibility, and excellent drug delivery, magnetic resonance imaging and hyperthermia formulation [119]</td>
</tr>
<tr>
<td>Fe₃O₄ nanoparticles coated with oleic acid and F127 polymer</td>
<td>Doxorubicin and Paclitaxel</td>
<td>The F127 impart steric stability, aqueous dispersity, and decreases uptake in macrophages thus slows down the rapid clearance by the reticuloendothelial system (RES). Comparative to the Feridex IV™ the clearance of the nanoparticles is slower thus aids imaging of the tumor [120]</td>
</tr>
<tr>
<td>Magnetic nanoparticles modified with oleic acid and L64 polymer</td>
<td>Enzyme</td>
<td>Pluronic coating stabilizes magnetic nanoparticles and offers adsorption (continued)</td>
</tr>
</tbody>
</table>
Table 9.2 (continued)

<table>
<thead>
<tr>
<th>Type of Pluronic-based nanoparticle and composition</th>
<th>Therapeutic molecule</th>
<th>Improvement achieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>of lipase from <em>Candida cylindracea</em> via strong hydrophobic interactions and hence increases the enzymatic production and thus reusability [121]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg phosphatidylcholine, α-tocopherol and poloxamer 407 liposome</td>
<td>Ibuprofen</td>
<td>The liposomal poloxamer gel prolongs release and targeted permeation of ibuprofen [122]</td>
</tr>
<tr>
<td>Soybean phospholipids and F127 based liposome.</td>
<td>Paclitaxel</td>
<td>Liposomal serves as a reservoir for paclitaxel and improves anticancer efficiency [123]</td>
</tr>
<tr>
<td>Dipalmitoyl phosphatidylcholine and poloxamer 188 liposomes</td>
<td>Doxorubicin</td>
<td>Incorporation of P188 into DPPC liposome exhibits thermosensitive property, which results in controlled drug delivery for lung cancer [124]</td>
</tr>
<tr>
<td>Polyethylenimine (PEI)/DNA/F127 complex nanogel</td>
<td>Thermo-responsive gene carrier</td>
<td>Inherent intracellular gene delivery and disrupting the endosome in the cell, retained even after F127 modification. The cytotoxicity of the nanogel increased as the temperature decreased from 37 to 20 °C, suggesting the Pluronic chain collapsed with temperature and plays a role in modulating cytotoxicity [125]</td>
</tr>
<tr>
<td>Heparin grafted F127 polymer nanogel</td>
<td>Vehicle for intracellular protein delivery</td>
<td>This formulation can be loaded up to 80–99% RNase A, maintain stability and offers significant cytotoxicity which suggests Heparin nanogel can be used as a high efficient delivery system for various proteins [126]</td>
</tr>
<tr>
<td>LMW heparin conjugated F127 nanogel</td>
<td>Low weight heparin</td>
<td>Minimize the adverse effects of Heparin and to enhance the therapeutic effect at fibrotic area [127]</td>
</tr>
<tr>
<td>Thiolated F127 polymeric micelles in combination with gold nanoparticle</td>
<td>Diagnostic</td>
<td>These cross-linked network micelles are used to immobilize various thiolated specific ligands and achieve specific targeting [128]</td>
</tr>
<tr>
<td>F127 polymeric micelles in combination with ceramic nanoparticles</td>
<td>Curcumin</td>
<td>This supramolecular complex helps to deliver intact drug in the presence of ceramic nanoparticle and can be used for drug delivery purpose in periodontal and orthopedic fields [129]</td>
</tr>
</tbody>
</table>

(continued)
9.5 Role of Pluronic Nanotechnology in Reversing or Overcoming Drug Resistance

Pluronic® has been used extensively to enhance delivery of therapeutics and to improve bioavailability due to its ability to form pores, which can interact with the MDR cells and develop sensitization of these cells to anticancer drugs [7, 15]. Pluronic® has gained popularity in drug delivery system as it embodies into the membrane allowing translocation in cells and thus, changing cellular functions such as mitochondrial respiration, ATP synthesis, activity of drug efflux transporters, apoptotic signal transduction, and gene expression [16]. Pluronic polymers are used to improve drug transport of various drugs through the most favored routes and even through impervious barriers such as the blood–brain barrier and the intestinal barrier [7, 8]. The most common diseases where Pluronics are commonly used to inhibit MDR in cells have been presented in Fig. 9.3.
9.5.1 Cancer

Cancer is one of the leading causes of death worldwide and can be defined as a state of the body when the cells keep on dividing, to form tumors (solid tumors) and in some cases, leukemia (there is no solid tumor formed). The incidence of new cancer cases estimated in the US in 2016 was more than 1.6 million, as per the American Cancer Society (http://www.cancer.org/acs/groups/content/@research/documents/document/acspc-047079.pdf). It is highly challenging to remove tumor completely by conventional surgical procedures. Most often, reoccurrence of tumors and invasion in healthy tissues are observed with time. Therefore, chemotherapy is the necessary approach in cancer treatment [131, 132]. However, development of multidrug resistance is the most significant impediment encountered in the treatment of cancer. The most common drug efflux protein P-gp acts as an export “pump” for a wide variety of chemotherapeutic drugs such as vinca alkaloids, anthracyclines, epipodophyllotoxins, and taxanes [133]. A pluronic nanotechnology strategy to inhibit the P-gp-mediated drug efflux could be effective to enhance antitumor efficiency and to overcome MDR (Table 9.3).

