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Santwana Padhi
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Polymeric nanoparticles for the treatment of solid tumors

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Preface

Cancer is one of the main causes of death worldwide due to the limitations of classical therapies such as low solubility of active drugs, toxic side effects on healthy cells, and resistance of tumor cells. These issues are partly solved by the recent development of polymeric nanoparticles, which improve drug absorption and the therapeutic index, while reducing side effects. Drug carriers must be biocompatible, biodegradable, and non-immunogenic. Coupled to a ligand that has affinity for that particular cell, polymeric nanoparticles are used to target specifically malignant cells or tissues and, in turn, improve drug stability.

This book, published in the series *Environmental Chemistry for a Sustainable World*, presents the latest advances in the application of polymeric particles for cancer treatment. Chapter 1 by Alex et al. describes the solid tumor microenvironment, and explains the initiation, progression, and metastasis of solid tumors. Then, Padhi et al. discuss conventional and innovative approaches for manufacturing polymeric nanoparticles in Chap. 2. Chapter 3 by Kulkarni reviews natural polysaccharide and protein-based polymers that can be employed to formulate polymeric nanoparticles. Prasher et al. detail the polymeric nanoparticles properties that control nanoparticle distribution to solid tumors in Chap. 4. Chapter 5 by More et al. explores passive and active targeting techniques for anticancer medication delivery to solid tumors (Fig. 1). Chapter 6 by Das et al. focuses on the synthesis and the application of polymeric nanoparticles encapsulating chemotherapeutics intertwined in natural polymers, and presents current patents, regulatory compliance, and marketed cancer therapies. Chapter 7 by Kumar et al. summarizes the benefits of using polymeric nanoparticle delivery to administer a combination of anticancer drugs. Active targeting on over-expressed receptors of solid tumors is presented by Sahu in Chap. 8.

Chapter 9 by Ghosh et al. reviews tumor-targeting ligands coupled to polymeric nanoparticles for efficient drug delivery. Theranostic applications of polymeric systems are given in Chap. 10 by Gautam et al., with focus on gold nanoparticles, superparamagnetic nanoparticles, quantum dots, micelles, and carbon nanotubes. Chapter 11 by Gosh et al. explains how biodegradable polymers can be used for oral delivery of drugs. Applications of polymeric nanoparticles for glioblastoma tumors,

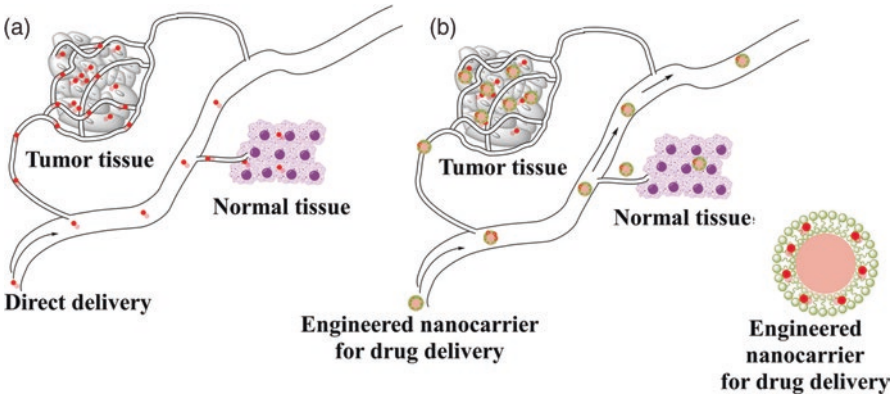

Fig. 1 Direct drug delivery (**a**) and delivery of engineered nanocarrier drug to extravasated vessel (**b**). Higher drug accumulation of the engineered nanocarrier is observed in tumor cells, without affecting the behavior of normal cells. (From Chap. 5 by More et al.)

lung cancer, and breast cancer are reviewed in Chaps. 12, 13 and 14 by Patil et al., Garg, and Tade et al. Chapter 15 by Behera and Padhi presents strategies to develop pH-responsive nanoparticles for the treatment of solid tumors. Polymeric nanoparticles for prostate cancer are discussed in Chap. 16 by Nangare et al. Chapter 17 by Padhi and Behera highlight the physicochemical properties of polymeric nanoparticles that influence the internalization and toxicological implications in target cells. Challenges and perspectives of the use of polymeric nanoparticles for treatment of cancer are discussed in Chap. 18 by Kumar et al.

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Santwana Padhi obtained her PhD from the Department of Pharmaceutics, Jamia Hamdard University, with a core expertise in cancer nanotherapeutics. Her research interest focuses on pre-clinical and clinical studies related to the drug entrapped targeted nanosystems for amelioration of solid tumors, design and evaluation of antibiotic loaded microparticles embedded polymeric scaffolds for tissue engineering and formulation, and optimization of different process variables for loading different drugs in biodegradable nanomatrices for enhancing the entrapment efficiency. Prior to her doctoral degree, she worked as a research assistant at the National Institute of Immunology (NII). She worked as a MSL in Biocon in oncology and dermatology domain. Presently she is working as a scientific officer at KIIT TBI and freelance editor at Manuscript Edit. She was granted senior research fellowship (SRF) from ICMR, junior research fellowship (JRF) from DBT, and Award of Excellence for Scientific Contribution from Jamia Hamdard University for 2 consecutive years. She has more than 4 years of research experience, mentored more than 5 postgraduate and 10 undergraduate students, and has published research articles and review articles in peer-reviewed journals. Email: ssantwanaa@gmail.com; https://scholar.google.com/citations?hl=en&user=OFKRgdQAAAAJ

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Chapter 1
The Tumor Microenvironment

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Abbreviations

ATP	Adenosine triphosphate
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid

1.1 Introduction

The origin of cancer is from stromal or parenchymal cells due to oncogenic type mutations in their deoxyribonucleic acid (DNA). The proliferation can be explained as when the cancer cells grow, they combine the normal or non-malignant cells with them and create a microenvironment which has greater importance in the local progression of the tumor as well as metastasis to the distant region. The microenvironment created due to interactions with the host system can be termed as an organ like

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skeleton for developing cancer (Marusyk et al. 2014; Tabassum and Polyak 2015). The reason behind the abnormal and unmatured immune components, fibrotic tissues and vasculature is the interaction between the stromal and cancer cells (Fukumura et al. 1998; Jain 2014; Apte et al. 2004; Bailey et al. 2008; Lin et al. 2001; Robinson et al. 2003).

The abnormal vascular network is created leading to compressed, leaky lymphatic vessels which lead to hypoxia in the tumour. Inside the tumour, every region is different and unique having different rates of multiplication, progression, proliferation, differentiation, vascularity, immunosuppressive components, inflammation, invasiveness all due to diverse epigenetic, genetic, phenotypic profile and at subclonal population level each tumour is different from other (Tabassum and Polyak 2015). The loss of effectiveness by the targeted anticancer therapy is all due to the diversity of each tumour cells (Bedard et al. 2013). Various past studies have reported that intercellular communication in the tumour microenvironment is due to chemokines, cytokines, inflammatory mediators, growth factors, enzymes which can remould the matrix, looking into recent studies which have highlighted the role of cell-free DNA, apoptotic bodies, circulating tumour cells as communicators not only to other tumour cells but with normal non-malignant cells (Denisenko et al. 2018; Balkwill et al. 2012).

Failure of integrity in the tissue, progression and carcinogenesis is due to tumour microenvironments cellular component interactions with non-cellular components present in the extracellular matrix (Jahanban-Esfahlan et al. 2019; Seidi et al. 2018). Using technologies such as various microfluid devices and advancement in the three dimensional area has laid the foundation to stimulate the tumour microenvironment and study its components and physiological conditions well (Ayoubi-Joshaghani et al. 2020; Sleeboom et al. 2018). Interactions between tumour microenvironment and tumour cells can be through two pathways, firstly it can be through the direct cell to cell contact and even cell-free contact using the extracellular matrix.

Secondly, it could be through the involvement of various mediators which horizontally transfer information between the cellular or non-cellular components (Baghban et al. 2020). Various components and physiological conditions of tumour microenvironment are depicted in Fig. 1.1. As we have discussed that microenvironment has an important role to play in the development of cancer, so it's necessary to neutralize the aggravating factors in the tumour microenvironment for better control of the disease, development of novel therapies are necessary for limiting tumour microenvironment effects but also the detection of the various abnormal components as well as physiological conditions present.

The concentration of apoptotic bodies, circulating tumour cells and circulating tumour DNA in the blood can be directly correlated with the advancement of the disease condition, helping to choose the best treatment option available according to staging. Another available option is liquid biopsies which are minimal non-invasive procedure and can detect not only above-mentioned components but also various other significant ones (Baghban et al. 2020). To use the components as inhibiting targets as well as in diagnosis of cancer, a better understanding is required about

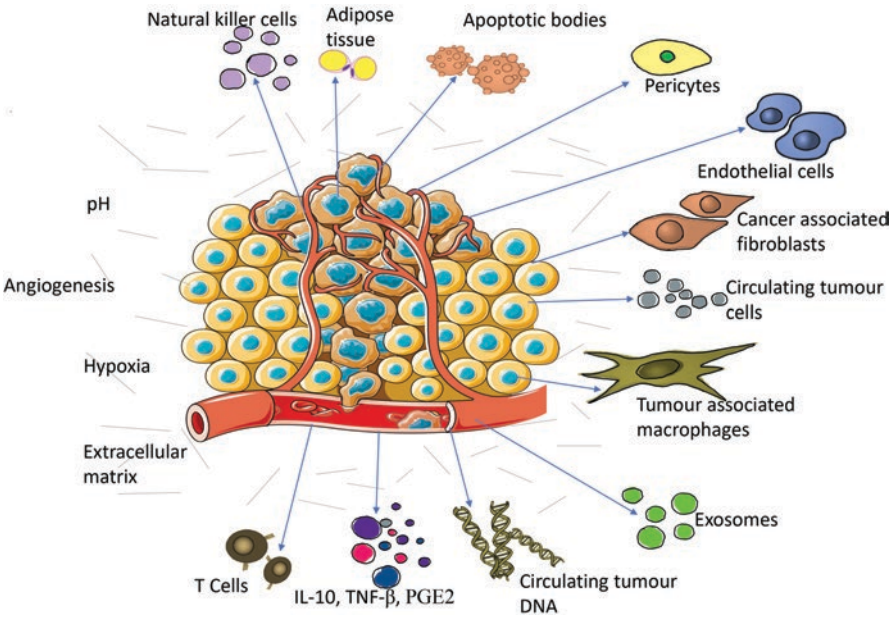

Fig. 1.1 The tumour microenvironment depicting several components comprising of cancer associated fibroblast, tumour associated macrophages, exosomes, apoptotic bodies, circulating tumor DNA, endothelial cells, immune cells, pericytes, adipose tissue, extracellular matrix, and physiological conditions. *IL-10* interleukin-10, *DNA* deoxyribonucleic acid, *PGE2* prostaglandin E2, *TGF-β* transforming growth factor beta

their origin, conditions under which they are released and their impact on proliferation, differentiation, and metastasis of the cancer cells.

The following chapter has been divided into two parts: abnormal cellular constituents of the tumour microenvironment and abnormal physiological conditions of the tumour microenvironment. Figure 1.1 gives an overview of the components involved in creating tumour microenvironment, and Fig. 1.2 gives overview about the therapies.

1.2 Abnormal Cellular Constituents of the Tumour Microenvironment

1.2.1 Pericytes

These cell type form contact with endothelium focally and are a type of vascular mural cells implanted in the basement membrane (Armulik et al. 2005). They are accounted for their role in maturation, development; remodelling and stabilization of the vasculature also maintain the vascularity of blood brain barrier. Cell repair

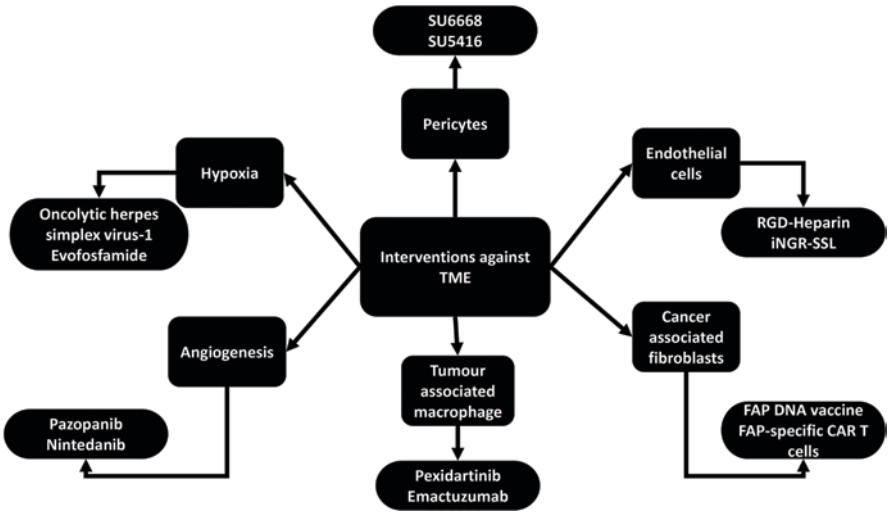

Fig. 1.2 Therapies against various components and physiological conditions of tumour microenvironment. Novel therapeutic approaches that can target various tumour microenvironment components such as pericytes include SU6668 and SU5416, Endothelial cells including tripeptide RGD-heparin and a tumor penetrating peptide iNGR-sodium stearoyl lactylate (SSL), Cancer associated fibroblasts include familial adenomatous polyposis (FAP) DNA vaccine and FAP-specific chimeric antigen receptor (CAR) T cells, tumour associated macrophages include pexidartinib and amactuzumab, therapies targeting angiogenesis include nintedanib and pazopanib, and in hypoxic state Oncolytic *Herpes Simplex* Virus-1and Evosfosfamide can be employed

due to pathological and physiological processes are also regulated by pericytes (Armulik et al. 2010). They surround pre-capillary arterioles, capillaries, collecting venules and postcapillary venules (Allt and Lawrenson 2001; Sims 1986). Their morphology as well as markers present are somewhat like that of vascular smooth muscle cells but can be distinguished by continuing pattern they form and their distribution right after and between capillaries, arterioles and venules and has also been observed next to the large vessel's endothelium (Andreeva et al. 1998; Hosono et al. 2017; Caspani et al. 2014).

They have been characterized as multifunctional type in case of the tumour microenvironment. During tumorigenesis and angiogenesis, endothelial cells, as well as pericytes, are involved in remodelling of the basement membrane (Kloc et al. 2015; Baluk et al. 2003; Ribeiro et al. 2017). Looking into their role in the immune system they are responsible for removing inborn leukocytes from the blood vessels, showing phagocytic activity directly and regulating the activation of lymphocytes (Birbrair 2018). Targeting these can show anti-angiogenic properties but clinically no such importance has been identified (Keskin et al. 2015; Hainsworth et al. 2007; Nisancioglu et al. 2010).

Certain studies indicate their low levels to be responsible behind the spread of cancer, to prove this a study conducted by Semb et al. concluded that deficiency of pericytes in case of platelet-derived growth factor Bret/ret. mouse model caused

escalated levels of metastatic insulinoma derived cells. Similarly, in another study conducted by Kalluri et al. showed that reducing the levels through genetic knockdowns of pericytes in case of breast cancer leads to escalating of pulmonary tumourigenesis when studied in NG2-TK type mouse model (Xian et al. 2006; Yonenaga et al. 2005; Hong et al. 2015; Cooke et al. 2012). Contradicting these two studies, it was reported that enhancement in the production of pericytes via cancer cells lead to glioma tumour development.

Analyzing the cytology of tumour cells it was revealed glioblastoma stem cells have a key role in the production of vascular pericytes and supporting tumor growth and vascular function by reshaping the perivascular area. Glioblastoma stem cells travel down towards the stromal cell-derived factor-1/chemokine receptor 4 axis and end at endothelial cells and are acted upon by transforming growth factor β for their development into pericytes (Cheng et al. 2013). It is also said that the blood vessels with a lower concentration of pericytes are likely to be vulnerable and sensitive for chemotherapy as well as radiations giving an idea that they have a key role in the protection of endothelial cells (Bergers et al. 2003; Franco et al. 2011).

The interaction between endothelial cells and pericytes are variable in normal cells and tumor cells (Winkler et al. 2011; Morikawa et al. 2002). It is said that tumour pericytes originate from normal surrounding tissues and there is a possibility that it can remain in the bone marrow derived cells after treatment to eradicate the tumour (De Palma et al. 2005; Du et al. 2008). A study conducted by in Lin Cheng et al. proved that vascular pericytes of glioblastomas are attained from glioma stem cells, noting down the property that glioblastomas are behind the pro generation of pericytes and have a key role to play in the formation of vascular structure inside glioma stem cells (Cheng et al. 2013).

1.2.2 Endothelial Cells

Trigger from the adjoining stromal cells in the tumour microenvironment can result in progression of the tumor (Li et al. 2007a, b; Egeblad et al. 2010). Endothelial cells are categorized as stromal cells of tumour microenvironment. These cells produce growth factors and cytokines when they are being penetrated by tumor cells (Brenner et al. 2010). In the case of growing tumor, the endothelial cells are accumulated in the innermost layer of the blood vessels (Salazar and Zabel 2019). The phenotype and morphology of the tumour endothelial cells are similar to the tumour and is significantly different from the normal endothelial cells (Dudley 2012; Aird 2009).

Metastasis gets initiated when various chemokines and adhesion molecules are expressed as well as when adhesiveness of tumour cells endothelium occurs (Iiizumi et al. 2007). Tumor cells have a stimulating effect on the endothelial cells due to which the formation of the tube is initiated as well as promoted leading to angiogenesis (Nishida et al. 2006). There are studies which not confine its role only to angiogenesis they can even lead to resistance of chemotherapy as well as metastasis.

Initial interaction between endothelium and cancer cells is due to the communication between carbohydrate to carbohydrate units (Dube and Bertozzi 2005; Raz and Nakahara 2008). This interaction trigger cancer cells as well as endothelial cells through free radicles, cytokines, growth factors and lipids which are physiologically active mediators (Orr et al. 2000).

The bonds are strengthened and formed via feedback of the listed mediators for expressing the adhesion molecules (Tang and Honn 1994; Kannagi 1997). Regulation of adhesion is through immunoglobulins, cadherins, integrins, selectins, thrombospondin (Nicolson 1988; Pauli et al. 1990). There are reports that resistant acquired for 5-fluorouracil and paclitaxel is due to overexpression of aldehyde dehydrogenase and multidrug resistance mutation (Akiyama et al. 2012; Hida et al. 2017). Tumour endothelial cells highlight significant characteristics of the developing tumour such as increased interstitial fluid pressure, acidosis as well as hypoxia of tumour, reduction in blood flow and leakage of the vascular system (Dianat-Moghadam et al. 2018).

Various receptors such as atypical chemokine receptor 1, atypical chemokine receptor 3 and chemokine receptor 4, chemokine receptor 7 are hall markers for progression of tumour endothelial cells (Salazar and Zabel 2019). As discussed, that these have a key role to play in the progression of cancer, so various strategies are applied to inhibit the proliferation, migration, growth, and survival of these cells thus controlling the angiogenesis (Daei Farshchi et al. 2018). Targeted therapy for tumor endothelial cells is the usage of gold nanoparticles, which are responsible for disrupting the signal between these cells and tumour cells (Zhang et al. 2019a, b, c). The components of vascular endothelial growth factors (vascular endothelial growth factors D, vascular endothelial growth factors B, vascular endothelial growth factors A, placental growth factor and vascular endothelial growth factors C) are responsible for showing angiogenesis by binding to the tyrosine kinase.

Vascular endothelial growth factors receptor accounting for signal initiation, endothelial cell metabolism, monocyte migration and haematopoiesis are due to activation of vascular endothelial growth factors receptor 1 via placenta growth factor and vascular endothelial growth factors B, the proliferation of endothelial cells along with an increase in vascular permeability and angiogenesis is accounted due to binding of vascular endothelial growth factors A with vascular endothelial growth factors receptor 2 (Simons et al. 2016).

Some studies have been published stating that there is a significant release of growth factors by the tumor endothelial cells and are termed as angiocrine factors (Butler et al. 2010). Notch ligand jagged1 is upregulated in these cells due to fibroblast growth factor 4 production by the B-cell lymphoma which further triggers fibroblast growth receptor 1. It has a decisive role to play in upregulation of notch ligand jagged1 which leads to the induction of Notch2–Hey1 in case of lymphoma cells enhancing tumor progression, infiltration by neutrophils, further metastasis as well as phenotyping of the stem cells (Cao et al. 2014, 2017; Zhu et al. 2011; Lu et al. 2013; Ghiabi et al. 2014; Pedrosa et al. 2015; Wieland et al. 2017). It is also responsible for downregulating a tumor suppression factor as well. The angiocrine factor for tumour suppression is ephrin type-A *receptor* 2 of tumor endothelial cells

and downregulates Slit2 leading to increment in cancer cell invasiveness (Brantley-Sieders et al. 2011).

Effective novel drug delivery systems targeting endothelial cells in various types of cancer has been summarized in Table 1.1.

1.2.3 Cancer-Associated Fibroblasts

These are the most abundantly available cellular component in the tumour microenvironment and show high expression of fibroblast activation protein, platelet derived growth factor α or platelet derived growth factor β, α-smooth muscle actin (Pietras and Östman 2010; Micke 2004). Not only these but surface markers such as myosin light chain kinase, myosin light chain 9, collagen type I alpha 2, decorin and matrix metalloproteinase can also be detected (Liu et al. 2019a, b, c; Nurmik et al. 2020; Nishishita et al. 2018; Brunel et al. 2018). It has been well investigated that cancer-associated fibroblasts have diverse functionality by having a key role in the progression and development of cancer as well as building up a scaffold for cancer (Quail and Joyce 2013). They are also responsible for increasing the viability of tumour cells, migration, proliferation, and reduction in apoptosis rate (Lee et al. 2018; Kalluri 2016).

Their normal physiological role in the body is wound healing but in case of cancer they are responsible for the production of extracellular matrix proteins leading to expression of immunosuppression cells, for example, tumour associated macrophages (PD-1+ TAMs) inducing immunosuppression, monocytes (Monteranand and Erez 2019; Liu et al. 2019a, b, c; Yavuz et al. 2019). The reason behind the active proliferation of cancer cells is due to the inducement of active stress leading to initiation in the autophagy pathway all due to cancer-associated fibroblasts (Zhou et al. 2017a, b). In the case of gastric cancer cancer-associated fibroblasts influence the expression of galectin-1, also have an important role in the production of vascular endothelial growth factor A and fibroblast growth factor 2 leading to angiogenesis (Wang et al. 2019a, b; Tang et al. 2016).

When cancer-associated fibroblasts are oxidized they lead to the release of cytokines and ketones leading to autophagy and biogenesis in the nearby mitochondria (Yang et al. 2016; Lisanti et al. 2010; Yan et al. 2019). In case of ovarian cancer the increased rate of metastasis and proliferation is all due to interleukin-6, chemokine ligand 5, C-X-C motif chemokine 10 which are a type of cytokines derived from cancer-associated fibroblasts, facilitate metabolism of tumour cells by escalating synthesis of nicotinamide adenine dinucleotide phosphate, mobilization of glycogen and phosphoglucomutase 1 phosphorylation as well as showing effects on tricarboxylic acid cycle (Curtis et al. 2019).

As discussed earlier those various biomarkers are expressed in case of cancer-associated fibroblasts and α smooth muscle actin being one of them expressed abundantly and its level in breast cancer tissue is notable (Sugimoto et al. 2006; Tchou et al. 2013). In a study conducted by Becker and team proved α smooth muscle actin

Table 1.1 Nanoparticles targeting endothelial cells

Type	Carrier used	Ligand	Drug/Therapeutic molecule used	Effective against	References
RGD-heparin	Heparin	Cyclized (Arg-Gly-Asp) peptide	Heparin	Ovarian cancer	Wang et al. (2019a, b)
iNGR-SSL	Liposome	iNGR peptide	Doxorubicin	Glioma	Zhou et al. (2017a, b)
Cell penetrating NGR-LP	Liposome	CPP peptide, NGR	siRNA against c-Myc	Fibrosarcoma	Yang et al. (2015)
NGR-SSL-CA4	Liposome	NGR peptide	Combretastatin a4	Glioma	Huang et al. (2016)
iNGR-PLGA	Poly(lactic-co-glycolic acid)	iNGR peptide	Paclitaxel	Glioma	Kang et al. (2014)
MSN-DOX-PDA-NGR	Mesoporous Silica Nanoparticles	NGR peptide	Doxorubicin	Glioma	Hu et al. (2016)
SSLD	Liposome	cRGDyC, cRGDfK	Doxorubicin	Melanoma, colon	Amin et al. (2015)
iRGD-MSN	Mesoporous Silica Nanoparticles	iRGD	Combretastatin a4, doxorubicin	Cervical cancer	Li et al. (2016)
RGD-MEND	Lipid nanoparticle	cRGDfK	siRNA against VEGFR2	Lung metastasis, renal carcinoma	Sakurai et al. (2018)
c(RGDyC)-LP	Liposome	cRGDyK	Sodium borocaptate	Glioma	Kang et al. (2017a, b)
PLGA	Liposome	cRGDyK	Galbanic acid	Colon cancer	Nik et al. (2019)
Cyclic RGD-PAMAM	Poly(amidoamine) dendrimer	cRGDyC	Arsenic trioxide	Orthotopic glioma	Li et al. (2016)
RGD-KLA/PTX-Lips	Liposome	cRGDyK	KLA peptide, PTX	Breast cancer	Sun et al. (2017)
P-(F56)-DOX	N-(2-hydroxypropyl) methacrylamide	F56	Doxorubicin	Colon cancer, lung cancer	Shamay et al. (2016)

DOX Doxorubicin, *PLGA* Poly (lactic-co-glycolic acid), *LP* Liposome, *RGD* (arginine-glycine-aspartate) peptide, *NGR* asparagine-glycine-arginine peptide, *CPP* cell penetrating peptide, *CA4* Combretastatin a4, *PAMAM* poly (amidoamine), *MEND* multi-functional envelope-type nano-device, *MSN* mesoporous silica nanoparticles, *PTX* Paclitaxel, *KLA* acetyl-(KLAKLAK)2-NH2, *siRNA* Small interfering Ribonucleic acid, *SSLD* Sterically stabilized liposomal doxorubicin, *F56* Copolymer targeting, VEGFR-1, *VEGFR* Vascular endothelial growth factor receptor

role in promoting tumour growth of mammary cells and by testing it on mice induced with tumour they showed that reducing α smooth muscle actin levels genetically decreased the tumour size without showing any changes to metastasis profile

(Becker et al. 2020). In a study conducted by Fang et al. on the role of cancer associated fibroblasts in causing resistance to pancreatic cells for gemcitabine, showed that the key reason behind inducement of resistance is via secretion of micro RNA-106b exosomes by the cancer-associated fibroblasts and uptake of this component by the other cancer cells, leading to increase in levels of the secreting product giving way for gemcitabine resistance and cancer cell survival (Fang et al. 2019).

1.2.4 Tumour-Associated Macrophages

These types of macrophages are derived from mononuclear cells and is one of the most abundantly infiltrating types tumour immune cells (Dehne et al. 2017). There are various key findings to prove the role of tumor associated macrophages in cancer-associated inflammation decreasing the antitumour immunity of the host system and leading to cancer progression. Important in remodelling as well as building up of extracellular matrix skeleton which helps the tumour cells in invasion and interaction with various stromal cells by releasing chemokines, cytokines, and growth factors (Noy and Pollard 2014; Gonzalez et al. 2018; Mantovani et al. 2017) M1 and M2 are two different types of polarization states of macrophages (Ostuni et al. 2015).

M1 is attained when T helper 1 cytokines interleukin-18, interleukin-12 or even toll-like receptors in the activated form have a key role in polarizing the macrophage to M1 state. M1 polarized macrophages can be labelled as antitumour macrophages because they produce reactive nitrogen as well as oxygen species, tumor necrosis factor-α, interleukin-6 and interleukin-1β (Jeannin et al. 2018). M2 is responsible for the healing of wounds, humoral immunity through Th2 immune activation, a remoulding of tissues. Various anti-inflammatory mediators are secreted by M2 polarized states such as TGF-β, IL-13 and IL-10 having hand in the development of the tumour. It is present in four different types of phenotypes state M2b, M2a, M2-like and M2c (Sica et al. 2006). The tumor associated macrophage type is similar to M2 polarized state (Guerriero 2018).

In tumorigenesis, they initiate the tumour development, growth and also metastasis due to their ability to secrete various growth factors, cytokines, a proteolytic enzyme, inflammatory components (Chen et al. 2019). In advanced stages of cancer, it has been seen that a high amount of tumor associated macrophages is present in the hypoxic area having defective antigen-presenting capability as well as good pro-angiogenic property (Laoui et al. 2014; Hinshaw and Shevde 2019). In a study conducted by Wenes et al. concluded that endogenous inhibitor for f MTORC1 and upregulation of REDD1 are all due to tumor associated macrophages which lead to the reduction of glucose uptake by these hypoxic macrophages thus increasing the availability to endothelial cells leading to advancement in metastasis and neoangiogenesis (Wenes et al. 2016).

Other components secreted by tumor associated macrophagess are C-X-C Motif chemokine ligand 8 a pro-angiogenic chemokine, matrix metalloproteinases,

cathepsins example of the proteolytic enzyme responsible in degrading the extracellular matrix secretions of angiogenic components (Mantovani et al. 2017; Cassetta and Pollard 2018; Prenen and Mazzone 2019). Recent approaches in controlling the tumor associated macrophages are through using a combination of antibodies blocking signal regulatory protein alpha (rendering the phagocytic ability to macrophages) and inhibitors for colony stimulating factor 1 receptor leading to repolarizing the tumor associated macrophages increasing their phagocytosis as well as rendering them with anti-tumour potential (Kulkarni et al. 2018; Ramesh et al. 2019). It is also responsible for suppressing natural killer cells and T-cells leading to further suppression of their anti-tumour activity and works synergistically with tumour associated neutrophils and dendritic cells in tumour microenvironment.

Enzymes secreted by tumor associated macrophages such as arginase and nitric oxide synthase leads to inhibition of T-cell anti-tumour property (Guo et al. 2016; Lohela et al. 2014; Angelis et al. 2015; Van Naarden Braun et al. 2015; Lu et al. 2011; Rodriguez et al. 2004; Zhou et al. 2020; Lin et al. 2019). The various targeting approaches for tumor associated macrophages include inhibiting the recruitment of tumor associated macrophages, depleting the viable tumor associated macrophages, reprogramming the tumor associated macrophages (Mantovani et al. 2017; Cassetta and Pollard 2018). Various therapies targeting tumor associated macrophages which are under investigation have been discussed in Tables 1.2, 1.3 and 1.4.

Table 1.2 Drugs under investigation for inhibiting the recruitment of the tumour-associated macrophages in tumour microenvironment

Drugs	Target	Monotherapy/ combination therapy	Type of tumour	Clinic trial phase	References
Pexidartinib	CSF-1R	Monotherapy	Melanoma	Phase 1 Phase 2	Butowski et al. (2016)
		Paclitaxel	Solid	Phase 1	
		Durvalumab	Pancreatic and colorectal cancer	Phase 1	
CCX872	CCR2	Folfirinox	Pancreatic cancer	Phase 1b	Noel et al. (2017) and Linehan et al. (2018)
Carlumab	CCL2	Monotherapy	Solid	Phase 1b	Yang and Zhang (2017)
PF-04136309	CCR2	Folfirinox	Pancreatic cancer	Phase 1b	Nywening et al. (2016)
Emactuzumab	CSF-1R	Monotherapy	Solid	Phase 1	Gomez-Roca et al. (2015)

CSF-1R Colony stimulating factor 1 receptor, *CCR2* C-C chemokine receptor type 2, *CCL2* chemokine (C-C motif) ligand 2

Table 1.3 Investigational drugs reducing the survival of tumor-associated macrophages

Drugs	Target	Monotherapy/ combination therapy	Type of tumour	Clinic trial phase	References
Lurbinectedin	Macrophages	Gemcitabine	Solid	Phase 1	Paz-Ares et al. (2017)
		Monotherapy	Solid	Phase 1	Elez et al. (2014)
		Monotherapy	Ovarian cancer	Phase 1	Poveda et al. (2017)
Trabectedin		Durvalumab	Solid	Phase 1	Lin et al. (2019)

Table 1.4 Drugs under investigation for polarizing tumour-associated macrophages to the M1 state

Drugs	Target	Monotherapy/ combination therapy	Type of tumour	Clinic trial phase	References
CP-893	CD40	Gemcitabine	Pancreatic cancer	Phase 1	Vonderheide et al. (2007)
CP-870		Monotherapy	Solid	Phase 1	Beatty et al. (2013)
Zoledronic acid		Monotherapy	Breast cancer	Phase 2	Gnant et al. (2011)

CD40 Cluster of differentiation 40, *CP* Chicago Pneumatic

1.2.5 Circulating Tumour Cells

These are the cells which are shed by the primary tumour and make their way into the blood supply (Krol et al. 2018). Relapse of cancer, multidrug resistance as well as metastasis can be correlated with circulating tumor cells (Shishido et al. 2019). They move around in body fluids and get attached to a particular site causing metastasis; their presence has also been noted in the primary stages of cancer (Yap et al. 2014). They are present in small amounts when analysed in blood but their presence has been noted in blood for quite long before attaching to a new surface such as capillary walls and then after entering new tissue, sometimes their size is responsible for clogging the various capillaries (Plaks et al. 2013).

Prognostic value of circulating tumor cells is well established as there is n number of evidence proving that these cells are shed from the primary tumour even before it becomes detectable using imaging technologies (Rhim et al. 2012). It has been observed that circulating tumor cells have self-seeding property in case of melanoma, breast cancer, colon cancer, interleukin-8 and interleukin-6 act as bait for these cells and in case of breast cancer their penetration into the mammary cells attracted by fascin-1 which is associated with the actin cytoskeleton and matrix metalloproteinase 1/collagenase-1. The various outcomes of self-seeding are involvement of stroma, growth of the tumour and enhancement of angiogenesis.

Even after the removal of the tumour or through eradication chemotherapy, the relapse is due to self-seeding process of circulating tumor cells (Kim et al. 2009; Jayatilaka and Phillip 2019).

As we have discussed its role in the progression of cancer, thus it becomes important to target this component for effective control in the development and metastasis of primary tumour which can be achieved using neo-adjuvant, adjuvant, combinational and anti-metastatic therapy (Yan et al. 2017). Circulating tumor cells as a biomarker can be evaluated through biopsy in case of advance urothelial cancer; a sampling of blood in colorectal, breast, gastrointestinal stromal tumour, ovarian, hepatocellular, pancreatic, oesophageal squamous cell, head and neck, small cell lung, skin and rectal cancer; in case of prostate cancer it can be evaluated from plasma (Abrahamsson et al. 2017; Magbanua et al. 2018; Germano et al. 2018; Zhang et al. 2019a, b, c; Kang et al. 2017a, b; Li et al. 2018a, b; Strati et al. 2017; Wang et al. 2018; Lou et al. 2018; Effenberger et al. 2018; Nanou et al. 2018; Troncarelli et al. 2019; Riethdorf et al. 2019; Messaritakis et al. 2018).

1.2.6 Exosomes

The average size of any exosome can vary between 30 and 100 nm and can easily be detected in any body fluid even from cell cultures, their outer covering is a lipid bilayer and levels get raised in a pathological disease state such as cancer (De Toro et al. 2015; Lee et al. 2012; Théry et al. 2002). It consists of nucleic acids, proteins, and lipids. Their release follows different mechanism and methods, in many cases, their release is associated with Rab proteins, pH-dependent proteins, calcium dependent proteins and p53 increased expression (Zhao et al. 2016).

The physiological importance of exosomes, in the case of stem cells, is their survival, preserving them, differentiation, involved in the reduction of inflammation and increase in the ability of wound healing. Their role can be described from the cells they originate, for example, if they are originating from tumour cells, they have a role in the advancement of cancer and if it is from immune cells they increase and maintain immunity (Mathivanan et al. 2010; Raposo and Stoorvogel 2013; Record et al. 2014). The various surface proteins of exosomes are cluster of differentiation 81, cluster of differentiation 63, tumour susceptibility gene 101 which can be detected in early stages of cancer when the tumour has not even undergone metastases or is in detectable size (Li et al. 2017).

5′-triphosphorylated components of exosome originating from stromal fibroblast have RNA component of signal recognition particle 7SL1 which is unshielded and if breast cells uptake these components leads to activation of viral ribonucleic acid pattern recognition receptor-retinoic acid-inducible gene I further development of cancer growth, resistance to chemotherapy and metastasis (Nabet et al. 2017). In advance cases of ovarian cancer, the spread can be noted in the omentum adipose tissue and all the exosomes of cancerous adipocytes, as well as fibroblasts, accommodate a large number of microsomal RNA21 responsible for chemotherapy resistance and aggressiveness of cancer (Zhao et al. 2016).

Exosomes are exploited as potential biomarkers, drug delivery system, as an antigen carrier all because they are natural, biodegradable, can be engineered and non-toxic (Khodashenas Limoni et al. 2017; Xie et al. 2019). Targeting exosomes can be through inhibiting syndecan heparan sulfate proteoglycans or axis heparanase/syndecan-1 (Ramani et al. 2013; Wu et al. 2019; Baietti et al. 2012; Thompson et al. 2013). In a study conducted by Sento and team concluded that heparin can reduce the uptake of exosomes and reducing the metastasis rate (Sento et al. 2016).

1.2.7 Apoptotic Bodies

Extracellular membrane-derived vesicles are produced by various cells which undergo apoptosis and are termed as apoptotic extracellular vesicles (Gregory and Dransfield 2018). It has been stated that they control immunoregulatory properties during the development of cancer (Caruso and Poon 2018). Not every apoptotic extracellular vesicles is constant in size; instead, it varies both in composition and in size (Gregory and Dransfield 2018). Those with greater size and having a diameter which varies from one to multiple microns are labelled as apoptotic bodies consisting of auto-antigens which are modified by caspase, organelles, are well defined like endoplasmic reticulum and mitochondria as well as condensed chromatins which are remnants of nuclear material, smaller ones having a size ranging between 50 and 1000 nanometre is ectosomes or microvesicles (Lynch et al. 2017).

In apoptotic bodies restricted molecular exchange has been noted due to membrane integrity loss and leading to release of some selective molecules that are capable of modulating innate pathways of inflammation. Phagocytes uptake the apoptotic antibodies and can be used to process the cycle of phagocytosis (Wickman et al. 2013). Apoptotic bodies are thought to accelerate intercellular connectivity through the transition of cellular factors in the production of molecular memory by macrophages (Gordon and Plüddemann 2018). Their importance has also been noted by saving nucleic acid from degradation by enzymes (Samos et al. 2006).

1.2.8 Circulating Tumour DNA

Apoptotic and necrotic tumour cells release cell-free DNA and the mechanism behind the release is unknown till now. Increment in cell-free DNA levels can be seen in cases of sepsis, inflammatory condition and doing too much of physical activity, if levels are compared between healthy individuals and cancer patients then increment is seen in the latter category (Diaz and Bardelli 2014; Wan et al. 2017). In normal tissues the release is due to normal cellular turnover, the cell-free DNA which is released from the tumour cells is termed as circulating tumour DNA. Some studies suggest that hypoxia is the driving factor for the release of circulating tumour DNA (Vietsch and Wellstein 2019).

It has potentiated as an upcoming biomarker as its increased levels can easily be observed even before the tumour gets detected using imaging technology and levels

can be processed using blood or fluid samples of the patient (Fiala and Diamandis 2018). Fragment size of circulating tumour DNA is between 120 and 180 bp and the peak below is 150 bp (Vietsch and Wellstein 2019). Circulating tumour DNA consists of the genetic alterations same as the tumour from which it is excreted such as variability in copy number of chromosomes, complex variations of structures, point mutations, etc. Levels are usually increased in advance cases and metastatic cases as compared to localized and early stages.

The success rate is 75% in detecting advance cases of ovarian cancer, pancreatic cancer, bladder cancer, colorectal cancer, liver cancer, gastric carcinoma, head and neck cancer, breast cancer. In the case of prostate cancer, thyroid cancer, kidney cancer and brain cancer the success rate for detection reduces down to 50% (Li et al. 2020). When compared to circulating tumour cells, circulating tumour DNA is easily detected from the body fluids. The ease of collection makes it a favourable detecting tool as compared to tumour biopsies. Using liquid biopsy it can be collected and can be accessed through Cobas, Super-ARMS, CAPP-Seq, next-generation sequence, droplet digital polymerase chain reaction which is more sensitive for circulating tumour DNA as compared to circulating tumour cells in case of epidermal growth factor receptor mutation (Liang et al. 2018; Watanabe et al. 2018).

Various patients suffering from the same type of cancer and same stage may exhibit different levels of circulating tumour DNA and the levels can be very well correlated to the stage of the disease as well as treatment response (Diehl et al. 2008).

1.2.9 Inflammatory Mediators and Immune Cells

A dual role inflammatory mediator such as intermediates of nitrogen, reactive oxygen species, prostaglandin E_2 suppresses as well as aggravates the inflammation. Anti-inflammatory process due to apoptosis and phagocytosis is initiated by phagocytes, macrophages and dendritic cells. Aggravation of the inflammatory process leads to the release of a certain component such as nitrogen species, cytokines, growth factors, oxygen reactive species from macrophages, neutrophils, B and T lymphocytes, myeloid-derived suppressor cells and mast cells. Instability on the genomic level, increase in cell survival, the proliferation of the tumour cells, angiogenesis, immunosuppression and invasiveness as well as metastasis of the tumour induced by certain components such as signal transducer and activator of transcription 3, hypoxia-inducible factor 1-alpha and nuclear factor kappa-light-chain-enhancer of activated B cells can be accounted due to aggravation of inflammatory response (Mittal et al. 2014; Dunn et al. 2002; Waldhauer and Steinle 2008; Shrihari et al. 2016).

Theories are stating that during the time of inflammatory conditions if there is low production of reactive oxygen species and reactive nitrogen species when oxidative stress is present leads to activation of the signalling pathway NF-kB inducing cell proliferation, role in angiogenesis, apoptosis and invasion as well as metastasis (Aivaliotis et al. 2012; Shrihari 2016). In the case of CD4 T helper cells (cluster of

differentiation 4), they activate B cells increasing the humoral response and is linked with enhancing the cytotoxic profile whereas CD8 cytotoxic T cells promote the elimination of the tumour cells via interferon gamma production (Korniluk et al. 2017).

Vascular endothelial growth factor A secreted by tumour inhibits activation of dendritic cells by the immune system leading to loss of antitumour response generated by the dendritic cells. Not only vascular endothelial growth factor A is responsible for the loss of this response but other factors such as increased lactic acid production, hypoxic condition, interleukin-10 and transforming growth factor beta – β can also be held responsible (Gabrilovich et al. 1996). The immune response can generate an antitumour effect but the recruitment of the immune cell in the deregulated environment leads to the growth of the tumour. The immune suppression is through myeloid-derived suppressor cells, immature dendritic cells, suppressive myeloid cells, tumour associated macrophages, immunosuppressive neutrophils (Gabrilovich et al. 2012).

1.2.10 Adipose Tissue

These tissues are categorised as loose connective tissues and their primary function id to store energy, insulation and cushioning. Recently there are studies which indicate their role in the expenditure of energy, lymphopoiesis, haematopoiesis, endocrine signalling (Trottier et al. 2012; Trujillo and Scherer 2006). The various constituents of adipose tissues are stem cells, pericytes, endothelial cells, fibroblasts, progenitor cells which help in tumour microenvironment signal transduction. It is considered an abundant source of vascular and stromal components consist of dendritic cells, eosinophils, mast cells, neutrophils, macrophages, lymphocytes (Bourin et al. 2013).

In case of obesity which has been linked with increased chances of poor prognosis in case of cancer and high risk in further development. Obesity and various types of cancer have been linked such as thyroid, gallbladder, pancreatic, oesophageal, kidney, liver, endometrial, colorectal, myeloma, non-hodgkin's lymphoma and postumour microenvironment nopausal breast cancer (Basen-Engquist and Chang 2011; Calle et al. 2003; Kyrgiou et al. 2017; Lauby-Secretan et al. 2016; Vainio et al. 2002). More adipocyte mass results in obesity condition and it has been observed that occurrence of metabolic disorders, alterations in various steroidal hormone levels as well as chronic inflammation is directly linked with obesity and these factors are contributors in tumour development (Iyengar et al. 2015; Howe et al. 2013; Rosen and Spiegelman 2014; Van Kruijsdijk et al. 2009).

Cancer associated with visceral organs and breasts arise from the adjacent adipose tissues and are considered as obesity-associated cancer. It has been noted when these cells undergo hypertrophic changes which result in the hypoxic surrounding or intracellular hypoxia. The generated hypoxic condition leads to increase in fibric content in the extracellular matrix due to overexpression of collagen I, collagen III and collagen VI (Sun et al. 2013; Trayhurn 2013). Further advancing of the rigidity of the adipose tissues leads to apoptosis as well as necrosis, rendering impairment

of the cellular function of adipocytes (Nishimura et al. 2008). There are pieces of evidence proving abnormal wound healing, activation of fibroblast process and myofibroblasts, as well as infiltration of macrophages, are associated with obesity, impacting the tumour microenvironment (Cozzo et al. 2011).

1.2.11 Neuroendocrine System Involvement

There is a correlation between the progression of cancer and chronic stress. In the biological system, the stress is mediated through the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. Activation of both results in the release of glucocorticoids and catecholamines. Epinephrine and norepinephrine are linked with catecholamine release, leads to binding on the immune cells, activation of hypothalamic pituitary adrenal axis results in the release of glucocorticoids as well as activation of the glucocorticoid receptor. Cell mediated and humoral immunity is rendered dysfunctional due to chronic stress, affecting proliferation of lymphocytes, suppression of immune cells such as T lymphocytes, macrophages, dendritic cells, natural killer cells.