Pluronic polymers are known to have low systemic cytotoxicity and weak immunogenicity [134]. Pluronics are known to decrease the membrane microviscosity by inserting in the plasma membrane to inhibit the P-gp efflux pump. Xiao et al. [135] investigated the ability of F127 to increase the therapeutic efficacy of camptothecin (CPT)-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticle by sensitizing tumor cells to apoptosis. It is also evident from in vivo studies, toxicity reduces and thus, reduces the adverse systemic effects of CPT, achieving better antitumor efficacy. Biodistribution study demonstrates longer retention of CPT nanoparticles in the body and also their tumor targetability. This was attributed to the functionalization with F127 which prolonged release when compared to the free drug, also improved cellular uptake in contrast to the non-functionalized nanoparticles. It is evident that F127 inhibits function of P-gp, increases the cellular uptake of CPT by the tumorous cells, thus improving the therapeutic efficacy [135]. Pluronic P85 successfully inhibits the P-gp drug efflux system, decreases ATP levels, and promotes apoptosis in MDR cells, Lewis lung carcinoma (3LL-M27), and T-lymphocytic leukemia (P388/ADR and P388) tumors [136] during doxorubicin treatments.

Another strategy of reversing MDR effects is to conjugate drug(s) chemically to Pluronic polymers. Upon conjugation of hydrophobic drug ruthenium (Ru) with Pluronics F127/folic acid, selective induction of intrinsic and extrinsic apoptosis in liver cancer cell apoptosis was achieved, while exhibiting minimal cytotoxicity towards human normal cells [137]. Poloxamers are utilized as a pore-forming agent and drug-releasing enhancer, which can induce drastic sensitization effects to various anticancer drugs. In a PLGA and d-α-Tocopheryl polyethylene glycol 1000 succinate (TPGS or vitamin E), poloxamer 235 stretches out from the aqueous phase and creates a porous structure on the surface of the nanoparticles [22]. This porousness enhances cellular uptake in docetaxel-resistant human breast cancer cell
line, MCF-7/TXT, and shows a higher cytotoxicity level in contrast to nanoparticle formed from PLGA, a TGPS nanoparticle, and the marketed formulation Taxotere® in vivo model.

Table 9.3 Selective P-gp and MDR inhibitory role of Pluronic drug nanoformulations in therapeutics

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pluronic P85-doxorubicin</td>
<td>Pluronics induces a selective inhibition of P-gp, which is a result of energy reduction in ATP levels (ATP depletion), particularly in MDR cells. The ability is accompanied by cytotoxic activity and sensitization in the MDR cells [10]</td>
</tr>
<tr>
<td>Pluronic P105 F127, c(RGDyK) (cyclic RGD [arginine-glycine-aspartic acid] peptide) with doxorubicin or paclitaxel</td>
<td>This study was evident that Pluronic P105/F127 mixed micelles have the ability to enhance drug accumulation in MDR tumor cells and c(RGDyK) play vital role for anti-angiogenesis which could be a potential delivery system for attaining superior tumor grown inhibition [139]</td>
</tr>
<tr>
<td>Pluronic P105 and P85 with doxorubicin</td>
<td>Pluronics are capable of inhibiting the respiration, as a result of which accumulation of reactive oxygen species occurs and release of cytochrome c which causes cell death via apoptosis [41]</td>
</tr>
<tr>
<td>Poly(e-caprolactone)/Pluronic F68 with docetaxel</td>
<td>This formulation of Pluronics enhances drug transport across blood–brain barriers and intestinal barriers and enhances cellular accumulation in MDR MCF-7/TAX30 cells/tumors [74]</td>
</tr>
<tr>
<td>Poloxamer 407 (P407), d-α-tocopheryl polyethylene glycol succinate and folic acid with doxorubicin</td>
<td>Pluronics reduces the drug retention in the plasma, reverses MDR effect, induces apoptosis, and reduces antitumor activity. Due the conjugation of P407 to FA intracellular trafficking study shows that targeted and selective delivery is achieved in contrast to the unconjugated moiety [138]</td>
</tr>
<tr>
<td>Pluronic F-127, folic acid and doxorubicin</td>
<td>The presence of folic acid, along with Pluronics, targets and causes drastic sensitization of tumor cells, as well as inhibition of P-gp ATPase activity, and thus high therapeutic efficacy is attained [139]</td>
</tr>
<tr>
<td>Pluronic F-127, PAMAM dendrimers and doxorubicin</td>
<td>Conjugation of Pluronic F-127 to the PAMAM dendrimers forms unimolecular micelles, which is believed to have greater stability, thus preventing aggregation in the blood. The drug conjugate with dendrimer showed higher apoptosis and degraded nuclei of cancerous cells (MCF-7 and ADR) when compared to free drug Additionally, the conjugated formulation showed a higher cytotoxicity than the free drug, which is believed to have the potential ability to reverse MDR [89]</td>
</tr>
</tbody>
</table>
9.5.2 Bacterial Infection

Anti-microbial agents/drugs, used since the 1940s, are the first drug of choice for treating any bacterial infections. They can be defined as a strain of bacteria that is not killed or inhibited by the required concentration of antimicrobial agent that kills or inhibits the majority of strains of that organism [140]. When the anti-microbial agents/antibiotics that are used to treat the microorganisms are no longer effective, it is termed as antimicrobial resistance. According to World Health Organization (WHO) antimicrobial resistance develops when “Resistant microorganisms (including bacteria, fungi, viruses and parasites) that are able to withstand attack by antimicrobial drugs, such as anti-bacterial drugs (e.g. antibiotics), antifungals, antivirals, and antimalarials, so that standard treatments become ineffective and infections persist, increasing the risk of spread to others”. As per the records by WHO, the higher proportion of antibiotic resistance in bacteria are common in infections like urinary tract infection, pneumonia, and bloodstream infections. One such highly resistant bacteria is Staphylococcus aureus (MRSA) or multidrug-resistant Gram-negative bacteria. Herein, antibiotics that are primarily used for treating bacterial infection mainly are discussed.

A highly refined poloxamer CRL-1072 has been used (alone or in combination) with antibiotics and was able to produce therapeutic efficacy against drug-sensitive and drug resistance organisms, especially Mycobacterium tuberculosis [141]. When CRL-1072 was used alone, it was found to be bactericidal to M. tuberculosis, but when used in combination with an antmycobacterial drug, the synergistic antibacterial effect was attained, even on the resistant strains of M. tuberculosisis in macrophage culture and even in mice. These drugs include isoniazid (INH), rifampin, PAS, ethambutol, and ethionamide, which exhibited complete reverse resistance when used in combination with CRL-1072 against their respective M. tuberculosis resistant strains. However, CRL-1072, when used against streptomycin-resistant strain, indicated that it could partially overcome the resistance. Therefore, poloxamers were able to enhance the effectiveness of selected drugs against M. tuberculosis resistant strains.

Drug-resistant pathogens such as methicillin-resistant Staphylococcus aureus (MRSA) have become a serious impediment to drug delivery due to its acquired ability to develop high levels of resistance against several classes of antibiotics. Methicillin-resistant Staphylococcus aureus (MRSA) is treated with vancomycin. Lee et al. [142] demonstrated that vancomycin, when used with Pluronic F127 polymer (Pluronic hydrogel, 25% w/v), could enhance drug delivery to the infected inner ear, and also could inhibit the MRSA growth. It is believed to achieve a higher patient compliance due to prolonged release, and helps to attain complete therapeutic efficacy. The biodistribution study (in vivo) indicates no sign of inflammation, fibrosis or open space on day 50. Therefore, this Pluronic hydrogel had the potential not only to treat the resistant strain of S. aureus, but also was able to sustain drug release, and most importantly, inhibit the growth of the MRSA. Similarly, MRSA growth in S. aureus has been effectively controlled using
photodynamic therapy (PDT) and has been an effective alternative to treat antibiotic resistance. For enhanced efficacy, the photosensitizer-hematoporphyrin (Hp) was encapsulated in PEGylated liposomes and Pluronic P127 micelles [143]. With a low Hp concentration, Pegylated liposome/Pluronic micelle formulation exerted complete bactericidal activity in contrast to liposome, which is caused by prolonged action in the plasma membrane of the microbes.