Pro-inflammatory mediators such as tumour necrosis factor, interleukin-6 are generated on a larger scale, enhancing the proliferation of tumour. Other factors induced by stress such as vascular endothelial growth factor and matrix metalloproteases are considered as pro-tumoral factors, involved in metastasis as well as enhanced survival of tumour cells (Scanzano and Cosentino 2015). The correlation between the dysregulations of neuroendocrine system and progression of cancer due to its detrimental effect on immune cells is depicted in Table 1.5.

Table 1.5 Effects on certain components of the immune system due to dysregulation of the neuroendocrine system

Types of immune cells	Result of activation	References
Macrophages	Increases gene expression of MMP9, MMP2, Mmp12, ctsl proteases leading to the invasiveness of TAMs Transformation of macrophage from M1 to M2 Stimulation by catecholamines results in the release of cytokines from TAMs Tumour progression due to infiltration of macrophages which were stimulated by the adrenergic system	Black (2002), Szelenyi et al. (2000), Van Miert (2002), Elenkov and Chrousos (2002), Qin (2015) and Lamkin (2016)
T cell	Helps in further proliferation, apoptosis as well as altering the distributing pattern Tumour progression and immune suppression due to increment in activity of T cell regulation Suppresses the antitumor activity of CD8 + T	Frick et al. (2009), Andersen et al. (1998), Thornton et al. (2007), Nan et al. (2004), Schmidt et al. (2016) and Nissen et al. (2018)
Natural killer cells	Cytotoxicity profile and alteration in the expression of receptor membrane	Ah (2007), Varker (2007) and Lamkin (2008)

TAMs Tumour-associated macrophages, *MMP* Matrix metalloproteinases, *CD8+* Cluster of differentiation 8+, *ctsl* Cathepsin L

1.2.12 *Tumour Microenvironment Components*

Components	Functioning in normal cell	Functioning in tumour microenvironment	Dysregulating factors	Pharmacological intervention/potential biomarker	References
Pericytes	Sprouting of new blood vessels Regulates capillary blood flow by controlling vasoconstriction and vasodilation Component of the blood-brain barrier Prevent disarrangement of the blood-brain barrier in hypoxia state Show phagocytosis Activation of lymphocytes and thus having a role in controlling blood coagulation	Initiation of Angiogenesis Helps in the survival of the endothelial cells Metastasis of tumour Stabilizing the newly formed blood vessels inside the tumour In case of renal cancer and melanoma if pericytes coverage is more leads to the aggressiveness of the cancer Immunomodulation	Expression of PDGF β/PDGFR β, TGF- β and Ang-1/Tie2 signalling pathway leads to employing of pericytes to the neovessels inside the tumour	Tyrosine kinase inhibitor SU6668 for inhibiting PDGFR signalling Tyrosine kinase inhibitor SU5416 for inhibiting VEGFR-2 and PDGFRB signalling	Bergers and Song (2005), Ahmed and El-Badri (2017), Raza et al. (2010) and Ribeiro and Okamoto (2015)
Endothelial Cells	Line the blood vessels, capillaries and sinusoids Responsible for exchange of material between the surrounding tissue and bloodstream Can form hollow tubes which act as capillaries and thus helping in new blood vessel formation Prevent adhesion and aggregation of platelets Act as an anti-coagulant agent t-PA released by endothelial cells leads to fibrinolysis	Progression of cancer, metastasis, angiogenesis Resistance to chemotherapy Makers for tumour endothelial cells are lysyl oxidase and suprabasin	It is said that origination of tumour endothelial cell is from cancer stem cells, vascular progenitor cells or tumour cells. Another mechanism stating that normal endothelial cells through exosomes, apoptotic bodies or through phagocytosis intakes oncogenes released by tumour cells They can even originate after endothelial cells fuse with tumour cells They can even arise from genetic instability due to hypoxic environment, cytokines, growth factors or reactive oxygen species.		Alberts et al. (2002), Félétou (2011) and Ciesielski et al. (2020)

(continued)

Components	Functioning in normal cell	Functioning in tumour microenvironment	Dysregulating factors	Pharmacological intervention/potential biomarker	References
Cancer Associated Fibroblasts	Wound closure by the production of the extracellular matrix, homeostasis of tissues, generation of cell traction forces	They have a role in immunosuppression via the release of various cytokines and chemokines such as TGF-β, IL-13, IL-8, IL-6, CXCL-12, CXCL-14, VEGF which are responsible for inhibiting both adaptive and innate immunity They have the ability to synthesise extra cellular matrix, production of more structural components of ECM leads to increase in its density making infiltration of T-cells	Additional reprogramming of fibroblasts into cancer associated fibroblasts takes place for their activation They get transformed from normal fibroblasts to cancer associated fibroblasts via chemical messengers secreted by tumour cells as well as immune cells such as various growth factors, cytokines, metabolites.	Controlling via immunotherapy, deleting FAP by genetic alterations initiating a mechanism leading to the infiltration of CD8+ T cells in various cancer models Usage of FAP DNA vaccine initiates immune response by CD4 and CD 8 T-cells In mouse models, anti-tumour CAR T along with anti-FAP cells has shown increment in tumour immunity Using vaccine SynCon FAP DNA with other anti-tumour vaccines can show benefits in developing immunity In case of pancreatic cancer, administration of FAP-specific CAR T cells can show anti-tumour effects Effect against metastases was shown in breast cancer mouse models when nanoparticles having the power to target stromal α-SMA^{+} were combined with docetaxel Using endothelial cell precursor bevacizumab for targeting CAFs is an ongoing study and presently in a phase-3 clinical trial It has proved that vitamin A and D have the capacity to transform activated CAFs to quiescent ones	Harper and Sainson (2014), Kalluri (2016), Roy and Bera (2016) and Liu et al. (2019a, b, c)

Tumour Associated Macrophages	Wound healing, are immunocytes, maintain tissue homeostasis, maintain the natural defence system of the body	The TAMs lack phagocytosis and help in metastasis of the tumour, they release IL-6, IL-10 and IL-8 helps in the development of cancer, chemoresistance, angiogenesis, they help in formation of the pre-metastatic environment	It has been studied that TAMs originate from M2 macrophages, and these get activated into TAMs through secretory molecules by secreted lipopolysaccharides from the outer membrane of the bacteria and various immune cells such as GM-CSF, INF-γ	Discussed in Tables 1.2, 1.3 and 1.4	Zhou et al. (2020)and Lin et al. (2019)
Circulating tumour cells	NA	Cell sheds, role in metastasis and seeding of cancer, resistance to chemotherapy as well as apoptosis		As a biomarker, describe mutations and genetic alteration as well as levels can be co-related with advancement of tumour, effectiveness of the tumour microenvironment can be checked from these, drug targets can be checked from detecting their origin These are detected via specific expressive molecules such as cytokeratin and EpCAM which are present on the surface of the cells using cell search system, this is used in diagnosis of breast cancer Mutation on CTCs such as CK19 and TP53 can be detected for outcome of the breast cancer In case of prostate cancer its levels along with prostate specific antigen can be checked for better diagnosis Disease progression state in case of lung cancer, colon cancer, bladder cancer, hepatocellular cancer can easily be correlated	Potdar and Lotey (2015)

(continued)

Components	Functioning in normal cell	Functioning in tumour microenvironment	Dysregulating factors	Pharmacological intervention/potential biomarker	References
Exosomes	Intercellular communication via the transfer of proteins, lipids, RNA, DNA, miRNA and mRNA, removal of waste from the cell, antigen presentation, transducing of signal	Released by cancer cells and aids in transferring the oncogenic material to other non-cancerous cells Tumour development and growth, drug resistance, metastasis, tumorigenesis and angiogenesis, the polarization of macrophages into TAM, induces apoptosis	NA	These can be used in target drug delivery, siRNA, recombinant proteins, anti-inflammatory drugs and antagomirs are being delivered Exosomes derived from dendritic cells are being studied for immunotherapy for cancer In case of MDA MB-231 cancer cell lines, exosomes were studied for carrying staphylococcal enterotoxin B which further activates T-cells	Crenshaw et al. (2018), Othman et al. (2019) and Zhang et al. (2019a, b, c)
Circulating Tumour DNA	NA	Released by cancer cells mainly under hypoxic condition Contains genetic information about the tumour	NA	It has potential to be used as a biomarker for early detection and management of cancer at various stages of the disease in colorectal, breast and lung cancer BRAF positive breast cancer can be diagnosed by identifying KRAS p. G13D and BRAF p.V600R mutations present on the ctDNA Acquired resistance can also be studied and evaluation of tumour microenvironment response	Calapre et al. (2019) and Cescon et al. (2020)

TGF- β Transforming growth factor beta, *PDGFR β* Platelet derived growth factor receptor beta, *PDGF β* Platelet derived growth factor beta, VEGFR- Vascular endothelial growth factor receptor, *IL* Interleukin, *CD8+T* Cluster of differentiation 8, *ECM* Extracellular matrix, *FAP* Fibroblast activation protein, *IFNγ* Interferon gamma, *GM-SCF* Granulocyte-macrophage colony-stimulating factor, *EPCAM* Epithelial cellular adhesion molecule, *CK19* Cytokeratin-19, *TP53* Tumour protein-53, *KRAS* Kirsten rat sarcoma virus, *ctDNA* Circulating tumor DNA, *miRNA* micro Ribonucleic acid, *mRNA* messenger Ribonucleic acid, *CAFs* Cancer associated fibroblasts, *CTCs* Circulating Tumour Cells, *α-SMA+* α-smooth muscle actin, *SynCon FAP* Synthetic consensus Fibroblast activation protein, *CAR-Tcells* Chimeric antigen receptor T-cell, *CXCL* C-X-C motif chemokine ligand, *t-PA* Tissue plasminogen activator

1.3 Abnormal Physiological Conditions of the Tumour Microenvironment

1.3.1 pH

The extracellular as well as intracellular pH has an important role to play in the development of cancer and is also used as a target to kill the viable cancer cells; extracellular pH has a decisive role to play in proliferation and expression of T-cell and interleukin-2 receptor (Carswell and Papoutsakis 2000). The extracellular pH of the cancer cell is on the lower scale when compared to non-cancerous cell ranging between 6.4 and 7.0. In previous studies conducted it was observed that there is more lactic acid production as compared to glucose which might be the reason behind the variability of pH in cancer and normal cells (Ashby 1966; Warburg et al. 1927).

Till the 1980s it was assumed that both intracellular, as well as the extracellular environment of the cancer cell, is acidic but with technology advancement, testing was done using positron emission tomography, optical imaging, magnetic resonance imaging and nuclear magnetic imaging sensitive to pH, it was observed that intracellular pH slightly alkaline to neutral in cancer cells which are alike to normal cells (Griffiths 1991; Oberhaensli et al. 1986). Cancer-specific imaging is developing nowadays, the acidic conditions of the tumour have helped not only in diagnostic are but also in therapeutics by designing of target-specific medicines and glycolytic rate of the extracellular tumour environment has the key role behind the acidic pH of the extracellular environment.

For detecting the pH, invasive techniques such as invasive microelectrodes can be used and in area of non-invasive chemical exchange saturation transfer magnetic resonance imaging, *in vivo* magnetic resonance spectroscopy, fluorescence imaging as well as positron emission tomography has been developed (Anderson et al. 2016; Zhang et al. 2010; Toy et al. 2014). After evaluating various studies, it can be concluded well that not only glycolytic rate can be made responsible for acidic tumour microenvironment but also low blood perfusion, inflammation, and hypoxia too (Vaupel et al. 1989; Gatenby and Gillies 2004; Cairns et al. 2006; Yang et al. 2012).

1.3.2 Angiogenesis

It is generally agreed that angiogenesis has a greater role to play in the development of the tumour, as it refers to the pullulating and extension of freshly created vessels (Yang et al. 2013). It helps in providing nutrition to the growing tumour cells and helps in the removal of waste and this may act to future spread to the distal parts. Formation of new blood vessels occurs around the tumour cells due to hypoxic environment and further angiogenesis help in the commencement of hypoxia-inducible factor, also increasing the transcription rate of platelet-derived growth

factor, angiopoietin 2, angiopoietin 1 and vascular endothelial growth factor (Yang et al. 2013). Initiation of angiogenesis begins with the degradation of the basement membrane and next step is endothelial cell monolayer disruption, the endothelial cells invade in the neighbouring stroma and lead to reorganization of the structure as well as tube formation (Carmeliet 2000). Notable involvement of pericytes is through secretion of platelet derived growth factor BB monomer secreted from the endothelial tip cells which attract the pericytes. Their attachment at the newly formed vessels helps in giving mechanical support and further fabrication of vascular endothelial growth factor (Carmeliet 2000; Gerhardt et al. 2003; Franco et al. 2011).

1.3.2.1 Vascular Endothelial Growth Factor Family

There are five members of vascular endothelial growth factor as discussed previously, vascular endothelial growth factor A, B, C, D, E; platelet-derived growth factor activating three receptors of tyrosine kinases named vascular endothelial growth factor R-1, R-2 and R-3. The role of vascular endothelial growth factor A in angiogenesis is the promotion of migration and proliferation of the endothelial cells, increasing the vascular permeability as well as having a key role in tube formation. Vascular endothelial growth factor B has been expressed in heart and skeletal muscles and considered as a growth factor for endothelial cells but its importance in angiogenesis is not yet clearly proven. Vascular endothelial growth factors D and C have a crucial role to play in lymph angiogenesis and act as lead in lymphatic metastasis (Ferrara 2002; Olofsson et al. 1996; Oklu et al. 2010; Adini et al. 2002).

In case of vascular endothelial growth factor receptors, the R-1 leads to chemotaxis of macrophages, from bone marrow it recruits endothelial progenitor cells into the blood vessels of the tumour, development of embryonic vessels, haematopoiesis, the R-2 stimulates angiogenesis by vascular endothelial growth factor, the R-3 has a binding site for vascular endothelial growth factor C and D which leads to the formation of lymphatic vessels not only in tumours but also in normal cells (Ferrara 2004; Korpanty et al. 2010; He et al. 2005).

1.3.2.2 Fibroblast Growth Factor and Fibroblasts

There are two types of fibroblast growth factors; fibroblast growth factors 1 and fibroblast growth factors 2. The role of the fibroblast growth factors is to maintain the functionality of the endothelial cells. These two components promote migration and proliferation of endothelial cells as well as an initiation for angiogenesis (Murakami et al. 2008). Fibroblasts are also highlighted as the initiator of angiogenesis and believed as suppressors for contact inhibition process due to secretion of transforming growth factor β, interleukin-6 and tumor necrosis factor α (Kirk et al. 1981; Paland et al. 2009; Degeorges et al. 1996). In cancer they promote growth and

invasion, the normal fibroblasts are converted to cancer ones through the action of micro RNAs such as micro-RNA-155 in case of pancreatic cancer and micro RNA-214 in case of ovarian cancer (Albrengues et al. 2015; Pang et al. 2015; Mitra et al. 2012).

1.3.2.3 Notch Signalling Pathway

This pathway controls the differentiation of various cells and has a key role to play in the cell-to-cell communication. It activates the dormant tumours and acts as a key element in the angiogenesis process. Endothelium specific notch ligand delta-like 4 is activated by vascular endothelial growth factor A, this moiety activates the notch signalling pathway in the adjacent cells leading to dorsal sprouting inhibition in endothelial tubes. In the case of tumour cells, the activation of the pathway in the stromal cells leads to increased functionality of the vasculature (Li et al. 2007a, b). If this pathway is inhibited it can show a reduction in branching as well as sprouting for the newly formed vessels. Though vascularity may increase but the tumour cells are hypoxic and poorly perfused (Noguera-Troise et al. 2006).

1.3.2.4 Transforming Growth Factor-β

It is present in three forms transforming growth factor β2, transforming growth factor β3 and transforming growth factor β1 (transforming growth factor beta). It is labelled as a proangiogenic element and has caused the arrest of growth as well as apoptosis in endothelial cells. It leads to activation of fibroblast growth factors 2 which further stimulates vascular endothelial growth factor and using the vascular endothelial growth factor R2, the mitogen-activated protein kinase pathway is activated (Barbara et al. 1999; Ferrari et al. 2009). In normal cells, due to tissue injury, this element is released into the normal cell microenvironment from the stromal component and the platelets but in case of cancer its presence in the initial stages have been accounted for preventing the malignant transformation, it's been proved as a stepping block for tumour progression (Xie et al. 2002). Tables 1.6, 1.7 and 1.8 represents various anti-angiogenesis moieties used and in pipeline.

1.3.3 Extracellular Matrix

The extracellular matrix is created by some stromal elements, cancer cells and cancer-associated fibroblasts. It is responsible for communication with other cancer cells via mechanical and molecular interactions (Lu et al. 2012; Tung et al. 2015). Composition of the extracellular matrix contains laminins, fibronectin, collagen, proteoglycans, and polysaccharides if it is synthesized by cancer-associated fibroblasts. If it is synthesized by stromal elements, then the composition is of

Table 1.6 Anti-angiogenesis receptor tyrosine kinase inhibitor under clinical trials (clinicaltrials.gov)

Drug	Indication	Clinic trial phase	ClinicalTrials.gov identifier
Sorafenib	Renal cell carcinoma Hepatocellular carcinoma Differentiated thyroid cancer	Phase-3	NCT00073307 NCT00105443 NCT00984282
Pazopanib	Renal cell carcinoma Soft tissue sarcoma	Phase-3	NCT00720941 NCT00753688
Sunitinib	Gastrointestinal stromal tumour Renal cell carcinoma Pancreatic Neuroendocrine Tumours	Phase-3	NCT00075218 NCT00098657 NCT00428597
Nintedanib	Idiopathic pulmonary fibrosis Non-small cell lung carcinoma	Phase-2 Phase-3	NCT00514683 NCT00805194
Fruquintinib	Colorectal cancer	Phase-3	NCT02314819
Anlotinib	Non-small cell lung carcinoma	Phase-3	NCT02388919
Apatinib	Gastric cancer	Phase-3	NCT01512745

Table 1.7 Drugs in use to target angiogenesis

Drugs	Mechanism of action	Cancer type	References
Cetuximab	EGFR targeting	Gastric cancer Colorectal cancer Human papillomavirus-associated head and neck cancer	Sihver et al. (2014)
Aflibercept	VEGFR1 and VEGFR2 targeting	Prostate cancer Colorectal cancer Non-small cell and small cell lung carcinoma	Mittal et al. (2014)
Endostatin	Target angiogenesis protein	Non-small cell lung carcinoma Colorectal cancer Nasopharyngeal carcinoma Melanoma	Cui et al. (2013)
Bevacizumab	Target VEGF	Renal cancer Breast cancer Colorectal cancer Ovarian cancer Prostate cancer Glioblastoma Non-small cell lung cancer	Heinemann et al. (2014)
Erlotinib	EGFR tyrosine kinase inhibitor	Hepatocellular carcinoma Pancreatic carcinoma	Dutton et al. (2014)
Dasatinib	Targets multi-BCR/ABL	Melanoma Adenoid cystic carcinoma Chronic myeloid leukaemia	Michaelson et al. (2014)

EGFR Epidermal growth factor receptor, *VEGFR* Vascular endothelial growth factor, *BCR/ABL* Breakpoint cluster region/Abelson

Table 1.8 Anti-angiogenic moieties in gene therapy

Anti-angiogenic moieties	Mechanism	References
Angiostatin	Endothelial cell migration and proliferation is inhibited	O'Reilly et al. (1994)
Arresten	$\alpha 1\beta 1$ integrin-binding property thus inhibiting angiogenesis	Li et al. (2018a, b)
Antiangiogenic metargidin peptide	α5β1 and αVβ3 integrin-binding property thus inhibiting angiogenesis	Spanggaard et al. (2013)
Endostatin	α5β1 integrin-binding property thus inhibiting angiogenesis	Kuo et al. (2015)

proteoglycans and fibrillar collagen (Santi et al. 2018; Socovich and Naba 2019). The physical state of the extracellular matrix is highly hydrated and charged giving tensile strength to the cancer tissue.

It has been well explained in various studies that the tumour cells are very stiff as compared to the neighbouring normal cells. Tumour-associated macrophages, cancer cells and cancer-associated fibroblasts work together in the formation of lysyl oxidase which is a cross-linking enzyme particularly lysyl oxidase-like 2 and lysyl oxidase 1, also the formation of collagen, transglutaminase family such as transglutaminase-2. The transglutaminase and lysyl oxidase redirect the elastin and collagen fibres which are further cross-linked by these two elements leading to rigidity enabling more cell migration (Balkwill et al. 2012; Levental et al. 2009). For further metastasis, the cancer cells need to cross extracellular matrix and for that various elements are secreted such as matrix metallopeptidase family proteolytic enzymes.

The density of the extracellular matrix is more as compared to any other medium, for crossing and invading matricryptins are released by proteases controlling the further progression (Brassart-Pasco et al. 2020). The density of the extracellular matrix has importance in the transport of the drug into the tumour tissues, high-density leads to a low distribution of the drug, the density of the microvessels are low in case of solid tumours having characteristic high levels of cross-linking in the extracellular matrix resulting in metabolic stress and hypoxia, relating to the hypoxia effect it can cause resistance to radiotherapy as well as an anti-cancer therapy (Moeller and Dewhirst 2004; Doublier et al. 2012; Jain 2014; Horsman and Overgaard 2016; Graham and Unger 2018).

1.3.4 Hypoxia

Hypoxia is viewed as the potential target for various developed and developing anti-cancer therapies because it is the key to the progression of metastasis, angiogenesis, drug resistance, heterogeneity of tumours and genetic instability. The characteristic features of hypoxia are low oxygen levels, depletion of nutrients, increased levels of

lactate and adenosine, extracellular acidosis (Multhoff and Vaupel 2020; Tamura et al. 2020). In the state of hypoxia, there is upregulation of hypoxia-inducible factors and transcriptional factors which directly control angiogenesis, genes of the cell cycles and metabolic cycles (Krock et al. 2011). The signalling in hypoxia is controlled by a heterodimer complex formed from cytoplasmic oxygen-dependent hypoxia-inducible factor components such as hypoxia-inducible factor -2α, 3α, 1α and 1β which are stabilized by hypoxia-inducible factor-prolyl hydroxylase domain enzymes such as prolyl-hydroxylase 1, prolyl-hydroxylase 2 and prolyl-hydroxylase 3, these are iron and oxygen-dependent enzymes.

In normal cellular conditions, hypoxia-inducible factor-α subunit is hydroxylated by the prolyl-hydroxylase enzymes on two propyl residues of this moiety leading to binding of Von Hippel – Lindau tumour-suppressor protein further its ubiquitination and degradation by proteasomes. When there are abnormal conditions leading to hypoxia which further advances suppression of prolyl-hydroxylase enzymes, the subunit of hypoxia-inducible factor-α gets translocated into nucleus and complexing with hypoxia-inducible factor-1β. The formed heterodimer that is hypoxia-inducible factor-α: hypoxia-inducible factor-1β transcription factor complex, which further works on target genes at the hypoxia-responsive elements leading to upregulation of transcription (Sormendi and Wielockx 2018; Koh and Powis 2012).