9.5.3 Immunodeficiency

Human immunodeficiency virus (HIV) causing acquired immune deficiency syndrome (AIDS) was first detected back in the 1980s. Since the early discovery of AIDS, 35 million cases have been reported worldwide (UNAIDS 2014). Antiretroviral treatment (ART) has made a gradual progression in reducing the mortality rate. It is estimated that 12.9 million people living with AIDS worldwide are receiving the antiretroviral therapy. However, WHO had conducted a surveillance between 2004 and 2010 and has reported that drug resistance to HIV treatment has scaled up over the years [144]. This suggests that HIV-drug resistance research is of paramount importance. The potent antiretroviral drugs have limited access in the body, resulting in formation of virus reservoirs (like macrophages). These HGIV-infected macrophages enter into the brain and spread the HIV in perivascular macrophages, microglia, and astrocytes [145]. The blood–brain barrier forms a major barrier and hinders antiretroviral drugs to traverse through it, forming an immunologic and pharmacological sanctuary site for HIV in the brain [146]. Transport proteins, such as P-gp, are expressed on the surface of the BBB, further impeding antiretroviral drug delivery into the central nervous system [145]. It was suggested by Bendayan et al. [147] that the cellular membrane of the brain macrophages might be another additional barrier to drug permeability. Thus, the P-gp expressed on the surface of the BBB depletes the energy generated by ATP hydrolysis and effluxes the drug out of the brain, decreasing concentration of the therapeutic agent reaching the brain. Also, toxicity, adverse drug reactions, low bioavailability due to poor physicochemical properties, rapid drug metabolism in the liver, impaired biodistribution in HIV reservoirs, emergence of drug resistance, requirement of drug monitoring, and lifelong adherence are some of the associated problems with antiretroviral treatment [148]. In this context, Pluronic copolymer aids in the passage of drugs to the CNS by inhibiting P-gp substrates by two mechanisms of action. First, most of the Pluronics (especially P85) decrease ATP levels in the brain endothelial cell monolayers, which is crucial for the last stage processing of HIV-1 [149]. Second, the lipid structure of the Pluronic micelles is adsorbed in the membrane causing destabilization of the P-gp [10]. To demonstrate the effect, Pluronics have on the endothelial cells of the brain that form the BBB, BBMEC (bovine brain microvessel endothelial cells) an in vitro model was employed [150]. This study reports two points: (1) Pluronic, used at a concentration below the CMC by increasing the uptake of the drug through the P-gp dependent
pathway, enhances the drug delivery of neuroleptic agents that interact with the P-gp efflux system; and (2) P85, when used above the CMC drugs, is entrapped within the polymeric micelles, which is able to penetrate the BBB due to vesicular transport. Thus, interaction of P85 with the brain endothelial cells seems to be an energy dependent process with an inhibitory effect.

To develop a new approach to improve drug delivery to the CNS and BBB and enhance the efficacy of the ART target drug delivery is pivotal. Spitzenberger et al. [145] demonstrated that Pluronic P85 (alone) and a combination of P85 (0.2 and 1%) with ART (AZT, 3TC and nelfinavir) could suppress the viral replication significantly more (8–22% of control) than the ART alone (38% of control) when conducted on a severe combined immunodeficiency animal model of HIV-encephalopathy (HIVE) expressing monocyte-derived macrophages. Thus, this study was able to demonstrate that P85 was efficient in enhancing penetration of antiretroviral drugs through the BBB and also had a significant effect on cells (macrophages) that have a viral reservoir in the CNS. Protease inhibitors (PI) are drugs used for the treatment of HIV-1, when combined with ART. The development of resistance to PI is a very common issue and may even last after ART is discontinued. To investigate the ability of the Pluronics to interact with the PI and the P-gp substrates, MDCKII and LLC-PK1 cells transfected with human MDR1 were examined for drug transport [151]. The cell accumulation study and ATPase assay implied that Pluronic P85 was efficient in inhibiting the interaction of P-gp with PIs such as nelfinavir and saquinavir. Pluronics inhibit multiple transporters, thus reducing efficiency of PI efflux pumps and allowing proper distribution and therapeutic concentrations of antiretroviral drugs reaching the brain. Hence, P85 is efficient in suppressing viral replication and reducing production of drug-resistant mutants. It is also suggested that oral delivery of antiretroviral molecules, such as, saquinavir, where absorption is limited by the efflux of P-gp, use of P85 in the formulation at concentration less than the CMC leads to enhanced bioavailability.

9.6 Conclusion

Pluronic copolymers have proven to be a promising nanotechnology tool in reversing multidrug resistance in many diseases such as cancer chemotherapy, bacterial infection, and resistance to antiretroviral drugs. The ability of Pluronics to inhibit several associated mechanisms of drug resistance has reinforced its use in enhancing drug bioavailability and targeting. Further, researchers have combined inhibitory mechanisms of Pluronics with the sensitization effects, which indeed inhibit several multiple drug resistance mechanisms. Thus, it is apt to conclude that ongoing research with Pluronic copolymers has the potential to develop new progress in one of the major impediments of drug delivery to reverse and/or prevent multidrug resistance phenomenon. Clinically, such an intervention helps to minimize dose and number of cycles required for treatment.
References

2. Prevention CDCa (2011) Antimicrobial resistance: no action today, no cure tomorrow


9 Pluronic Nanotechnology for Overcoming Drug Resistance 237