The metabolic changes due to hypoxia lead to the production of ATP through glycolytic pathway rather than the production of ATP through the tricarboxylic acid which generate 36 molecules of ATPs but in this case, there is a generation of 2 ATP molecules from a single glucose moiety. The production of ATP is faster as compared to tricarboxylic acid cycle, this conservation of energy due to differently reprogramming is for the formation of new moieties of amino acids, lipids, nucleic acids in support of increasing growth of tumour cells. The glycolysis is responsible for acidosis in the tumour microenvironment, high levels of lactate synthesis which increases the invasiveness of cancer and alterations in the tumour stroma (Shestov et al. 2014; DeBerardinis et al. 2008; Levine and Puzio-Kuter 2010; Cairns et al. 2011; Koppenol et al. 2011).

The hypoxia-induced immune suppression is through dendritic cells, macrophages, tumour cells and myeloid-derived suppressor cells show an expression of PD-L1 which is a ligand for immune checkpoints resulting in inhibition of T cell cytolytic activity (Noman et al. 2014). It induces fibrosis through macrophages, interfering with the antitumour property of the immune system, desmoplasia and vessel formation induced by hypoxia leads to vasculature compression and leakiness (Noman et al. 2015; Doedens et al. 2010; Motz and Coukos 2013; Chen et al. 2014). Table 1.9 summarizes various agents targeting the hypoxic state of tumour microenvironment which are under pipeline.

Table 1.9 Agents under investigation for targeting the hypoxic state (clinicaltrials.gov)

Agents	Cancer type	Clinical trial phase	ClinicalTrials.gov identifier
PR-104	Acute myelogenous leukemia Acute lymphocytic leukemia	Phase-1 Phase-2	NCT01037556
AQ4N	Solid malignancies Non-Hodgkin's lymphoma	Phase-1	NCT00090727
TH-4000	Non-small cell lung cancer Squamous cell carcinoma	Phase-2	NCT02454842 NCT02449681
Tirapazamine	Cervical cancer Head and neck cancer Lung Cancer	Phase-3	NCT00262821 NCT00174837 NCT00017459
Apaziquone	Bladder Cancer	Phase-3	NCT00598806
Evofosfamide	Pancreatic Cancer	Phase-3	NCT01746979

PET/CT Positron Emission Tomography/Computed Tomography

1.4 Conclusion

Tumour micro environment and unravelling of its milieu has emerged as a reliable tool to gauge the development and progression of tumors. The understanding has given a direction to the researchers as well as clinicians for better amelioration of this difficulty to conquer malady. Various techniques have been developed to gain control over ever-changing microenvironment and control the rate of tumour progression described in Tables 1.10 and 1.11. The oncolytic viruses are in much spotlight as immunotherapy, the oncolytic herpes simplex virus-1 has been approved by food and drug administration in the United States of America for the treatment of melanoma, certain studies have concluded that its replication increases when it is present in microenvironment having the hypoxic condition, one of the studies indicated its role in breast cancer resistant cells.

Using rapamycin for inhibition of the mammalian target of rapamycin pathway has shown evidence of increasing the immunosuppressive property on the immune cells, lowering down the infiltration of cluster of differentiation 8+ T cell inside the tumour (Vito et al. 2020). If we discuss paclitaxel, which works by decompressing the vessels reducing the hypoxic environment, cancer-associated fibroblasts and collagen in the extracellular matrix are also reduced by this drug. Nanoformulated paclitaxel has shown increased survival rate in pancreatic cancer patients when compared to gemcitabine therapy. Losartan in combination with angiotensin system inhibitors can lower down the production of transforming growth factor-β1 and connective tissue growth factors, increasing oxygen supply in the microvasculature, helping in drug delivery with improving circulation, another effect of losartan is blocking the recruitment of monocytes (Martin et al. 2016). The pathways for vascular endothelial growth factor can be inhibited by using multitargeted tyrosine kinase inhibitors and angiogenesis inhibitors (Carmeliet and Jain 2011).

Table 1.10 Novel hybrid interventions in clinical trials related to tumour microenvironment (clinicaltrials.gov)

Title of the study	Intervention	Status	Clinical Trials.gov identifier
Effects of MK-3475 (Pembrolizumab) on the Breast Tumor Microenvironment in Triple-Negative Breast Cancer	Merck 3475 Pembrolizumab	Phase-1	NCT02977468
Imaging of tumour microenvironment in patients with oropharyngeal head and neck squamous cell carcinoma using RGD PET/CT imaging	Gallium-68-DOTA-(RGD)2 PET/CT scan and a CT perfusion scan	Phase-2	NCT04222543
Modulation of the tumour microenvironment using either vascular disrupting agents or STAT3 inhibition in order to synergise with PD1 inhibition in microsatellite stable, refractory colorectal cancer	Nivolumab and BBI608/nivolumab and BNC105	Phase-2	NCT03647839
A study of ALKS 4230 on the tumor microenvironment	ALKS 4230	Phase-2	NCT04592653
Targeting the tumor microenvironment in R/M SCCHN	Combination of cyclophosphamide, radiotherapy and avelumab	Phase-1/2	NCT03844763
Preconditioning of tumor, tumor microenvironment and the immune system to immunotherapy	Dacarbazine	Phase-2	NCT04225390
Modulation of the tumor microenvironment by abemaciclib in operable HPV-negative head and neck cancer	Abemaciclib/ abemaciclib + nivolumab	Phase-2	NCT04169074

R/M SCCHN Recurrent and/or metastatic squamous cell carcinoma of the head and neck, *HPV* Human papillomavirus, *STAT3* Signal transducer and activator of transcription 3, *DOTA-(RGD)2 PET/CT* Derivatives of Octreotide Dicarba-Analogs-Arginine-glycine-aspartic acid positron emission tomography/computed tomography, *CT* computed tomography

As discussed in the section of the extracellular matrix that its density affects the drug uptake, thus targeting extracellular matrix would be helpful in the uptake of chemotherapy as well as increasing its effectiveness (Provenzano et al. 2012). Recent study approaches are to combine immunotherapeutic agent with antiangiogenic, an example is from a phase-3 clinical trial where bevacizumab (an antiangiogenesis agent) given with atezolizumab in case of non-small cell lung cancer improved survival rate in these patients. In the case of patients suffering from renal carcinoma, axitinib and avelumab is given as first-line treatment option and is in the phase-3 clinical trial (Datta et al. 2019). This is consistent with the previously drawn conclusions from various studies that radiotherapy and chemotherapy are not designed or they work just by targeting the malignant or tumour cells, they have an impact on tumour microenvironment and the success, as well as the failure of the treatment, depends upon the normalization or resistance of tumour microenvironment from the various treatment options available.

Table 1.11 Putative nanotools for targeting the components and physiological aspects of tumour microenvironment

Target	Nanoparticle	References
Extra cellular matrix	Extracellular matrix can be degraded leading to better penetration of drug into the cell by using PEGylated nanoparticles consisting of hyaluronidase Tumour growth suppression due to inhibition of lysyl oxidase by polymer nanoparticles consisting of anti-lysyl oxidase antibodies	Maldonado et al. (2015) and Gkretsi et al. (2015)
Angiogenesis	Using PLGA nanoparticles containing doxorubicin or combrestatin, angiogenesis can be inhibited Polymer nanoparticles carrying lodamin have also shown inhibitory effect on angiogenesis	Kishimoto et al. (2016) and Clemente-Casares et al. (2016)
Tumour Associated Macrophages	Paclitaxel loaded PLGA nanoparticles reducing the growth of tumour	Cao et al. (2018)
Exosomes	Exosomes sheathed doxorubicin loaded Luminescent porous silicon nanoparticles for targeting tumour microenvironment	Roma-Rodrigues et al. (2017)
Pericytes	Peptide conjugated liposomes loaded with doxorubicin Peptide conjugated liposomes for delivery of docetaxel	Kang and Shin (2016)
Endothelial cells	Liposomes carrying doxorubicin (various nanoparticles used to target endothelial cells are discussed in Table 1.1)	Sakurai et al. (2019)
Cancer Associated Fibroblasts	Lipid bilayer modified nanoparticles Carboxymethyl Cellulose based nanoparticles	Liu et al. (2019a, b, c)
Hypoxia	Ce6-PEG-CDDP-metformin nano drug delivery system to target hypoxia	Song et al. (2020)
pH	PEGylated hyaluronic acid carrying doxorubicin for delivery of drug in abnormal pH condition	Zhang et al. (2020)

PLGA Poly D,L-lactic-co-glycolic acid, *PEG* Polyethylene glycol, *Ce6-PEG-CDDP* chlorin e6-Polyethylene glycol-co-deliver of cisplatin

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Chapter 2
Methods to Formulate Polymeric Nanoparticles

Santwana Padhi, Sweta Priyadarshini Pradhan, and Anindita Behera

2.1 Introduction

Owing to the complexity of some disorders and the toxicity associated with some drugs, new drug delivery routes are becoming extremely relevant. A drug-delivery system is a formulation or device that enables active ingredients to be introduced into the body in order to increase their effectiveness and safety, by monitoring the drug load, time, and release of the drug at the site of action, navigating biologic membranes to reach the therapeutic target (Bruschi 2021).

Drug delivery systems are becoming more refined with time relying on a better controlled release, retaining therapeutic effectiveness, and the active ingredient targeting to the particular site of action, preventing systemic release of the active drug (Wang et al. 2016). Nanotechnology is gaining momentum in this regard, as it has the ability to address some of the issues associated with the aforementioned conventional administration paths (Macedo et al. 2020).

The formulation of polymeric nanoparticles plays a key role in providing therapeutic efficacy and could be improved to boost drug bioavailability, either by enhancing absorption through increased solubility or by enabling transport through biologic membranes (Jain 2020). The composition of the nanoparticulate system may also be adjusted to monitor and sustain drug release at therapeutic levels as well.

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Investigations into the use of different materials as nanocarrier components such as polymers and surfactants are an important prerequisite for determining the physicochemical attributes and also improving the applicability of these systems. The judicious choice of the formulation method and the associated parameters also influences the pharmacokinetics and biodistribution parameters which influences the drug retention pattern in the body (Begines et al. 2020)**.** Inappropriate understanding about nanotoxicity is a major concern, and it certainly necessitates further study to boost effectiveness while also increasing safety, allowing for safer practical application of these medicines. Hence it can be stated that a careful design of Polymeric nanoparticles can be beneficial in addressing the issues associated with their usage.

The present chapter presents an overview of the different formulation methodologies for the fabrication of polymeric nanoparticles. Different conventional and advanced techniques are discussed briefly.

2.2 Methods for Preparation of Polymeric Nanoparticle

Preparation of polymeric nanoparticles include two steps, the first step involves preparation of an emulsion of drugs to be encapsulated as polymeric nanoparticles followed by the preparation of nanoparticles in the second step (Vauthier and Bouchemal 2009). Sometimes, the nanoparticles are produced in a single step during emulsion preparation. Following section discuss the different conventional and advanced techniques for preparation of polymeric nanoparticles (Table 2.1) (Vauthier and Bouchemal 2009; Zielińska et al. 2020; Rao and Geckeler 2011).

2.2.1 Nanoprecipitation

Nanoprecipitation method also known as solvent displacement method requires two immiscible solvents for its separation process. The internal phase contains a polymer which is mainly dissolved in organic solvents such as acetonitrile or acetone (Araújo et al. 2009; Cañadas et al. 2016; Sánchez-López et al. 2017). Because of its immiscibility properties, it is easily removed by the process of evaporation. The principle of formulation relies on interfacial deposition of the polymer after the displacement of organic solvent from lipophilic phase to aqueous phase. This method is used majorly for drug entrapment of hydrophobic drugs, but it has also been adapted for hydrophilic drugs as well. Polymer as well as the drug is suitably dissolved in polar, water miscible solvents. The resultant solution is thereby poured in a controlled manner under stirring into an aqueous solution containing a surfactant. The resultant nanoparticles are formed rapidly by rapid solvent diffusion method. Finally, the solvent is removed under reduced pressure and the polymer

Table 2.1 Conventional and advanced methods for preparation of polymeric nanoparticles

Methods	Polymers	Drug	References
Nanoprecipitation	Poly(L-lactide)-*b*-polyethylene glycol	Erlotinib and doxorubicin	Zhou et al. (2017)
	Poly ethylene glycol- poly (lactic-co-glycolic acid)	Doxorubicin	Almoustafa et al. (2021)
	Poly (lactic-co-glycolic acid)	Ibuprofen	Sahin et al. (2017)
Solvent diffusion	Poly(2-hydroxyethyl methacrylate)	Curcumin	Kumar et al. (2014)
	Chitosan coated poly-dl-lactic-coglycolic acid	Albendazole	Kang et al. (2017)
Emulsification solvent evaporation	Poly (lactic-co-glycolic acid)	Curcumin	Chen et al. (2014)
		Hesperitin	Ersoz et al. (2019)
		Epirubicin	Esim et al. (2020)
	Poly (ε-caprolactone)	Ellagic acid	Mady and Shaker (2017)
	Poly(ethyleneglycol)-*block*-poly(ε-caprolactone)	Nimesulide	Sengel-Turk et al. (2017)
Supercritical fluid expansion	Hydroxypropyl methylcellulose, poly (vinyl alcohol), chitosan and polyethylene glycol	Sunitinib malate	Razmimanesh et al. (2021)
Interfacial polymer deposition	Poly (D,L-lactide-coglycolide acid)	Lomustine	Mehrotra and Pandit (2015)
Salting out	Human serum albumin	Cabazitaxel	Qu et al. (2016)
Complex coaservation	Chitosan and gelatin	5-flourouracil and epigallocatechin gallate	Wang et al. (2019)
	Bovine serum albumin and poly-D-lysine	Curcumin	Maldonado et al. (2017)
Polymerization method	Casein/gum tragacanth	β-carotene	Jain et al. (2016)
Ionotropic gelation method	Sodium alginate	Doxorubicin	Khalid et al. (2018)
Spray drying	Phytic acid-chitosan	Methotrexate	Ciro et al. (2020)
Phase separation techniques	Chitosan	Lomustine	Mehrotra et al. (2010)
	β-carotene loaded nanoparticle of zein	Methotrexate	Jain et al. (2018)

precipitates resulting in the formation of nanoparticles (Fessi et al. 1989; Dinarvand et al. 2011). Surfactants are used to maintain the stability of the colloidal suspension. The obtained nanoparticles possess narrow size distribution.

Mainly water insoluble drugs have been incorporated into nanoparticles employing the nanoprecipitation technique with typical drug content values. However, this technique suffers the drawback of a low incorporation efficiency of highly water-soluble drugs owing to rapid migration and therefore loss of drug into the aqueous phase.

2.2.2 Solvent Diffusion

In the present method, the water-soluble solvent containing polymer and drug along with the water insoluble organic solvent like dichloromethane or chloroform is used as an oil phase (Niwa et al. 1993). The internal phase involves the use of partial water-miscible organic solvents such as ethyl acetate or benzyl alcohol which previously hydrolyzes with water molecules in order to warrant a primary thermodynamic stability of both the phases at room temperature (Souto et al. 2012). The successive dilution with large expanse of water persuades solvent diffusion from the dispersed droplets into the external phase, resulting in the formation of colloidal particles. Due to the spontaneous diffusion of water-soluble solvent (acetone or methanol), an interfacial turbulence is created between two phases leading to the formation of smaller particles. This method produces nanoparticles with a rough morphology, according to prior studies. Despite the fact that it necessitates a large amount of aqueous phase, which is usually excluded from colloidal dispersion, and given the possibility of hydrophilic drug diffusion into the aqueous phase, this method is still widely used for the synthesis of polymeric nanoparticle (Quintanar-Guerrero et al. 1998; Vasile 2018).

2.2.3 Emulsification Solvent Evaporation

It was the first and most widely used method for the preparation of polymeric nanoparticles. The preparation of nanoparticles by this classical method follows the widely used protocol of dissolving the polymer in a water immiscible, volatile organic solvent which is thereby emulsified with an aqueous phase to effectively stabilize the system. The organic solvent is then evaporated inducing the rapid formation of polymeric particles from the organic phase droplets. The solvent evaporation method has been widely employed to formulate particles from a range of polymeric materials, particularly poly (lactic acid) and poly (lactic-co-glycolic acid) (Song et al. 1997). This technique has been widely used for encapsulating hydrophobic drugs and the results for incorporation of hydrophilic drugs have been poor. A modification of this procedure has led to the development of formulation protocols which is favored for encapsulating hydrophilic compounds and proteins.

(a) Single emulsion technique

This method is based upon the emulsification of an organic solution containing the polymer and the aqueous phase containing the drug, followed by the evaporation of the organic solvent (Rosca et al. 2004). Different surfactants such as polyvinylalcohol, sodium dodecyl sulfate, pluronic F68 dissolved in the aqueous phase are used in the process of formulation. The size reduction of the emulsion droplet is done by sonication. The evaporation step is required to eliminate the organic solvent present in the organic phase. This leads to subsequent precipitation of the polymer as nanoparticles with an appreciable diameter in the nanometer range.

(b) Double emulsion technique

The single emulsion method suffers from the disadvantage of poor entrapment efficiency of hydrophilic drugs hence double emulsification method is applied (Iqbal et al. 2015). This method involves preparation of water in oil primary emulsion between the internal aqueous phase and the organic phase. The internal aqueous phase containing the drug is dispersed into the organic phase containing polymer dissolved in organic solvent under sonication. This water in oil emulsion is then slowly dropped into the external aqueous phase containing a suitable concentration of surfactant under sonication and a water in oil in water emulsion is formed (Zambaux et al. 1998). The secondary emulsion is then kept for stirring for 6 h to evaporate the organic solvent. The resulting particles are washed thrice with water by centrifugation at 13000 rpm for 20 min. This is done to remove excess of surfactants. Every time the supernatant is removed, fresh water is added and further centrifuged. The pellet obtained after final centrifugation is then redispersed in 2–3 ml of water. The dispersion is then kept for lyophilization to get the nanoparticles (Vandervoort and Ludwig 2002).

It has been reported previously that water soluble drugs are efficiently encapsulated using this method with a good encapsulation efficiency and a good release rate profile (Panigrahi et al. 2021). The main problem with trying to encapsulate a hydrophilic molecule like a protein or peptide-drug is the rapid diffusion of the molecule into the outer aqueous phase during the emulsification.

Several parameters can influence the properties of the particles produced these parameters include:

- nature of polymer
- polymer molecular weight
- nature of organic phase
- polymer concentration in the organic phase
- volume ratio of organic: aqueous phase
- nature of surfactant
- surfactant concentration and molecular weight
- stirring speed.

The main drawback is the removal of organic solvents and the excipients associated with the nanoparticles post production. Any residual organic solvents may have toxicological implications. In addition, the excess surfactant used is also difficult to

remove. Another limitation is that surfactant must be present for preparation of nanoparticles in order to stabilize the system. Particles therefore cannot be produced naked and then post adsorbed with a surfactant. Polyvinyl alcohol is most frequently used as a stabilizing emulsifier to fabricate nanoparticles. However, polyvinyl alcohol has some problems in that as it remains at the surface of nanoparticles which is difficult to remove subsequently. It is known that polyvinyl alcohol existing on the surface of nanoparticles changes biodegradability, biodistribution, particle cellular uptake, and drug-release behavior (Vauthier and Bouchemal 2009).

2.2.4 Interfacial Polymer Deposition

The technique involves addition of polymer dissolved in water miscible solvent (usually acetone) into an aqueous non solvent under stirring. The non-solvent is usually an aqueous surfactant or drug solution without surfactant. The rapid diffusion of solvent into the aqueous phase causes a decrease in the interfacial tension between the two phases which together with the increased interfacial surface area created by the turbulence results in the formation of small droplets of organic solvent without the need for high shear mechanical stirring. The solvent then diffuses further into the aqueous phase and water concurrently diffuses into the solvent droplets, resulting in the formation of polymer particles from the droplets. Particles are stabilized by a layer of polymer deposited at the interface (Lambert et al. 2001). Properties of the polymer may alter the physicochemical properties at the interface. Decreased miscibility of organic solvents with water is associated with an increase in their resultant interfacial tension and thus increases the size of the particles. The higher the viscosity of the organic phase, the greater the surface tension and hence the size of the particles. An increase in molecular weight of polymers is associated with a decrease in the number of end carboxyl groups and hence lowers the zeta potential of the resulting particles. Additives present in the formulation may also significantly affect this surface charge. Poly (lactic acid) with high molecular weight (109 and 251 kDa) yielded poorly stable nanocapsules larger in size and susceptible to aggregation. In the presence of lecithin, polymer charges were masked and zeta potential was determined by amount of lecithin present on the outer surface either mixed with or surrounding the polymer film. The presence of surfactant in the system acts as a stabilizer to prevent coalescence of the droplets (Mosqueira et al. 2000).

2.2.5 Salting Out

This technique avoids the use of chlorinated solvents. This method involves the use of water-miscible polymer solvents such as ethanol or acetone and an aqueous phase containing gel i.e., salting out agent such as calcium chloride, magnesium chloride, magnesium acetate as well as non-electrolytes, sucrose with a colloidal stabilizer. Briefly, a saturated salt solution containing a stabilizing agent such as polyvinyl

alcohol is added under stirring to a solution of acetone containing the polymer. The emulsion mixture is then diluted by using approximate volume of deionized water or an aqueous solution in order to allow diffusion of organic solvent to external phase resulting in precipitation of polymer leading to nanoparticle formation. An oil in water emulsion forms as the salt prevents the water and acetone mixing. Sufficient water is then added to allow the acetone to diffuse into the external aqueous phase and induce particle formation (Allémann et al. 1993; Jung et al. 2000). From the perspective of drug encapsulation, this method is most appropriate for water insoluble compounds; although the loading of water-soluble compounds can be improved by techniques such as altering the pH of the aqueous phase. It possesses an advantage of not requiring any temperature for preparation and can be further utilized for heat sensitive substance (Lambert et al. 2001).

2.2.6 Supercritical Fluid Expansion

The use of rapid expansion of supercritical solution using supercritical carbon dioxide was first introduced for preparation of polymeric nanoparticles. The use of supercritical carbon dioxide was due to its easy availability, cost-effectiveness, non-toxicity, non-flammability with low critical temperature of 31.06 °C (Franco and De Marco 2021). This process involves the dissolution of both core material and wall material in supercritical fluid. Then, the obtained solution is released into small nozzle with reduction in pressure which results in desolvation and deposition of polymeric material on the core (Keshavarz et al. 2012). Recently the field of supercritical fluids has been investigated as an approach for the preparation of sub-micron sized particles. The rapid expansion of supercritical solutions results from saturating a supercritical fluid with the substrate(s) followed by depressurizing this solution through a heated nozzle into a low-pressure chamber in order to cause instant nucleation of the substrate (s) in form of very small particles or fibers, or films when the jet is directed against a surface which are further collected from the gaseous stream (Kumar et al. 2021) The major merits of this process includes the production of particles free of organic solvent, mild operating temperatures for processing biological materials and easier micro-encapsulation of drugs for sustained release of the therapeutic agents. Fine particles of compounds such as cholesterol acetate, griseofulvin, and megestrol acetate were produced by extraction of the internal phase of oil-in-water emulsions using supercritical carbon dioxide (Shekunov et al. 2006).

2.2.7 Complex Coacervation

Complex coacervation is a phase separation process that spontaneously occurs when two oppositely charged polyelectrolytes are mixed in an aqueous solution. Compared to other methods, this process can be carried out entirely in an aqueous solution and at low temperature and thus has a better chance to preserve the

bioactivity of the encapsulated substances. The colloidal particles produced are in the nanometer or micrometer scale depending upon the substrates or the processing parameters used such as pH, ionic strength and polyelectrolyte concentrations (González-Monje et al. 2021). The major shortcoming of this technique is that complex coacervates have low drug loading efficiency and poor stability. Therefore, crosslinking of the formed complex by chemical reagents such as toxic glutaraldehyde is necessary. Complex coacervation mainly involves 3 steps i.e., mixing of core and coating material with continuous liquid phase for developing of immiscible phases. Secondly, several parameters such as pH, molecular weight, temperature, ionic strength, concentration of coating material should be maintained properly in order to produce the encapsulating layer around the bioactive compound and lastly different techniques like desolvation, heating and crosslinking should be followed for proper solidification of nano and microcapsules (Bakry et al. 2016). Recently, this process is used for manufacturing of various nutraceuticals and phytochemical products. It is also used in producing particles with increased encapsulation efficiency and controlled release products of bioactive compounds (de Souza et al. 2017).

2.2.8 Polymerization

In polymerization method, monomers are polymerized to form nanoparticles in an aqueous solution. Drug is incorporated either by dissolving in the polymerization medium or by adsorption onto the nanoparticles after polymerization is completed. The resultant nanoparticle suspension is then purified to remove various stabilizers and surfactants employed for polymerization by the process of ultracentrifugation and re-suspending the particles in an isotonic surfactant-free medium (Inagaki et al. 2018).

2.2.9 Ionotropic Gelation

It is a very simple technique which involves no chemical crosslinking, thus avoiding toxic effects. The method utilizes a mixture of two aqueous phases, of which one is the polymer chitosan, a di-block co-polymer ethylene oxide or propyleneoxide and the other is a polyanion sodium tripolyphosphate (Sarmento et al. 2006). In this method, positively charged amino group of chitosan interacts with negative charged tripolyphosphate to form coacervates with a nanosize range. Coacervates are formed as a result of electrostatic interaction between two aqueous phases, whereas, ionic gelation involves the material undergoing transition from liquid to gel due to ionic interaction conditions at room temperature. Insulin loaded nanoparticles has been reported to be prepared by ionotropic gelatin method by mixing insulin with tripolyphosphate solution and slowly adding

chitosan molecules by stirring continuously (Wu et al. 2005). It rapidly enhances the intestinal absorption and pharmacological bioavailability significantly with particle size ranging from 250 to 400 nm (Serpe et al. 2004; Colonna et al. 2008; Debnath et al. 2011).

2.2.10 Spray Drying

The method involves either dissolving or suspending the drugs in the polymer/solvent mixture and then sprays drying through nozzle (Hans and Lowman 2002). It is suitable for both water-soluble and water insoluble drugs, and produces nanoparticles with high entrapment efficiencies. A modification of this method is spraying the drug/polymer/solvent mixture into frozen ethanol overlaid with liquid nitrogen (cryogenic method). It is used to prepare microspheres of recombinant human growth hormone that releases drug over a period of 1 month (nutropin depot). It is most widely and accepted method for the preparation of nano/microencapsulation of bioactive compounds. The spraying is done by using an atomizer in which the slurry mixture is sprayed into drying chamber and the liquid gets evaporated by the supply of hot air thereby resulting in entrapment of drug in a powder formulation containing nano or microcapsules which are mainly collected by cyclone recovery (Ray et al. 2016; Shishir and Chen 2017). This process is cost effective, less complex and requires less energy. It is also used to prepare phytopharmaceutical products and stable nutraceuticals. For example: *Nigella sativa* which is extensively used as fortifying agent for various dairy products, processed food and possess nutraceutical application is formulated using spray drying method by higher efficiency in which the active volatile oil content is turned into active powder formed nano/microcapsules (Edris et al. 2016). Using spray drying process, different phytoconstituents such as *Curcuma longa, Euginauni flora, Cinnamomum zeylanicum, Bidens pilosa, Agaricus bisporus* etc. are encapsulated with bioactive compounds to achieve several advantages like masking bitter and astringent properties, enhanced solubility, positive physical parameters with oxidative stability, improved bioavailability and reduced hygroscopic character with better shelf-life (Cortés-Rojas et al. 2016; Yingngam et al. 2018; Gopi et al. 2018; Ostroschi et al. 2018).

2.2.11 Phase Separation Technique

(i) Phase separation by solvent partitioning: The method was used by Leelarasamee et al., to encapsulate hydrocortisone in poly lactic acid and dichloromethane solution. Slow injection of this suspension into a stream of mineral oil resulted in precipitation of polymer around solid hydrocortisone, because dichloro-

methane is insoluble in mineral oil but not in the polymer (Leelarasamee et al. 1998).

(ii) Phase separation by non-solvent addition: This technique involves suspending the drugs (either solid crystals or aqueous solution) in an organic solution of polymer and subsequent phase separation by addition of second organic solvent (Chen et al. 2003).

2.3 Advanced Techniques for Preparation of Nanoparticles

Besides solvent evaporation, nanoprecipitation, salting out, supercritical fluid technology, membrane dialysis, phase separation techniques etc. there are some advanced methods or technologies introduced for the preparation of polymeric nanoparticles. Drug loading in the polymer can be achieved by two processes: firstly, when the drug is absorbed in the polymeric nanoparticle after being formed and secondly when the drug is instilled in the polymeric nanoparticle at the preparation time. The different advanced techniques used for preparation of polymeric nanoparticles are ring opening polymerization, desolvation of macromolecules and electro-hydrodynamic atomization.

2.3.1 Ring Opening Polymerization

In this method, a long chain is formed where at the terminal end there is presence of a polymer and it acts as reactive center for the reaction of chain opening process of cyclic monomer. It is the method of electrophilic monomer activation, nucleophilic monomer activation and end-chain activation. Caprolactone synthesis is an example for ring-opening polymerization technique. Firstly, diketopyrrolopyrrole, toluene and 6-mercapto-1-hexanol is stored in the ampoule then to that caprolactone is added by mixing at temperature 50 °C. Triethylamine is added to the aliquot and the reaction comes to an end with the addition of cold methanol. Then the obtained product is filtered and finally dried at vacuum (Xu et al. 2018; Xu et al. 2019).

2.3.2 Electro-Hydrodynamic Atomization

This process was first introduced by Xie et al. using poly (lactic-co-glycolic acid) solution and acetonitrile and occurs due to electro-hydrodynamic polymerization phenomenon (Xie et al. 2006). This can be achieved by the diffusion process of fluid which leads to the development of nanoparticle with compact size and narrow size

distribution. It is a dynamic process where the where the fine droplets are achieved from conical meniscus of liquid under the effect of electrostatic stress which termed as electro-hydrodynamic polymerization (Parhizkar et al. 2016; Rai et al. 2017).

2.3.3 Desolvation of Macromolecules

In this method, the main aim was that to add a desolvating agent like polysaccharide, which can be desolvated by change in temperature and pH by adding appropriate quantity of counter ion. This method does not require high temperature means thermolabile molecules or drug substances are suitable for this method. For e.g., bovine serum albumin, a thermolabile substance can be prepared by desolvation technique by adding acetone as a desolvating agent (Park et al. 2015; Nosrati et al. 2018).

2.3.4 Mussel-Inspired Chemistry for Polymerization

Mussel inspired chemistry uses synthetic polymers for its polymerization process by forming an excellent polymeric coating on the natural derived products. Huang et al. invented polyacrylamide immobilized molybdenum disulphide composites by using mussel inspired chemistry technique. Polyacrylamide sheets were immobilized into molybdenum disulphide nanosheets and nanosheets of molybdenum disulphide was firstly improved by polydopamine. Polydopamine was obtained from dopamine by oxidative stress process in alkaline solution. After modification of molybdenum disulphide nanosheets with polydopamine, polyacrylamide was immobilized by surface-initiated polymerization method. This method of preparation widely used for adsorption of heavy metals such as copper, iron etc. (Huang et al. 2018a, b). This process is the most promising chemical technologies which involves surface modification phenomenon and generally derived for drug delivery system. It includes many implications in the environment field as well like catalysis, oil-water separation process, adsorption for purification of compounds etc. (Zhang et al. 2017; Zeng et al. 2018).

2.3.5 Self-Polymerization

Polydopamine, a natural compound mainly contains melanin in it and due to its presence; it is used in the electricity, magnetics and optics. Moreover, it possesses excellent biocompatibility and also contains catechol, amine and imine functional groups for which it has wide applications in the field of biology and biomedicine.

The best property of dopamine is that it is a monomer and can self-polymerized itself in the alkaline solution by changing pale brown to deep brown color. It has an immense use in the polymer coating and for target delivery system (Hickey et al. 2015). It is also reported that molybdenum disulfide when treated with gold nanocomposites incorporated with polydopamine produces an excellent anti-bacterial activity against microorganism *Staphylococcus aureus* (Zeng et al. 2018). Now-a-days fluorescent nanoparticles are widely used due to their improved drug delivery profile and applications in bio-imaging. Biological imaging is an important tool of monitoring several biological processes such as metastasis, tumor growth and many more. However, inorganic and traditional fluorescent materials have some limitations and highly toxic in nature so organic fluorescent are preferred (Shi et al. 2017). When polydopamine are immobilized with organic fluorescent materials then it produced great properties for cell imaging (Zhang et al. 2012). The nanocomposite materials are also developed with the conjugation of polydopamine and carbon tubes functionalized with chitosan particles. This nanocomposite material also utilizes for copper ion removal (Zeng et al. 2016). Self-polymerization of polydopamine with polyethyleneimine has excellent bioimaging applications with enhanced biocompatibility with improved biodegradability. Therefore, from the above examples it is cleared that polydopamine due to its self-polymerization properties it is hugely used in the fields of bio-imaging, thermal therapy and drug delivery system as well (Huang et al. 2018a, b) (Table 2.2).

2.4 Conclusion

Polymeric nanoparticles are a cutting-edge technology that necessitates the selection of an appropriate technique from a variety of options. Polymeric nanoparticles can now be constructed using simple, secure, and repeatable techniques. It is feasible to select the best method of preparation to generate nanoparticles with the optimal size range and appreciable drug entrapment performance based on the physicochemical attributes of a drug. The formulation method for fabrication of Polymeric nanoparticles employs less-toxic reagents, simplified process to allow for cost-effective scale-up and optimization protocols to increase yield and entrapment performance. Limitations such as one method or procedure not being appropriate for all drugs, involvement of complex post-preparative steps such as purification and preservation and insufficient stability of some active components remain to be addressed in order to enhance the efficacy of the engineered nanoparticles. However Polymeric nanoparticles have shown tremendous potential for the advancement of drug delivery systems, amid these technical hurdles.

Table 2.2 Anticancer drugs in the form of polymeric nanoparticles for the treatment of different types of solid tumors

Name of the method	Name of the anticancer drug	Polymer (s) used	Type of targeted cancer cells	References
Emulsification solvent evaporation	Docetaxel	Poly (lactide-*co*-glycolide)		Rafiei and Haddadi (2019)
	Imatinib	Poly(ε-caprolactone)-methoxy poly(ethylene glycol)	Human fibroblast (L929), leukemia (K-562), osteosarcoma (SAOS-2), and breast carcinoma cells (MCF-7)	Ilkar Erdagi and Yildiz (2019)
	Daunorubicin	Poly(lactic-co-glycolic acid and poly(d,l-lactic acid)	HL-60 cells	Liu et al. (2010)
	Doxorubicin and Sorafenib	Poly(lactide-co-glycolide) and polyethylene glycol-poly (lactide-co-glycolide)	Human cancer cell line HT-29	Babos et al. (2018)
	Cisplatin, doxorubicin, and 5-fluorouracil	Polycaprolactone-polyethylene glycol	Breast cancer cells (T47D and MCF7)	Eatemadi et al. (2015)
	Docetaxel	Poly(sarcosine) and poly (ethylene glycol) coated poly (lactic-*co*-glycolic acid)	Human cancer cell lines (U-87 MG, HeLa. C2BBe1, HCT-116, NCI–N87, NCI–H929-Luc-mCh-Puro)	Bhattacharya (2021)
	Curcumin and 7-ethyl-10-hydroxy camptothecin	Polyethylene glycol and poly (lactic-co-glycolic acid	Human cervix cancer cells (HeLa) and human ovarian cancer cells (A2780)	Li and Gao (2020)
	Epirubicin	Poly (lactide-coglycolide acid)	Human lung squamous cell carcinoma (SK-MES-1) cells	Esim et al. (2020)
	Cytarabin	Poly (ε-caprolactone)	KG-1 leukemic cell line	Jan et al. (2021)
	Docetaxel and bevacizumab	Carboxymethyl chitosan and poly(lactic-*co*-glycolic acid)– for docetaxel methoxy polyethylene glycol-poly (β-amino ester) – for bevacizumab	Caco-2 cells	Feng et al. (2021)
	Camptothecin	Chondroitin sulfate	CT – 26 Colon cancer cell lines	Zu et al. (2019)
	Gefitinib and yttrium 90	Folic acid-poly ethylene glycol- 1,2-distearoyl-sn-glycero-3-phosphoethanolamine and poly (lactide-co-glycolide) – hydroxide	HONE 1 nasopharyngeal cancer cells	Yugui et al. (2019)

(continued)

Table 2.2 (continued)

Name of the method	Name of the anticancer drug	Polymer (s) used	Type of targeted cancer cells	References
Nanoprecipitation	Curcumin	Poly (ethylene glycol)-block-poly (lactide-co-glycolide)		Li et al. (2021)
	Curcumin	Shellac		Baby et al. (2021)
	N-trimethyl chitosan chloride – Curcumin polymeric nanoparticle	Poly(2-methacryloyloxyethyl phosphorylcholine)-*b*-poly(2-(diisopropylamino)ethyl methacrylate)		ElzhryElyafi et al. (2017)
	Doxorubicin	Poly(ε-caprolactone) and poly(trimethylene carbonate)	Breast cancer cells (MCF-7)	Brzeziński et al. (2021)
	Sorafenib	Poly(lactide-co-glycolide), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine –polyethylene glycol –2000	Breast cancer (MDA-MB-231) cells	Tahir et al. (2020)
	Curcumin – bortezomib	Methoxy-poly(ethylene glycol)-*block*-polylactic acid diblock copolymers	Breast and cervix cancer cells (HeLa, MCF-7 and MDA-MB 231)	Medel et al. (2017)
	Paclitaxel	(PEGylated) poly (lactic acid-*co*-caprolactone)		Lallana et al. (2018)
	Paclitaxel	Poly (lactic-co- glycolic)-polyethyleneglycol	Intestine epithelial carcinoma cell lines, carcinoembryonic antigen -expressing Caco-2 clone and non- carcinoembryonic antigen -expressing SW480	Pereira et al. (2018)
	Cabazitaxel	Methoxypoly (ethylene glycol)-block-poly(D, L-lactic acid)	Human prostate cancer cell lines (DU145 and 22RV1)	Shuai et al. (2020)
	Glycoalkaloidic extract	Poly(D,L-lactide)	Breast (MDA-MB-231) and bladder (RT4) cancer cells	Miranda et al. (2020)
	Rapamycin and piperine	Poly(lactide-co-glycolide)	Human-derived breast cancer cell line (MDA-MB-231)	Katiyar et al. (2015)
	Oxilaplatin	Methoxy poly(ethylene glycol)-b-poly(D,L-lactide)		Kadina et al. (2021)

Solvent diffusion method	Docetaxel	All-trans retinoic acid grafted poly β-amino ester copolymer	Human umbilical cord vascular endothelial (HUVEC) and breast cancer cell lines (MCF-7)	Karimi et al. (2020)
	Gemcitabine and 5-fluorouracil	Poly(lactic-co-glycolic acid)-polyethylene glycol	Human lung cancer cells (A549 and HEL-299)	Zhou et al. (2021)
	Ellagic acid	Poly (ε-caprolactone)	Caco-2 and HCT-116 cell lines	Mady and Shaker (2017)
	Curcumin	Chitosan and gum arabic	Human colorectal adenocarcinoma cell line (HT29) and a human colon carcinoma cell line (HCT116)	Udompornmongkol and Chiang (2015)
Supercritical fluid expansion method	Disulfiram	Polyvinylpyrrolidone and methoxy *b*-poly(L -lactide) 2000- poly(ethylene glycol) 2000	MDA-MB-231 cells (a human breast cancer cell line	Tang et al. (2020)
Interfacial polymer deposition	Cyclophosphamide	Gliadin-gelatin composite nanoparticles	MCF – 7 breast cancer cells	Gulfam et al. (2012)
Complex coacervation method	5-fluorouracil and (−)–epigallocatechin -3-gallate	Chitosan and gelatin	Colon cancer cell lines (HT 29 and CT 26)	Wang et al. (2019)
Spray drying	Doxorubicin	Sodium alginate	Lung carcinoma A549 cells	Mishra et al. (2021)
	Simvastatin	Poly(lactic-co-glycolic acid)	MCF-7 breast cancer cells	Anzar et al. (2018)
	α-galactosylceramide	Dimethylaminoethyl methacrylate, butyl methacrylate and methyl methacrylate	JAWSII and B16F10 cell lines, iNKT hybridoma DN3A4-1.2 and theB16CD1d+cell lines	Gonzatti et al. (2019)
Supercritical fluid expansion method	Sunitinib malate	Hydroxypropyl methylcellulose, poly (vinyl alcohol), chitosan and polyethylene glycol		Razmimanesh et al. (2021)
Polymerization method	Doxorubicine	Poly (N-vinylcaprolactam)		Morfin-Gutierrez et al. (2021)

(continued)

Table 2.2 (continued)

Name of the method	Name of the anticancer drug	Polymer (s) used	Type of targeted cancer cells	References
Ionotropic gelation method	Imatinib	Chitosan	CT26 colon carcinoma cell lines	Bhattacharya (2020)
	Doxorubicin and small interfering RNA	β-cyclodextrin grafted trimethyl chitosan		Zhang et al. (2021)
	Doxorubicin	Maleimide-bearing chitosan and catechol-bearing alginate	MB49 bladder cancer cells	Sahatsapan et al. (2021)
Electrohydrodynamic atomization method	Cisplatin	Poly (lactic-co-glycolic acid)		Parhizkar et al. (2016)
Desolvation of macromolecule	Cardamom extract	Gelatin	Human glioblastoma cancer U87MG cells.	Nejat et al. (2017)
Mussel inspired chemistry for polymerization	Doxorubicin	PEGylated, mussel-inspired polydopamine	4T1 Breast cancer cells	Hou et al. (2017)
	Doxorubicin	Poly(lactic-co-glycolic acid) /polydopamine core-shell nanoparticle	Human head and neck squamous carcinoma cell line (UMSCC 22A)	He et al. (2017)

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Chapter 3
Natural Polymers-Based Nanoparticles Targeted to Solid Tumors

Shrikaant Kulkarni

3.1 Introduction

The natural polymers are originated from plants, animals, bacteria and fungi. They are categorized into two major types such as polysaccharides and protein-based polymers. Both of them have been exhaustively explored for their applicability in solid tumors. Natural polymers tend to form scaffolds as a viable extracellular matrix. The solid tumors targeted drug delivery accompanied by high loading efficiency and abysmally low invasive tendency can be attained (Dragan and Dinu 2019; Garg et al. 2012). Moreover, the functional groups can be attached to the polymer backbones. Application of natural polymeric nanoparticles has shown a lot much of promise and potential in drug delivery (Ulbrich et al. 2016; Kumar et al. 2017). The frequently used natural polymers in the form of their nanoparticles for drug delivery are discussed in this chapter elaborately.

The application of nanoscience and nanotechnology targeted drug delivery front has witnessed a major upsurge over the last two decades, primarily with an intention to maximize therapeutic efficacy and minimize toxicity (Padhi et al. 2015; Patnaik et al. 2021). Polymeric nanoparticles enabled targeted drug delivery systems have showed numorous benefits over traditional large sized drug delivery systems (Padhi et al. 2018). Natural biodegradable polymers-enabled nanoparticle drug delivery systems hold a lot much of promise and potential by throwing open, a viable, and safe alternative for regulated release of drugs. These nanoparticle-enabled formulations are superior to macroformulations or conventional formulations in terms of controlled drug release, targeted delivery and therapeutic effects (Behera and Padhi 2020). Moreover, they can contain dosing frequency, while retaining stability and

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the structure. This is attributed to their capability to target specific tissues and organs, boost bioavailability, solubility and permeability of potent drugs, work as DNA carrying containers in gene therapy and deliver macro molecules like proteins, peptides and genes via oral administration. The availability of host of natural biodegradable polymers has made it possible to develop safe and efficient drug delivery systems provided problems in the research, such as design and development are addressed with care. It covers adequate drug introduction in the nanoformulation with efficiency in release, enough biodistribution, biocompatibility, specificity in targeting, stability and shelf life. Still there is an ample scope to explore this research area to give an impetus and tap maximum potential of nanomedicines.

This chapter provides an overview of the current development and future scope of natural biodegradable polymer- driven nanoformulations for drug delivery systems. Moreover, various methods for synthesis and characterization are elaborated with sufficient details in addition to prominent features of representative nanoformulations for drug delivery.

3.2 Formulation and Characteristics of Natural Polymers

3.2.1 Polysaccharides

Polysaccharides consist of long chains polymers of carbohydrates made up of monosaccharides as monomers (sugar) bonded with glycosidic bonds. Polysaccharides possess more than ten monomeric sugar units. Naturally, the genesis of polysaccharides lies in animals (e.g., chitosan, chondroitin), plants (e.g., pectin, guar gum), algae (e.g., alginate) and microbes (e.g., dextran, xanthan gum) (Hamman 2008). They are characterized by the properties like non-toxicity, biocompatibility and excellent stability which can be used to advantage such as drug delivery to solid tumors (Hamman 2008; Liu et al. 2008). Chitosan, dextran, alginates and hyaluronic acid etc. are frequently used candidates for the said purpose.

3.2.1.1 Chitosan

Chitosan is a linearly shaped, positively charged amino polysaccharide which is produced by deacetylation of chitin. It is obtained by condensation of β–(1-4) linked -d-glucosamine and N-acetyl-d-glucosamine monomers having 1, 4 glycosidic linkages. Chitosan possesses excellent properties such as biocompatibility, mucoadhesivity, antibacterial and antifungal properties and nontoxicity (Motiei et al. 2017; Benhabiles et al. 2012; Alburquenque et al. 2010). It is decomposed by enzymes to shorter chain polymers like oligomers and monomers (Motiei et al. 2017). However, the low solubility of chitosan in either a neutral or more pH medium limits its applications in biology. This problem can be overcome by

suitably functionalizing its backbone; while controlling its other biological properties using this method (Argüelles-Monal et al. 2018). On functionalization, chitosan can be transformed into hydrogels, nanoparticles, micro- or nano-spheres and micelles that are used in cancer treatment.

Formulation of Chitosan-Based Nanoparticles

Chitosan and its derivatives are used extensively in anticancer drug delivery by encapsulating anticancer agents with the nanoparticles or binding directly. Chitosan ascorbate nanoparticles were found to exhibit inhibitory effects on cervical cancer (Sekar et al. 2018). He synthesized chitosan ascorbate for the first time from chitosan and ascorbic acid by salification followed by their nanoparticles using ionotropic gelation with the help of pentasodium tripolyphosphate as a crosslinking agent in the acidicenvironment. The particle size of the nanoparticles was of the order of 170 nm, which further could be regulated by controlling the concentration of ascorbic acid. The nanoparticles exhibited antioxidative tendency. They checked the viability of cervical cancer cells (HeLa cells) without affecting the survival rate of healthy cells (human fibroblasts WI-38). The study demonstrated the potential of the chitosan ascorbate nanoparticles to work as nanocarriers for targeted drug delivery in cancer (Sekar et al. 2018).

However, additional data, covering in vivo study, is required to validate the applicability of such formulation. The surface functionalization of chitosan-based nanoparticles can be done so as to use it for broad range of applications. Nascimento et al. showed epidermal growth factor receptor – targeted chitosan nanoparticles for delivery of small interfering RNA in treating non-small cell lung cancer (Nascimento et al. 2014). Epidermal growth factor receptor is overexpressed in the non-small cell lung cancer conditions, which serves as a target in the drug delivery. In the study, epidermal growth factor receptor binding peptide and poly (ethylene glycol) 2000 were conjugated with chitosan to form derivatives. Further, the chitosan derivatives were chelated with Mad_2 small interfering RNA genes to obtain the desired nanoparticles. Chitosan with varying molecular weights were used. Lower molecular weight (50 kDa) chitosan yielded lower particle size than that by the high molecular weight ones (90 kDa) (Nascimento et al. 2017). 100% encapsulation efficiency was observed in both formulations. Both epidermal growth factor receptor – targeted chitosan nanoparticles exhibited better selective uptake efficiency with non-small cell lung cancer cells (A549 cells). The encapsulated small interfering RNA quite successfully reduced Mad_2 (a mitotic checkpoint unit), leading to cancer cell death on a massive scale by apoptosis. A chitosan formulation with low molecular weight exhibited higher activity than a high molecular weight counterpart (Chen et al. 2018). The lower molecular weight formulation was explored further in cisplatin – sensitive and – resistant rats models. The targeted formulation demonstrated better inhibition effect in tumor growth as against a nontargeted one. The effect shown by a platinum-resistant model was further more (Nascimento et al. 2017).

Chitosan-based nanoparticles are used for the delivery of smaller molecules. Boroujeni et al. prepared folate-functionalized chitosan nanoparticles for encapsulating curcumin in the breast cancer therapy (Esfandiarpour-Boroujeni et al. 2017). Folate can link with the folate receptors overexpressed by cancer cells. Thus, it can be made use of as a targeting ligand to drug delivery systems. Curcumin was delivered by the folate-functionalized chitosan nanoparticles consistently over a span of 1 week. The release rate in acidic environments was rapid because of the protonation of amino groups in the chitosan main chain, bringing about the swelling of the polymer matrix (Vivek et al. 2013). The formulation bettered therapeutic effect as compared to free curcumin in breast cancer cells (MCF-7), and the nanoparticles in isolation didn't show any toxicity to healthy cells (L928 cells) (Esfandiarpour-Boroujeni et al. 2017).

Chitosan nanoparticles too could release doxorubicin in hepatic cancer therapy. Tian et al. synthesized a chitosan nanoparticle linked with glycyrrhetinic acid, which could bind with mouse hepatocytes and glycyrrhetinic acid receptors on human hepatic cells for targeting liver (Tian et al. 2010; Mao et al. 2007). The glycyrrhetinic acid – functionalized chitosan nanoparticles demonstrated better cellular uptake efficiency in case of human hepatic carcinoma cells (QGY-7701). Loading doxorubicin along with the nanoparticles formulation, doxorubicin exhibited higher cytotoxicity towards human hepatic carcinoma cells and presented improved performance in inhibiting tumor growth in H_{22} cell-bearing rats, as against the free drug (Tian et al. 2010).

Niu et al. prepared a pH and thermosensitive liposome with cell-penetrating peptide functionality for encapsulating doxorubicin in triple-negative breast cancer therapy. Poly (N-vinylcaprolactam) was first bonded with the chitosan backbone. Poly (N-vinylcaprolactam) is a biocompatible polymer that shows reversibility in phase transition from hydrophilic to hydrophobic on raising the temperature. Chitosan was instrumental in producing the pH-sensitive drug delivery system due to its slight increase in solubility in acidic conditions. The conjugate was then altered using cell-penetrating peptide for enhancing the permeability of drug delivery system. Ultimately, the copolymers self-assembled into nanoparticles in water solution and thereby loaded doxorubicin into the hydrophobic core. Drug release rate was exacerbated in acidic and hyperpyrexic environments from the nanoparticles. The formulation selectively improved the cellular uptake efficiency of doxorubicin by breast cancer cells (MCF-7) while healthy human umbilical vein endothelial cells (HUVEC) are not affected. The cell-penetrating peptide promoted the formulation to mount at the solid tumor site on administration. The drug delivery system showed better tumor inhibition effect in the xenograft mice model with minimal off-target fallouts and lesser systemic toxicity as compared to treatment with doxorubicin in isolation (Niu et al. 2019).

3.2.1.2 Hyaluronic Acid

Hyaluronic acid (hyaluronan) is a linear negatively charged glycosaminoglycan formed by condensation of glucuronic acid and N-acetylglucosamine as monomeric units with -1,4 and -1,3 glycosidic linkages. Hyaluronic acid has the capacity to bind with numerous cell surface receptors like CD_{44}, which is present necessarily in tumor extracellular matrix and is connected with tumor growth (Misra et al. 2011). Consequently, hyaluronic acid can be used to produce drug delivery systems for targeted therapy. Hyaluronidases are the enzymes to break down hyaluronic acid. Hyaluronidases are largely expressed in environments at micro level of tumors (McAtee et al. 2014; Lokeshwar et al. 2001). Hence, regulated drug release of hyaluronic acid drug delivery systems is accomplished on degradation of hyaluronic acid using hyaluronidase enzyme (Chen et al. 2018). However, hyaluronic acid also has a role to play in tumor cell growth and angiogenesis with associated complex bio-activities. It should be used preferably in developing drug delivery systems with use of hyaluronic acid as a carrier in the treatment of cancer (Misra et al. 2011).

Formulations of Hyaluronic Acid-Based Nanoparticles

Hyaluronic acid nanoparticles have the ability to encapsulate a host of molecules namely, drugs with small molecules, imaging agents as well as small interfering RNA for targeted delivery. Ganesh et al. prepared a hyaluronic acid – based nanoparticles formulation that could encapsulate cisplatin, small interfering RNA or near infrared dye indocyanine green on the frontiers like tumor imaging and combinational therapy (Ganesh et al. 2013a, b). Because of its negative charge, hyaluronic acid can't readily encapsulate the anionic small interfering RNA due to repulsion. Hence, different fatty amines or cationic polyamines were introduced into the hyaluronic acid backbone to minimize the negative charge density; followed by the use of the conjugates for encapsulating small interfering RNA. Small interfering RNA thus encapsulated exhibited selective uptake efficiency by models of solid tumor cells (breast cancer MDA-MB468 cells) as well as metastatic tumor cells in mice. However, the *in vitro* gene-silencing activity finding was not in harmony with mice models (Ganesh et al. 2013a, b).

Hyaluronic acid nanoparticles were functionalized and explored further for minimizing cisplatin resistance. Indocyanine green was encapsulated by the hyaluronic acid nanoparticles formulation for complete body imaging; cisplatin and small interfering RNA were loaded in the matrix in order to target CD_{44} receptors in the mice model having human lung tumors showing resistantance to cisplatin. Findings demonstrated encouraging efficacy combined with treatments against cancer (Ganesh et al. 2013a, b). Zhone et al. too synthesized a hyaluronic acid – based nanoparticles formulation to deliver doxorubicin in a drug-resistant model pertaining to over expression of CD_{44} tumor. Hyaluronic acid was modified by using L-lysine methyl ester and lipoic acid followed by the cross linking of hyaluronic acid – conjugates to develop the nanoparticles. The delivery of doxorubicin of the

nanoparticles was induced by glutathione, which is present in the cytoplasm. The nanoparticles formulation exhibited better targeting tendency and antitumor activity towards CD_{44} receptor instrumental in the overexpression of doxorubicin – resistant breast cancer cells (MCF-7/ADR cells). Nanoparticles loaded with doxorubicin accumulate largely in the tumors of MCF-7/ADR xenografted mice. The tumor proliferation was controlled, and subsequently prolonging the life of mice (Zhong et al. 2015).

Other modified forms hyaluronic acid – based nanoparticles too have been prepared to deliver doxorubicin. Yan et al. conjugated a positively charged amphiphilic copolymer made up of PEGylated cationic quaternary amine and n-octyl acrylate combined with hyaluronic acid to synthesize the nanoparticles. The cationic copolymer was employed to minimize the negative charge of the system on the whole to ensure cell endocytosis. The acidic nature of the endosome gave an impetus to the release of doxorubicin from the carrier. The drug delivery carrier exhibited reasonable antibacterial ability. Apart from the release of doxorubicin, the drug delivery system exhibited a lot much of promises to get over the bacteria-induced tumor resistance (Yan et al. 2019).

Bi-functionalized hyaluronic acid nanoparticles were synthesized for delivering doxorubicin by Tian et al. Hyaluronic acid was functionalized by using a liver targeting ligand such as glycyrrhetinic acid and pH-responsive L-histidine. The delivery of doxorubicin by the system was improved under low pH conditions due to the protonation of imidazole groups of histidine, leading to increase in particle size. The cytotoxicity of the so developed formulation was assessed with the help of human hepatoma cell line $HepG_2$ and H_{22} in mice bearing tumors. The targeted formulations were quite successful against tumors over the drug in isolation (Tian et al. 2019). Han et al. developed a formulation to bring about regulated release and targeted delivery of doxorubicin to squamous cell carcinoma cells (SCC_7 cells).

Hyaluronic acid on crosslinking with 2-(pyridyldithio)-ethylamine and polycaprolactone the nanoparticles were fromed. Hyaluronic acid was instrumental in targeting the CD_{44}-ligand receptors overexpressed in the cancer cells; 2-(pyridyldithio)-ethylamine was a disulfide crosslinker for regulated release of the therapeutic agent; while the polycaprolactone hydrophobic in nature ensured that the doxorubicin is encapsulated. These crosslinkages were broken down and brought about the intracellular release of doxorubicin of the delivery system when glutathione is present. Both in vitro and in vivo therapeutic performance of doxorubicin was enhanced by the use of such targeted nanoparticle (Han et al. 2015). Cadete et al. synthesized docetaxel-loaded hyaluronic acid nanoparticles by using emulsification technique in absence of organic solvents or heat. Sodium hyaluronate was conjugated with dodecylamide to derive derivative of hyaluronic acid that is amphiphilic in nature, which doesn't need the application of cationic surfactants in the synthesis of nanoparticles.

Thus, the safety of the drug delivery system could be improved a lot. This formulation had an ability to check the proliferation of A549 cells, as well as to boost the intracellular uptake efficiency by cancer cells. The nanoparticles structurally ramained stable in human plasma after treatment for 24 h (Cadete et al. 2019).

Because of the targeting capability of hyaluronic acid, it can be used as a vehicle in drug delivery systems, apart from a targeting ligand in other nanoparticles-based systems. Zhang et al. used hyaluronic acid to lace the surface of poly (lactic-co-glycolic acid) nanoparticles to codeliver docetaxel and naphthoflavone. The core of the nanoparticle was changed in the first place with polyethyleneimine to acquire a positive charge. Next, the nanoparticles were deposited with hyaluronic acid to boost the cellular uptake performance of payloads through hyaluronic acid induced endocytosis. The formulation could easily get over the resistance of multi-drugs in breast cancer cells (MCF-7/1B1 cells), and exacerbating cell apoptosis. The pharmaco-kinetic study shows that, the bioavailability of docetaxel was substantially improved in the hyaluronic acid – coated nanoparticles systems as against the docetaxel or docetaxel-loaded poly (lactic-co-glycolic acid) nanoparticles in isolation (Zhang et al. 2019).

Further some other hyaluronic acid – coated inorganic nanoparticles, such as gold, silica nanoparticles and metal–organic frameworks, too were used in the targeted delivery for the treatment of cancer. Use of hyaluronic acid in a drug deliver system makes it possible to control the release, targeted delivery, formulation stabilization, diagnostics, imaging and bioavailability, biocompatibility enhancement (Lee et al. 2018a, b; Cao et al. 2018; Zhao et al. 2015; Shu et al. 2018; Xu et al. 2017; Shi et al. 2019).

3.2.1.3 Alginates

Alginates are linear in shape and negatively charged or anionic polymers consisting of irregularly arranged monomeric units of (1→4′)-linked β-d-mannuronic acid and α-l-guluronic having 1, 4 glycosidic linkages. Alginic acid is not soluble in water however its salt with monovalent metals is soluble; such as sodium alginate (Rehm and Valla 1997). Alginate settles in the form of a gel when subjected to mild reaction conditions (pH, temperature, etc.) and doesn't demand toxic organic solvents. The gel quality is influenced by the gelation rate which is governed by kind of ions available in the solution, temperature and the chemical structure acquired by the alginate (Rehm and Valla 1997; Augst et al. 2006). The gel formation is followed by the migration of water molecules into the gel matrix. However, since the alginates are derived from seaweeds, heavy metals and protein may be captured in the body of alginate molecules as impurities (Tonnesen and Karlsen 2002). Alginates hold a lot much of potential in designing drug deliver systems, preferentially hydrogels, in cancer treatment.

Formulation of Alginate-Based Nanoparticles

Alginate is used for the development of nanoparticle-based drug delivery systems. A pH-sensitive and reduction-responsive nanoparticles made up of derivative of alginate was formed by Chiu et al. Sodium alginate was subjected to thiolation to

produce nanoparticles of disulfide derivatives. Cationic fluorescein-labeled wheat germ agglutinin was conjugation with the nanoparticles used for targeted delivery of docetaxel in treating colon cancer cells (HT-29 cells). The formulation so prepared exhibited selective uptake and showed higher cytotoxicity toward HT-29 cells unlike towards healthy mouse fibroblast cells (L929 cells) (Chiu et al. 2020). It was showcased that sodium alginate is insensitive towards enzymatic and hydrolytic break down in the top gastrointestinal tract. Thus, the break down of encapsulated drugs can be prevented (Bhattacharyya et al. 2016). Disulfide derivative nanoparticles were broken down by glutathione overexpressed in cancerous cells, leading to controlled release at the target site or in induced gastrointestinal environments (Chiu et al. 2020).

A regulated release strategy of similar kind was applied to deliver doxorubicin and paclitaxel. Gao et al. used disulfide crosslinked alginate nanoparticle formulations for encapsulating doxorubicin in cancer treatment. The drug-loaded nanoparticles exhibited significant inhibition towards the human liver cancer cells (HepG2) and human cervical cancer cells (HeLa), while they brought about the proliferation of human liver healthy cells (L-O2). The formulation also led to selective penetration inside the cancer cells. Further, the cardiotoxicity of doxorubicin in treating zebrafish was decreased encapsulating the drug by the alginate nanoparticles (Gao et al. 2017). Ayub et al. synthesized disulfide crosslinked sodium alginate to enhance the delivery potential of paclitaxel.

Surfaces chemistry of the nanoparticles was changed using polymers namely, poly (allylamine hydrochloride and poly (4-styrenesulfonic acid-co-maleic acid) sodium salt with a layer-by-layer process to lengthen the release of payload. Paclitaxel was uptaken selectively by human colorectal adenocarcinoma cancer cells (HT-29) although not healthy human colon cells (CRL 1790), and it led to death of cancer cells (Ayub et al. 2019). Zhang et al. used alginate nanoparticle formulations as vehicles for combinatorial therapy. A hydrophobic photosensitizer, such as pheophorbide A, on conjugation with alginate through disulfide linkage produces a nanoparticle formulation in an aqueous medium. Doxorubicin was loaded to such the nanoparticles in combination therapy.

A glutathione dose-based release of doxorubicin and pheophorbide A of the nanoparticles was found. The nanoparticles carried doxorubicin and pheophorbide A inside murine melanoma cells (B16 cells) readily. Irradiation with light, cytotoxic atomic oxygen created by pheophorbide A and doxorubicin exhibited higher antitumor effect as against doxorubicin in isolation or nanoparticles without photoirradiation. The nanoparticles were found to get accumulated at the tumor site of B16 tumor-possessing mice with not much off-target effect. Tumor cells proliferation was checked by combining chemo- and photodynamic therapy (Zhang et al. 2017). There are examples of similar kind wherein alginate is used as a drug vehicle in cancer treatment (Jayapal and Dhanaraj 2017).

3.2.1.4 Dextran

Dextran is consisting of branched chain glucan structure linked by the -1,6 linkage between d-glucose units; side chains at -1,2, -1,3, or -1,4 positions may be available. The molecular structure of dextran is dependent upon the source from which it is derived. Molecular weight and branching degree may influence its biological behavior (Khalikova et al. 2005). Dextran has significant water solubility and flexibility to bring about functional alterations. Dextran helps stabilize the drug delivery systems or drug conjugates and exacerbate the bioavailability of a given drug on is administration with not much toxicity (Yee et al. 2019). Hence, dextran and its derivatives are amenable to develop as drug vehicles like micelles and hydrogels.

Formulation of Dextran-Based Nanoparticles

Dextran self-assembles to form nanoparticles which can be used in numerous anticancer drugs. Thambi et al. showed a carboxymethyl derivatized dextran nanoparticle can be employed for delivering doxorubicin. Carboxymethyl dextran on conjugation with lithocholic acid through a disulfide linkage could be used for nanoparticle formulation preparation followed by encapsulation of doxorubicin. With the help of the disulfide bond, controlled drug release from the matrix of the nanoparticles was ebhanced by the availability of glutathione. These bio-reducible carboxymethyl dextran nanoparticles showed better toxicity with squamose cell carcinoma cells (SCC7) as against the doxorubicin-loaded nanoparticles with no disulfide linkage. Doxorubicin was delivered quite effectively in the nuclei of squamose cell carcinoma cells cells. The drug delivery systems delivered doxorubicin through the enhanced permeativity and retentivity in a mice model with tumor. They exhibited higher therapeutic efficacy and bio-distribution as against nanoparticles loaded with doxorubicin (Thambi et al. 2014).

Curcumin is a polyphenol organic species which has the ability of forming conjugates with various polymers (Kumar et al. 2018; Kim et al. 2011). It is used as a therapeutic agent too in the treatment of a broad range of cancers (Giordano and Tommonaro 2019). Curio et al. prepared a dextran–curcumin conjugate and used it to develop nanoparticles in aqueous environments through self-assembly. The dextran–curcumin conjugated nanoparticles were used for the encapsulation of methotrexate employed in breast cancer therapy. Controlled release of methotrexate was done consistently from the delivery carrier, while the uptaking of the nanoparticles by breast cancer cells (MCF-7) was fast. In addition, methotrexate, coupled with the curcumin, exhibited a synergy in the treatment of breast cancer cells. This work peeped into the combination therapy wherein drug–polymer conjugates are employed as drug carriers for delivering therapeutic agents to treat cancer (Curcio et al. 2019).

Dextran nanoparticles on encapsulating chlorin e6 were used in cancer photodynamic therapy. Gold nanoparticles were coated to enhance the stability of the dextran-based nanoparticles. The system on the whole was quite stable even on

keeping it in serum for 6 days. The amount of nescant oxygen produced by the metallic-polymeric nanoparticles was not equivalent to that of free chlorine e6 or chlorine e6-loaded dextran nanoparticles *in vitro*. However, the nanoparticles were uptaken with efficiency by squamose cell carcinoma cells (SCC7) even if gold deposition is present. *In vivo* imaging showed that the outstanding stability of gold-deposited nanoparticle which brought about chlorin e6 targeted delivery to tumor sites with no leakage. Antitumor activity was observed due to an adequate quantity of chlorin e6 mounted at the tumor sites; this quantity was far more than either free chlorine e6 or chlorine e6-loaded dextran nanoparticles with gold deposition (Lee et al. 2017).

A lipid-based dextran nanoparticle formulation was developed by Zhang et al. to encapsulate the micro-RNAs for treating osteosarcoma cells (KHOS and U-2OS cells). Dextran acrylate was changed to stearyl amine to accomplish self-assembly of nanoparticles. The microRNAs-loaded nanoparticles, acquire greater stability due to micro-RNAs. These nanoparticles could deliver quite effectively micro-RNAs inside carcinoma cells for transfection, leading to reduction of osteosarcoma cell growth and proliferation (Zhang et al. 2015).

Foerster et al. synthesized PEGylated dextran nanoparticles to target myeloid cells of the liver. Property profile of PEGylated and non-PEGylated nanoparticles for small interfering RNA delivery was compared. PEGylated dextran nanoparticles avoided aggregation of the particles to generate small sized particles. Moreover, PEGylated nanoparticles could bring about change in biodistribution and cellular uptake without causing any cytotoxicity. PEGylated dextran nanoparticles decreased their uptake using peripheral blood mononuclear and murine cell lineages *in vitro* and substantially affected dextran accumulation of nanoparticles from lungs to liver. The low molecular weight PEGylation couldn't make any change in the plasma circulation time. These nanoparticles were removed within 1 day after administration. Overall, PEGylated dextran nanoparticles exhibited lot much of promise in using them in liver macrophage targeting (Foerster et al. 2016).

Tang et al. synthesized a dextran nanoparticle vulnerable to pH-dependent self-assembly. Folic acid on conjugation with dextran produced the desired nanoparticle formulation system. In such system, folic acid promoted pH-dependent self-assembly because of its hydrophobicity and ensured doxorubicin loading by virtue of electrostatic attraction. Folic acid was also instrumental in targeting folate receptors overexpressed in particular by cancer cells. These nanoparticles demonstrated the maximum tumor inhibition effect both *in vitro* and *in vivo*, and the sustenance time of murine breast carcinoma 4T1 cell subcutaneous tumor-bearing mice was extended (Tang et al. 2018).

3.2.2 Protein-Based Polymers

Proteins consist of amino acids. The amino acids are linked with peptide bonds, in a three-dimensional structure which is stabilized by hydrophobic interactions, hydrogen, disulfide bonding and salt bridges. The genesis of protein-based polymers lies in natural tissues. They are characterized by biocompatibility and biodegradability. They break down naturally such that after drug delivery the accumulation of byproducts is abysmally low (DeFrates et al. 2018). Proteins, collagen, albumin and gelatin are the materials made use of quite frequently in the drug delivery application.

3.2.2.1 Collagen

Collagen is one of the abundant proteins in vertebrates. Type I collagen predominates in extra cellular matrix. The properties such as biocompatibility and biodegradability make collagen useful in tissue engineering and in numerous kinds of drug delivery systems. However, collagen is vulnerable to dissociation during separation and refinement. Moreover, it degrades naturally thereby the stability of formulations gets affected. To get over such problems and better its mechanical properties, crosslinking of collagen is done frequently with a host of other natural or synthetic polymers (Chaubaroux et al. 2012; Sionkowska and Kozłowska 2013). Right choice of crosslinking agents and techniques, the property profile of collagen can be tuned in accordance with the therapeutic needs. The necessary alterations, help in controlling their stability and the release behavior profile.

3.2.2.2 Gelatin

Gelatin is a biopolymer and is characterized by biocompatibility and biodegradability obtained by the hydrolysis of animal collagen. Gelatin dissolves rapidly in water; such solubility may limit its effectiveness in drug delivery when used on a long-term as a drug carrier. The mechanical and physiochemical properties of gelatin can be tuned by combining with various crosslinking and targeting agents, thereby modifying their release profile (Bigi et al. 2001). In addition, gelatin is a potential candidate in the production of thermoreversible gels, which opens up new horizons for the application areas of gelatin in pharmaceutical industry (Mad-Ali et al. 2017).

Formulation of Gelatin-Based Nanoparticles

Gelatin is used as a drug vehicle in the treatment of a broad range of cancers (Khan et al. 2018). Lu et al. synthesized gelatin nanoparticles encapsulating paclitaxel used in intravesical bladder cancer therapy. Drug delivery system was used to shield

paclitaxel thereby preventing it from getting diluted on urine production, causing therapeutic failure. The release rate of paclitaxel was controlled by the solubility of drug in the medium. Hence, the paclitaxel concentration remains unchanged irrespective of the urine volume. The dog model study shows that the systemic absorption of the nanoparticles was poor; the drug targetes and accumulates in bladder tissues, with active concentration in pharmacological study is retained at least for a week (Lu et al. 2011).

Wang et al. developed surface-functionalized gelatin nanoparticles for targeted delivery of doxorubicin. 3-carboxyphenylboronic acid as a targeting ligand was employed as a surface attachement moiety to the gelatin nanoparticles. The ligand targets sialic acid which is overexpressed by the tumor cells. The stability and the release rate of doxorubicin nanoparticles increased substantially in the acidic conditions. The 3-carboxyphenylboronic acid facilitated penetration of the nanoparticles in the cells. Doxorubicin was internalized by tumor cells, and gets deposited in the nuclei of the cells. The 3-carboxyphenylboronic acid – functionalized gelatin nanoparticles exhibited the maximum accumulation in the tumors and the highest antitumor activity in the hepatocellular carcinoma cells (H_{22}) – containing mice against free drug or gelatin nanoparticles (Wang et al. 2016).

Hu et al. developed nanoparticle drug delivery system that can shrink with the help of gelatin and poly-L-lysine with dendritic morphology (Hu et al. 2015). Poly-L-lysine is a dendrimer with a size of the order of 5 nm, has an ability to penetrate strongly into tumors (Yevlampieva et al. 2012); while gelatin is biodegraded by using enzyme gelatinases covering matrix metalloproteinase – 2, which is overexpressed in tumor cells (Xu et al. 2013a, b). Doxorubicin was conjugated first with dendritic poly-L-lysine, followed by attaching it to surface of the gelatin nanoparticles. The system on the whole was kept intact in the tumor through the enhanced permeability and retention effect. Matrix metalloproteinase – 2 is available in the tumor microenvironment which hydrolyzes the gelatin nanoparticles to bring about release of the tiny doxorubicin/poly-L-lysine conjugates, which promoted the deep internalization of doxorubicin into the nuclei of tumor cells (Hu et al. 2015).

Moreover, matrix metalloproteinase – 2-responsive gelatin nanoparticles codelivering doxorubicin and 5-aminolevulinic acid were prepared by Xu et al.; 5-aminolevulinic acid is a photosensitizer used in photodynamic therapy. The release of payloads was induced by the availability of metalloproteinase – 2 in the tumor microenvironment. Collective and cumulative effects of chemotherapy and photodynamic therapy were accomplished subjecting to laser irradiation for *in vitro* and *in vivo*. Moreover, the gelatin nanoparticles didn't exhibit any cardiotoxicity when compared with doxorubicin (free) in S180 sarcoma cell-bearing mice (Xu et al. 2019). Application of gelatin nanoparticles in drug delivery for treating non-small cell lung cancer was published.

Karthikeyan et al. encapsulated resveratrol by using gelatin nanoparticles and assessed its therapeutic efficacy particularly in non-small cell lung cancer cells (NCI-H460). The *in vitro* study revealed that consistent release of resveratrol from the nanoparticles was obtained after a burst release. The uptake efficiency by the cells of the formulation was rapid. The resveratrol nanoparticles exhibited better

antitumor activities particularly in the lung cancer cells and swiss albino mice in comparison with the free drug (Karthikeyan et al. 2012, 2015).

Kuo et al. altered the surface chemistry of gelatin nanoparticles by adhering phytohemagglutinin erythro agglutinatine for targeted delivery of gemcitabine in the treatment of non-small cell lung cancer cells cells. Phytohemagglutinin erythro agglutinatine has an ability to improve both targeting and inhibition activity towards overexpressed epidermal growth factor receptors in the tumor environment at micro level. In a nutshell, gelatin on conjugation with with NeutrAvidin-tetramethylrhodamine yielded gelatin nanoparticles showing fluorescence which are used for tracking purpose. Phytohemagglutinin erythro agglutinatine and gemcitabine on grafting to the nanoparticles formed the drug delivery system. The targeting nanoparticles thereby stifle the growth of non-small cell lung cancer cells (A549 and H292) on catalyzing epidermal growth factor receptors phosphorylation and bringing about cell apoptosis (Kuo et al. 2016).

Sanlier et al. developed another magnetic drug delivery system which used gelatin nanoparticles for targeted delivery of gemcitabine. The magnetic gelatin nanoparticles were prepared by combining gelatin and iron oxide suspension, followed by physical adsorption of gemcitabine on the surface of the magnetic nanoparticles. Release of gemcitabine from the nanoparticles was influenced by pH and can be controlled. Release in a sustained manner was accomplished as against the free drug (Hamarat Sanlıer et al. 2016). Gemcitabine is delivered by gelatin nanoparticles as vehicles in the treatment of pancreatic cancer. Xu et al. synthesized epidermal growth factor receptors – targeting gelatin nanoparticles that are thiolated for delivering wt-p53 plasmid or gemcitabine. Both of these gelatin nanoparticles formulations demonstrated excellent antitumor activity in human pancreatic adenocarcinoma (Panc-1)-possessing mice. When these formulations were used in tandem, therapeutic effects were far better as compared to solitary anticancer agents (Xu et al. 2013a, b, 2014).

3.2.2.3 Albumin

The various types of albumin cover human serum albumin, bovine serum albumin, rat serum albumin and ovalbumin. Human serum albumin and bovine serum albumin are used extensively for the development of drug delivery system. These proteins are characterized by high solubility without any toxicity and poor immunogenicity. These protein molecules offer a number of binding sites; therefore, a drug is conjugated with albumin with covalent linkages or by adsorption on their surfaces (Li et al. 2013; Adabi et al. 2017). Albumin can be used as a drug delivery vehicle for cancer therapeutics. It was presented that albumin-bearing nanoparticles have an excellent ability to cross the blood brain barrier. The blood brain barrier penetration can be improved a lot by altering the surface chemistry of albumin nanoparticles by using peptides (Lin et al. 2016).

Formulation of Albumin-Based Nanoparticles

Albumin is the protein-based natural ingredient used commonly in cancer drug delivery. Aljabali et al. applied bovine serum loaded albumin nanoparticles to enhace the properties such as solubility and bioavailability of piceatannol in the treatment of colon cancer. The nanoparticle formulation was stabilized by using a crosslinking agent like glutaraldehyde and it could be uptaken by colon cancer cells (CaCo-2 and HT-29) through endocytosis, because of the size effect shown by nanoparticles. The anticancer activity of piceatannol was enhanced on its fabrication into the nanoparticles. It checked the overexpression of nuclear p65 and hypoxia-induced factors in colon cancer cell lines with greater effectiveness as compared to the piceatannol in free-state. The nanoparticles further exhibited significant inhibition of tumor growth in the murine model ascribed to colon cancer that is induced chemically (Aljabali et al. 2020). Results of similar kind were presented earlier, on attaching other naturally occurring compounds namely, curcumin or caffeic acid phenethyl ester to the surface of polymeric nanoparticles comprising of albumin or artificial polymer (Tambuwala et al. 2019; Khan et al. 2018).

Jithan et al. synthesized bovine serum albumin nanoparticle encapsulating curcumin for treatment of breast cancer. The rate of dissolution and solubility of curcumin were enhanced by the drug delivery system. The nanoparticle system offered consistent release of curcumin both *in vitro* and *in vivo*. In comparison with the free curcumin, drug delivery system – delivered curcumin exhibited better antitumor activity in the treatment of the breast cancer cells (MDA-MB-231 cells). Greater intracellular drug concentration levels were observed with the drug-loaded nanoparticles. The bioavailability of curcumin was modified by incorporating in albumin nanoparticles, based on pharmacokinetics study conducted in mice (Jithan et al. 2011).

Motevalli et al. used bovine serum albumin nanoparticles in order to encapsulate curcumin and doxorubicin for minimizing the adaptive treatment tolerance shown by resistant breast cancer cell lines (MCF-7). The release of drugs from the nanoparticles was evaluated in an acidic buffer environment that simulates the microenvironment prevailing in lysosome. The release of both the drugs from the nanoparticles followed the first-order kinetics. Curcumin exhibited release at slower pace as against doxorubicin, primarily due to more hydrophobicity than doxorubicin. Hence, curcumin interacts with the albumin more vigorously (Motevalli et al. 2019; Chen et al. 2015). Experimentation showed that curcumin and doxorubicin on co-encapsulation in albumin nanoparticles acquired an ability to reduce the adaptive treatment tolerance effect. In contrast, if curcumin and doxorubicin were loaded separately on nanoparticles, curcumin shows aggregation tendency and is vulnerable to entrap by lysosomes, forcing it redundant to prevent P-glycoprotein in the cytosol (Neerati et al. 2013). Consequently, any doxorubicin uptaken by the cancer cells would be released (Motevalli et al. 2019).

Lu et al. synthesized another drug delivery system based on bovine serum albumin nanoparticles for the co-delivery of doxorubicin and cyclopamine. Cyclopamine can enhance doxorubicin piling by resisting expression of p-glycoprotein like

curcumin (Lu et al. 2019; Malhi et al. 2016). Antitumor activities of the formulation were assessed in doxorubicin-inhibitory breast cancer cells (MDA-MB-231) and tumor-carrying mice. The nanoparticles formulation substantially brought about the reversal of the drug resistance in the model of cancer cells. The distribution may take place at the tumors and lymph nodes in the xenograft model, resisting growth of tumors and decreasing distant metastasis (Lu et al. 2019).

Onafuye et al. brought about encapsulation of doxorubicin in human serum albumin so as to get over the transporter-induced drug resistance of cancer cells. Doxorubicin is a substrate of ATP binding cassette subfamily B member 1 (p-glycoprotein). The albumin-based nanoparticles enhanced the anticancer activities of doxorubicin in the drug-resistant cells (Onafuye et al. 2019). The application of albumin as a nanocontainer helps in improving the transporter-induced drug resistance as against synthetic polymers like poly (lactic-co-glycolic acid) and polylactic acid as the nanovehicles (Pieper et al. 2019). Kimura et al. synthesized doxorubicin-loaded human serum albumin nanoparticles to assess the antitumor activity. The cytotoxicity of the nanopaticles formulation system was poorer as compared to doxorubicin *in vitro*, while contradictory findings were found *in vivo* although there was not much of difference in the profiles of biodistribution. Moreover, the drug delivery system reduces tumor metastasis. Further investigations are required to study the underlying mechanisms of the way nanoparticles work when cancer cells both *in vitro* and *in vivo* are treated (Kimura et al. 2019).

Zhang et al. used human serum albumin nanoparticles for encapsulating the doxorubicin prodrug. The nanoparticle formulation system was sensitive to pH and vulnerable to aggregation in an acidic medium because of the protonation of carboxylic acid groups in doxorubicin prodrug, which further got accumulated and retained in tumors. The prodrug nanoparticles exhibited higher cellular uptake and cytotoxicity as compared to nanoparticles of doxorubicin; whereas the *in vivo* anticancer efficacy both formulations were similar. Both of these nanoparticles exhibited poorer cardiotoxicity as against the free doxorubicin (Zhang et al. 2020).

Wang et al. synthesized an effective cytotoxic derivative of maytansine. The safety profile of the prepared derivative was enhanced in an animal model. The release profiles could be controlled by maintaining the correct ratio of drug to albumin while preparing nanoparticle formulation. The formulation initially provided the protection of the drug molecules from an early release and fast body clearance (Wang et al. 2017). Lee et al. prepared human serum albumin nanoparticles of paclitaxel. PEGylated human serum albumin nanoparticles were synthesized for better therapeutic effect of paclitaxel in cancer chemotherapy. This formulation was subjected to lyophilization and rehydration before use. Therapeutic efficacy of the formulation was compared with the commercial drug, Abraxane® in numerous breast cancer cell lines. The same formulation showed a better cytotoxicity. In a tumor-carrying mice model,

PEGylated human serum albumin nanoparticles lengthened the time of circulation of paclitaxel beyond 96 h and paclitaxel was found to pile up in the tumors, leading to an excellent antitumor activity consequently enhancing the cells survival rate (Lee et al. 2018a, b).

Doxorubicin-loaded cationic albumin nanoparticles were synthesized by Abbasi et al. for treatment of breast cancer (Abbasi et al. 2012). Polyethyleneimine was used to acquire a positive charge on nanoparticles and thereby enhancing the stability of the nanoparticles (Wang et al. 2008). The incorporation of the positively charged polymers exacerbated the cellular uptake efficiency of breast cancer cells (MCF-7) and such formulation demonstrated more cytotoxicity in breast cancer as compared to free doxorubicin (Abbasi et al. 2012).

3.3 Application of Polymeric Nanoparticles in Targeting Solid Tumors

3.3.1 Active Targeting

Polymeric nanoparticles are primarily a class of polymer-based carriers in the nanoregime (Padhi and Behera 2020). The polymeric nanoparticles as a potential drug delivery system has acquired a lot much of importance by virtue of its characteristics such as biodegradability, biocompatibility, simple to design, and huge prospects on structural variation front (Padhi et al. 2020). Application of nanoparticles in biomedical and pharmaceutical fields is very broad particularly in the diagnosis and treatment of cancer (Afsharzadeh et al. 2018; Delouise 2012; Thambi and Lee 2019).

However, since it has more cost and batchwise differences in production output that limits its presence in market. Nanoparticles are classified as nanospheres or nanocapsules. Such nanoparticles find wide use in the area of nanomedicine, targeted drug delivery systems, sustainment in the release and as a theranostic vehicle. Very commonly applied methods in the production of nanoparticles are classified as "top-down" and 'bottom-up' approach. The top-down approach involves polymers used in manufacturing nanoparticles, while, the bottom-up approach refers to polymerization of monomers resulting into the formation of nanoparticles. The selection of right methods for preparation is influenced by the nature of solvent, drug properties and polymer type. The way it is released from polymeric nanoparticles is governed by drug trapping technique like adsorption, absorption, entrapment, and type of polymer used (Crucho and Barros 2017). A host of natural and synthetic derived polymers have been in the use for the preparation of nanoparticles. Polymeric nanoparticles have tremendous potential in overcoming the biological barriers. The penetrability of nanoparticles through the biological barriers is attributed to either active or passive pathway.

Tumor tissue has low vasculature in comparison with the normal healthy tissue because of angiogenesis which is responsible for enhancing vascular permeability at the tumor sites (Ganta et al. 2014). The weak lymphatic drainage of cancer cells results into a delayed macromolecular clearance. This is referred to as enhanced permeability and retention effect which helps in delivering many nanovehicles to

the tumor site. This retention by enhanced permeability and retention is largely caused by macromolecules covering lipids and drugs while small molecules diffuse into the blood circulation (Tripathi et al. 2019; Verma et al. 2017). The size of nanoparticles plays a vital role in the tumor cell uptake efficiency, phagocytosis and rapid renal clearance. This parameter influences circulation half-life and therapeutic effects of anticancer drugs. Change in surface chemistry by using a biocompatible hydrophilic polymer namely, poloxamer and polyethylene glycol can reduce the phagocytosis of nanoparticles from macrophages.

3.3.2 Passive Targeting

The surface charge held by nanoparticles play a significant role in passive targeting, for example, cellular uptake of cationic nanoparticles is easy and rentained for a longer time as against anionic ones. However, a major limitation of passive targeting is the inability to distinguish tumor cells. This is due to nonfeasibility of passive targeting across all tumors because different tumors possess a tumor vascularization and fenestration of blood vessels to different extents. It is revealed from the earlier study that every tumor cell is overexpressed by a particular type of receptor on its cell surface. Targeting such receptors by using ligands in order to recognize and internalize nanoparticles into tumor cells is considered as active targeting. The ligands frequently employed are monoclonal antibodies or fragments of antibody, antigen binding, and single chain variable fragments (Bregoli et al. 2016). However, such mechanism requires not ony the exclusive expression of receptors but also the uniform expression homogenous across targeted cells. The active targeting mechanism is responsible for increasing the cytotoxicity to tumor tissues while reducing the delivery of toxic drugs to healthy tissues (Bar-Zeev et al. 2017; Bregoli et al. 2016).

Folic acid conjugated with polyurethane nanocarrier which is loaded with paclitaxel exhibited improved apoptosis and therapeutic efficacy of folate–targeted nanoemulsion (Ajorlou et al. 2016). Moreover, cumulative stimuli sensitivity (magnetic field, temperature, radiation, and pH) with surface charge alteration is taken as active targeting. Jiang et al. conjugated the poly (lactic-co-glycolic acid) nanocontainer with positively charged chitosan and biotin for active targeting. The findings showed the synergistic effect of surface charge possessed by chitosan and ligand biotin reflected upon cellular uptake efficiency of the designed nanocarrier (Jiang et al. 2014).

The resistance offered by cancer cells is either intrinsic or extrinsic or acquired which facilitates the efflux of the drugs from the cells, leading to reduction in the cytotoxic potential of the chemotherapeutic agents and thereby enhance toxicity of the drugs effluxed at normal cells. This efflux results into reduction in the concentration levels of the chemotherapeutics, which further leads to nonresponsive recurrence, and then failure of the treatment as well as patient morbidity. With the very purpose of developing novel drug delivery system for improved targeted delivery

accompanied by lowered adverse effects, Su et al. have prepared cholic acid -functionalized- poly(lactic-co-glycolic acid) – D-α-tocopheryl polyethylene glycol succinate star-shaped nanoparticles to treat malignant melanoma (Su et al. 2017). *In vitro* and *in vivo* study results of this paclitaxel-loaded star-shaped cholic acid – poly(lactic-co-glycolic acid) – D-α-tocopheryl polyethylene glycol succinate nanoparticles showed better performance in the cancer cells (Su et al. 2017).

Other polymeric nanoparticles that are pH-sensitive were prepared to deliver curcumin and doxorubicin with monomethoxy – poly ethylene glycol-b-poly (lactic-co-glycolic acid) – b-P-(L-glutamic acid) polymer and have been used to target cancer stem cells in conjunction with the various cancer cells. pH-based release variation (at pH 5.0) of the chemotherapeutics from the nanoscale polymeric nanoparticles possessing negative charge at surface showed marked antitumor activity and efficacy towards *in vitro* and xenograft mice model too.

Numerous approaches have been employed get over the problem of multi drug resisance by using nanoparticle formulations of chemotherapeutics and genetic modulators. The problem of multi drug resisance in the lung cancer therapy was overcome by doxorubicin encapsulated poly(juglanin dithiodipropionic acid)-b-poly ethylene glycol-small interfering RNA Kras nanoparticles. These nanoparticles showed sustained release profile and were quite effective towards multi drug resisance cancer cells. Reduction in p-glycoproteinp and c-Myc expression (a multifunctional transcription factor that expresses during rapid cell division) and increase in p53 at the same time showed decrease in cancer cell proliferation which may be attributed to the cumulative effect of juglanin, doxorubicin and small interfering RNA Kras. Thus drug-resistant lung cancer proliferation may be targeted in the advanced research in the time to come.

Incorporation of pluronic in nanotechnology is one more strategy adopted to overcome the multi drug resisance. Pluronics comprise of a [poly (propylene oxide)] unit (hydrophobic in nature) sandwitched between two [poly (ethylene oxide)] units (hydrophilic in nature). Therfore to prevent or reverse mechanisms of multi drug resisance, pluronic has been reported (Chowdhury et al. 2017). Pluronic 85 in the similar way has been introduced in the polymeric nanoparticles against leukemia. Transferrin truncated in doxorubicin encapsulated pluronic 85/lipid-polymeric nanoparticles predicted high antitumor efficacy towards acute myeloid leukaemia and resistant cancer cell lines. Similarly, the polymeric nanoparticles have showed the maximum antitumor activity in resistant cancer – carrying experimental animal showing minimum systemic toxicity. Guo et al. used sericin polypeptide to prepare pH-sensitive sericin- poly (γ-benzyl-L-glutamate) micelles by facilitaing the cellular uptake of the anticancer drug (doxorubicin) through clathrin – driven endocytosis route. They observed that sericin – poly (γ-benzyl-L-glutamate) micelles check the drug resistance by boosting micelles internalization over inhibition of the P-glycoprotein receptors (Guo et al. 2018).

Ball milled nanoparticles deliver docetaxel and resveratrol mixture in conjugation with folic acid so as to treat prostate cancer. Cytotoxicity was improved a lot in addition to decontrol of survivin with enhanced Caspase-3 expression in human PCa cell lines (cleaved). Moreover, ATP-binding cassette transporters markers get

reversed when docetaxel resistant PCa cells are exposed to folic acid conjugated nanoparticles. Ball milled nanoparticles were internalized on incubation for 1 h, 30 mins, while acid conjugated nanoparticles loaded with docetaxel and resveratrol enhanced the concentration levels of drug from membrane to cytoplasm via the receptor-driven endocytic route. It was also found that conjugated nanoparticles counteract the multi drug resisance efflux pumps and enhanced the uptake efficiency of cells. Transmission electrom microscopic images confirmed the phenomenon wherein nanoparticles treated cells exhibited a roughened surface as against control brought about by the internalization of nanoparticles.

Cryotherapy has been in use to prevent multi drug resisance in cancer treatment. It involves use of responsive nanoparticles resulting into release of chemotherapeutic agents by bursting on exposing to ice cooling. Wang et al. studied the ability of cold responsive nanoparticles formed by the combination of phospholipid and polymer to get over the drug resistance in ovarian cancer. The probable mechanism underlying this therapy is burst release of drug from nanoparticles after cold exposure, where the drug released comes out from lysosome due to concentration gradient profile and damage the lysosomes by cryo dissembling of nanoparticles. The drug released in large quantity in cytoplasm easily attach to targeted molecules prior to expelling it using efflux pump. Cell viability findings are in harmony with this phenomenon. Another mechanism underlies a decrease in efflux pump activity with the help of ice cooling. In addition, the polymeric nanoparticle gets accumulated upto a greater extent at the tumor sites (Wang et al. 2018).

3.4 Conclusion

Natural material based drug delivery systems have shown promising results as compared to conventional therapies. They offer abundant benefits such as biocompatible, biodegradable, nontoxic and nonimmunogenic, and easy modification of the structure for functionalization. When designing drug delivery formulation, by using natural materials for cancer treatment several strategies can be considered. Different natural materials carry various electronic charges, which are highly associated with the encapsulation of therapeutic agents. Electronic charges can be adjusted by conjugating polyamines with the natural materials. Mechanical properties, targeting ability and drug release manner can also be controlled by modifying the structure of natural materials with polyamines, small molecules and targeting ligands. In order to optimize the therapeutic performance of drug delivery systems, more than one natural ingredient can be employed. In some cases, synthetic materials, metallic particles or hybrid liposomes are employed to achieve multiple purposes. Examples and recent advances have been discussed in the above sections. Preliminary results have shown the potential of combining organic and/or inorganic components in one system to maximize the functions of drug delivery systems. It may play an important role in development of drug delivery systems.

It is difficult to translate the experimental findings into clinical applications. Abraxane® is the only natural nanomaterial approved by the food and drug administration till now used in cancer therapy. Although some other naturally occurring materials namely, chitosan are identified by the food and drug administration as food under provisions of generally recognized as safe, however, they have not been approved as nanomaterials to be used in cancer drug delivery till now. The origin of natural materials lies in a plethora of sources; therefore, attaining high purity standards is a herculean task as there are variations in different batches. The safety profile of natural polymeric materials may be influenced by the contaminants present in the matrix of such molecules. Quality standards of every natural material have to be laid down and maintained so as to use them in clinical applications. Moreover, derivatives or crosslinkers are not amenable to complete degradation by natural enzymes. Such problems need to be addressed, though drug delivery systems made out of natural polymeric nanoparticles have shown a lot much of potential and promise in preclinical studies.

Other than the biological bottlenecks, there are some technical challenges too. The scalability of materials which involve conversion of natural polymer into nanomaterials is tedious with respect to transfer from laboratory to industrial scale production. Production and characterization of polymeric nanomaterials demands advanced and sophisticated equipments affecting high manufacturing cost. The stability of the drug delivery systems too is a major concern during synthesis. *In vivo* assessment is necessary for translating it into clinical applications. In few cases, *in vitro* and *in vivo* findings are found to be contradictory which need further study in exploring the disease mechanism at greater depth. Drug delivery systems with specific functions need to be designed to get over the problems based on the merits of the cases. Moreover, the wide gap between the patients and animal models needs to be ascertained for evaluating the efficacy of the therapeutics. Comprehensive testing and characterizations are required in all cases prior to transferring natural material-derived drug delivery systems from the laboratory scale to the bedside in cancer treatment.

This chapter summarizes a spectrum of natural polymeric materials that find use in drug delivery carriers in cancer therapy. The unique properties possessed by such materials make it possible to develop tailored or truncated drug delivery systems for better therapeutic effects of drugs. The clinical applications of natural polymeric nanomaterial-induced drug delivery systems are not limitless presently. Once the clinical translation and scalability issue is sorted out application of the advancement in nanotechnology, using natural material-driven drug delivery systems may become a quite meaningful strategy for cancer therapy.

Site-specific delivery of the anticancer drug is of vital importance from the point of view of the researchers and to enhance efficacy and survival of cancer patients. Therefore, research and development efforts globally are underway to develop targeted delivery of the chemotherapeutic agents to obtain patient friendly therapy. However till now, numerous patients treated by traditional therapy suffer from chronic systemic toxicity because of uneven distribution profile of the drug in normal healthy tissues which takes toll of millions of people every year. Despite

research is underway on cancer, still handful of nanoformulations are actually useful for treatment. Thus, huge medical demands of nanomedicine in therapy and diagnosis need to be met.

These constraints in applicability are attributed to cost and stability of natural polymeric nanoparticles as they possess very high surface free energy, which makes it to agglomerate. The other limitation is the cytotoxicity of nanoparticles which at times is unpredictable and differs from case to case. The interaction mechanism of nanoparticles with biological systems is yet to be unravelled and better details are required pertaining to the effect of morphology, surface charge and composition on toxicity of nanoparticles. Additionally, occupational hazard data and tolerance limits of new polymeric nanomaterials need to be available. The unprecedented future of nanotechnology holds lots of promises in cancer therapy. Continuous research initiatives for the development of novel polymeric nanomaterials-based drug delivery systems will pave the way in setting the tone to develop a safe, efficient and effective treatment of this fatal disease. Revolutions in this area of research will surely go a long way with cheaper, reliable and sophisticated technology for cancer diagnosis and targeted delivery of drugs to ensure cure of the disease on the whole.

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Chapter 4
Optimization of Physicochemical Properties of Polymeric Nanoparticles for Targeting Solid Tumors

Parteek Prasher, Mousmee Sharma, and Harish Mudila

Abbreviations

4 T1	Breast cancer cell lines
MCF7	Michigan Cancer Foundation-7

4.1 Introduction

The application of nanotechnology has gifted us with an array of nanocarriers such as lipid based nanoparticles, polymeric nanoparticles, metallic nanoparticles, nanoemulsions, carbon nanotubes, quantum dots and so on (Behera et al. 2020a, b; Hassan et al. 2021). Out of the pool of stated nanocarriers, polymeric nanoparticles have garnered wide attention of researchers. The physicochemical properties of polymeric nanoparticles including hydrophobicity, molecular weight, crystallinity, surface charge, nature of the co-polymer, and glass transition temperature markedly

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influence their ability to target the tumors (Morachis et al. 2012; Prasher et al. 2020a, b). Importantly, the biodegradability and stimuli responsiveness of drug carrying polymeric nanoparticles ensures a targeted delivery of the payload drug at the cancer site, without harming the surrounding healthy cells (Li et al. 2017). Specifically, the positive charges on cationic polymeric nanoparticles facilitate their electrostatic interactions with negatively charged phospholipid head groups expressed on the tumor cell endothelium (Farshbaf et al. 2018). However, the cytotoxicity of polymeric nanoparticles towards tumor cells largely depends on their internalization, and localization within the cellular environment to express an optimal cytotoxic effect (Sharma et al. 2020; Padhi et al. 2018).

The cationic polymer-based drug delivery nanosystems enable an efficient delivery of small interfering RNA, which silences the target genes in cancer cells, in addition to sensitizing the latter towards anticancer drugs such as paclitaxel (Conte et al. 2020). The polymeric nanoparticles act as carrier system for theranostic materials, contrast agents, and radionuclides where the enhanced permeability and retention effect of nanoparticles plays a critical role in achieving the desired effect (Kang et al. 2020). The fabrication of polymeric nanoparticles with macromolecules such as albumin enhances their interaction with tumor microenvironment, and enhances the lifetime of circulating nanoparticles by preventing their recognition and clearance by mononuclear phagocytic systems (Hyun et al. 2018). This phenomenon improves the pharmacokinetics of the cargo drug carried by the nanoparticle, thereby improving its therapeutic efficacy (Owens and Peppas 2006). However, only the native conformation of albumin serves as a favorable surface modification agent of polymeric nanoparticles for achieving an effective delivery of anticancer drugs to solid tumors (Prabhu et al. 2015). In this chapter, we discuss the effects of various physicochemical attributes of polymeric nanoparticles for targeted drug delivery to solid tumors.

4.2 Physiology of Solid Tumors

The solid tumors possess a distinctive physiology compared to the normal tissues arising mainly due to a poor vasculature, deprived oxygenation, abnormal pH and temperature, and a lack of essential nutrients required for an optimal functioning of cellular processes (Brown and Giaccia 1998; Tannock et al. 2001; Padhi et al., 2015). The tumor cells possess abnormal vasculature with distended capillaries and enervation of smooth muscles with poorly formed endothelia and basement membranes. The arterio-venous shunts result in leaky walls thereby causing a tortuous blood flow (Siemann 2010). The leakiness of the tumor blood vessels caused by discontinuous endothelia raises the interstitial pressure that further prevents the fresh blood supply to the tumor tissues (McDonald and Baluk 2002). Overall, these factors weaken the local action of the deliberated pharmaceuticals due to inadequate concentration to attenuate the target solid tumors (Schaaf et al. 2018).

The inadequate administration of the directed pharmaceutical to the tumor site causes the survival of residual tumor cells that manifest relapse of the malignancy (Forster et al. 2017). Similarly, a weak lymphatic drainage in solid tumor adversely affects the drug delivery to the solid tumors and its retention in the tumor vasculature for an effective action (Padera et al. 2016). Mainly, the erroneous lymphatic flow and a leaky vasculature in the solid tumors enhances the permeation and retention of high molecular weight drug molecules compared to smaller therapeutics (Shayan et al. 2006), which get readily redistributed in systemic circulation mainly by diffusion. Moreover, the small tumor volume ensures a high permeability and retention due to high density of vasculature, compared to large solid tumors with heavy vasculature at the periphery but avascular regions at the center (Mohammed et al. 2019; Alitalo and Detmar 2012). However, the impaired lymphatic drainage causing the enhanced drug retention in solid tumors overrides the enhanced accumulation of high-molecular weight therapeutics caused by vascular permeability and extravasations in tumor vasculature (Miyamoto et al. 1990; Krishan et al. 1997). Importantly, the absence of functional lymphatic system raises the interstitial fluid pressure in solid tumors that prevents the loss of therapeutic macromolecules from the leaky microvasculature of solid tumors (Heldin et al. 2004; Ferretti et al. 2009; Azzi et al. 2013; Kelin et al. 2018).

Reportedly, the heterogeneously distributed hypoxic tissues with $pO_2 \leq 2.5$ mm of mercury represent solid tumors, which arise due to imbalance between the supply and consumption of oxygen (Jing et al. 2019). Generally, the solid tumors consume higher oxygen content compared to the normal tissues, which exceeds the supply (Muz et al. 2015). The structural deformities in the tumor vasculature impede the microcirculation, thereby limiting the oxygen supply in the affected areas (Tameemi et al. 2019). Similarly, the higher diffusion distances between the nutritive vasculature and tumor cells result in poor perfusion followed by oxygen deficiency eventually causing diffusion-limited hypoxia (Petrova et al. 2018). Therapy-induced anemia and functional anemia caused by deterrence in the oxygen transport ability of blood serves as a major cause of anemic hypoxia that manifests malignancy (Dunst and Molls 2002; Varlotto and Stevenson 2005).

Hypoxia-inducible factors monitor the cellular response to oxygen levels by regulating angiogenesis, cell differentiation and the mediation of apoptosis (Brown 2007). In hypoxic conditions, the dimerization of hypoxia inducible factor-α and its association with the hypoxia-responsive elements causes metabolic reprogramming and tumorigenesis (Vaupel 2009; Sorensen and Horsman 2020). The higher glucose uptake by cancer stem cells causes preferential glycolysis over oxidative phosphorylation that lowers the susceptibility of solid tumors towards chemotherapy and radiotherapy (Kanniappan and Geschwind 2013; Pelicano et al. 2006). The enhanced lactate release caused by higher rate of glycolysis promotes DNA repair mechanism in solid tumors that provides protection against chemotherapeutics (Jose et al. 2011).

The increased lactate release in solid tumors, in addition to glutaminolysis, bicarbonate depletion (Huber et al. 2017) and ATP hydrolysis cause tumor acidosis (Villar et al. 2015; Lopez et al. 2019; Kennedy and Dewhirst 2010). This creates a pH gradient across the tumor cell membrane, where the extracellular pH remains

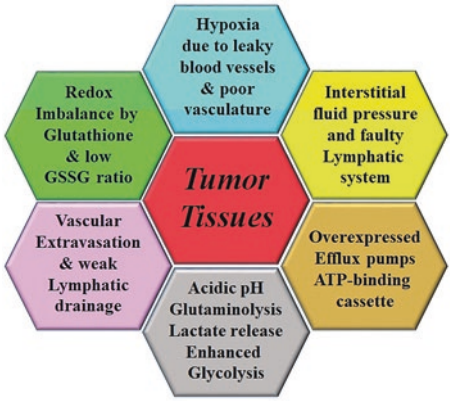

Fig. 4.1 Physicochemical conditions of a typical tumor microenvironment. The tumor tissues show redox imbalance, suffer hypoxia, have a high interstitial fluid pressure, have faulty lymphatic system, display overexpression of efflux pumps, display acidic pH, and undergo vascular extravasations leading to weak lymphatic drainage

higher compared to the intracellular pH (Kato et al. 2013; Chiche et al. 2010). Nevertheless, the over-expression of membrane bound efflux pumps in the solid tumors possess potential to selectively reject the chemotherapeutics directed as anti-cancer drugs, that serves as a mainstay for offering drug resistance in the solid tumors (Ughachukwu and Unekwe 2012; Robey et al. 2018). Figure 4.1 illustrates the physicochemical conditions of a typical tumor microenvironment.

4.3 Physicochemical Properties of Polymeric Nanoparticles for Targeting Solid Tumors

4.3.1 Size and Molecular Weight

The physicochemical properties of polymeric nanoparticles significantly influence their biological applications for drug delivery and controlled release (Kamaly et al. 2016; Sanchez et al. 2020; Sonam et al. 2013; Verma et al. 2017). The polymeric nanoparticles constituted from high molecular weight polymers with a longer chain length decelerate the degradation rate of the drug encapsulated polymeric nanoparticles, thereby providing a sustained drug release (Su and Kang 2020; Kumari et al. 2010). In addition, the polymeric nanoparticles ensure a better drug loading, improved bioavailability, superior pharmacokinetics and ameliorated absorption in the intestine (Abdifetah and Na-Bangchang 2019; Zhao and Qiu 2013).

However, the reduction in size of polymeric nanoparticles results in lowered encapsulation efficiency (Parnell et al. 2009), which mainly arises due to the decrease in viscosity of the organic polymeric phase caused by decreasing molecular weight of the constituent polymer (Prasher et al. 2020b). This results in an increased diffusion of the encapsulated drug in the surrounding physiological aqueous phase thereby causing a burst release (Son et al. 2017). The molecular weight and size of the polymeric nanoparticles influences its hydrophobicity, which decides

absorption pattern and degradation kinetics of the drug delivery system (Fu and Kao 2010).

4.3.2 Hydrophobicity

Mainly, the polymeric nanoparticles possessing a lower hydrophobicity demonstrate a rapid degradation, while those possessing a higher hydrophilicity exhibit a prolonged drug release (Guncum et al. 2018; Sung and Kim 2020). Interestingly, the hydrophilic polymeric nanoparticles improve the absorption of encapsulated drug molecules by the absorptive enterocytes in gastrointestinal epithelia pertaining to their robust mucoadhesive characteristics (Taipaleenmaki and Stadler 2020; Ensign et al. 2012; Ghosh 2000).

Similarly, the polymeric nanoparticles made up of hydrophobic polymers undergo a rapid uptake by the peyer's patches lining the gastrointestinal epithelia (Bacchav et al. 2018; Ma and Willams 2017; Pridgen et al. 2015). The delivery of drug-loaded nanoparticles to the intestinal peyer's patches poses significant challenge for a rapid and intact passage through the stomach. The hydrophobized mucoadhesive nanoparticles of rifampicin – poly methylvinylether maleic anhydride copolymer (gantrez AN-110) successfully improved the uptake of the drug via peyer's patches due to an improved localization in the intestine. The 400–500 nm average particle size of nanoparticles and > 13% loading capacity towards rifampicin provided optimal transit through the lymph. The *in vivo* analysis suggested a higher intestine-to-stomach ratio for the drug carrying nanoparticles, hence confirming the intestinal availability for improved uptake by the peyer's patches, which improved the bioavailability by 182.4%. Eventually, the 28-day oral dose revealed a high safety profile of the nanosystem with a reduced hepatotoxicity (Bacchav et al. 2018).

Owing to a lack of affinity towards mucus, the lymphatic uptake of hydrophobic polymers via peyer's patch results in the improved bioavailability of encapsulated drug molecules carried by these polymeric nanoparticles (Snipstad et al. 2014; Gericke et al. 2020). Notably, a superior transcellular transport and a trivial P-glycoproteins efflux by microfold cell contribute to the bioavailability of hydrophobic polymeric nanoparticles thereby making them an attractive drug delivery vector (Werle 2008; Schnurch and Grabovac 2006). Conversely, the mucosal affinity of hydrophilic polymeric nanoparticles pertaining to an opposite surface charges increases their retention in the mucosal epithelia further ameliorating the bioavailability of the encapsulated drug molecules (Lai et al. 2009; Zhou et al. 2019; Rossi et al. 2019; Yu et al. 2012).

The nanoparticles made up of di-block biodegradable co-polymer poly (sebacic acid) and poly (ethylene glycol) enabled the crossing of mucus barrier. The analysis on human cervicovaginal mucus indicated that the nanoparticles displayed a rapid diffusion rate at 12-times higher speed as compared to the water. However, the nanoparticles made of poly (lactic-co-glycolic acid) diffused at much slower rate (3300-times slower) in the human cervicovaginal mucus when compared to water.

Notably, the nanoparticles showed instant penetration to the sputum of the patients suffering from cystic fibrosis (Tang et al. 2009).

4.3.3 Surface Charge

The surface charge plays a critical role in the determination of surface fabrication of polymeric nanoparticles for a better absorption of the encapsulated drug molecules (Du et al. 2018). The positively charged polymeric nanoparticles improve the uptake and absorption of the encapsulated drug molecules across the gastrointestinal tract mainly due to the mucoadhesive properties of these nanoparticles (Khachane et al. 2011). The positively charged polymeric nanoparticles improve the therapeutic efficacy of water insoluble therapeutics such as meloxicam. The drug-loaded positively charged nanoparticles made of biodegradable poly-epsilon-caprolactone and cationic surfactant such as di-dodecyl dimethyl ammonium bromide plays an important role in drug delivery applications with synergistic inhibition of ulcers and inflammation. These nanoformulations improved the encapsulation efficacy of meloxicam by 90%, in addition to a significant amelioration in the therapeutic efficacy.

Similarly, the negatively charged polymeric nanoparticles displayed a superior oral absorption due to their affinity towards microfold cell (Chen et al. 2018). The presence of hyperbranched polymers improved the *in vitro* immunoreactivity and hemocompatibility of the methacrylate based polymers in primary human blood cell assays. The analysis indicated that the charge played only a trivial role on the coagulation of nanosystem, or in the activation of platelet, and T lymphocyte cell. The higher concentration of cationic hyperbranched polymeric nanosystem resulted in the activation of dendritic cells, hence presenting applications as vaccine adjuvants. The biodistribution analysis of intravenously administered hyperbranched polymeric nanoparticles improved their retention time in systemic circulation, which accumulated in liver and spleen, eventually releasing in urine. Besides, the surface charge plays a critical role in determining the stability of colloidal solutions of drug delivery systems based on polymeric nanoparticles (He et al. 2010). The presence of a high surface charge on polymeric nanoparticles reduces the event of agglomeration in colloidal solution due to inter-particle electrostatic repulsions thereby stabilizing the nanoparticle solution (Shao et al. 2015).

The nanoparticles having a positive charge displayed a higher toxicity compared to those with a negative zeta potential. The nanoparticles having a higher ‘like’ charges showed robust interactions with the cells. The neutral polymeric nanoparticles demonstrated a trivial opsonization as compared to the charged counterparts; especially the presence of surface positive charged which led to a heightened immune response (Han et al. 2018). The polymeric nanoparticles with charged surface undergo a rapid phagocytosis, which extends their applications for managing the intracellular infections (Xiao et al. 2011).

The presence of primary amine (-NH_2) group at the surface of polymeric nanoparticles encourages their uptake by phagocytosis compared to the terminal sulphate (-SO_4^{2-}), hydroxyl (-OH^-) and carboxylic acid (-COOH) groups (Faroozandeh and Aziz 2018; Behzadi et al. 2017). The presence of positively charged groups on polymeric nanoparticles favor their interactions with membrane of target cells eventually improving the pharmacokinetics of the encapsulated drug by promoting its cellular internalization and biodistribution (Arvizo et al. 2010; Forest and Pourchez 2017).

4.3.4 Crystallinity

The crystallinity of polymer determines the kinetics of degradation and drug entrapment efficiency (Karavelidis et al. 2011). The polyester based on 1, 3-propanediol and aliphatic dicarboxylic acids led to the preparation of ropinirole hydrochloride – loaded polymeric nanoparticles. The constituent polyester polymers carry varying degree of crystallinity from 30% to 67.5% for applications as drug delivery vehicles. However, the toxicity of these nanocarriers towards human umbilical vein endothelial cells limits their applications in drug delivery. A typical drug loading method of polyesters involve solvent evaporation and emulsification that led to the mean particle size 164–228 nm, with drug loading capacity of 16–23%. The X-ray diffraction analysis revealed the existence of hydrochloride salt of ropinirole drug in amorphous state inside the polymer matrices of polymeric nanoparticles. The nanosystems displayed a burst release of the encapsulated drug for the first 6 h mainly due to the presence of drug molecules at the surface of polymeric nanoparticles. Eventually, the rate of drug released slowed.

Mainly, the polyesters having a low degree of crystallinity demonstrated higher drug release efficacy. These inferences concluded that the degree of crystallinity of polymeric nanoparticles presented an important parameter for drug release properties. These parameters get reduced in the presence of high crystallinity because the drug encapsulation mainly occurs in the amorphous regions since the crystallinity presents barrier to restrain the drug encapsulation and demonstrates high water impermeability (Tamboli et al. 2013). The rationally designed pentablock copolymers of polylactide – polycaprolactone – poly (ethylene glycol)-polycaprolactone-polylactide offered sustained delivery of steroids in a controlled manner. The drug delivery system provided an *in vitro* release profile of triamcinolone acetonide, based on the nanoparticle crystallinity. Similarly, the amorphous nature of polymer directly influences its degradation kinetics and hence the drug release properties (Glavas et al. 2013). The array of copolymers having semicrystalline or amorphous hydrophobic blocks constructed on ε-decalactone, ε-caprolactone, and L-lactide using poly (ethylene glycol) as initiator resulted in the formation of micelles with amorphous cores. The core crystallinity assisted in the tailoring of micelle properties and it facilitated the drug delivery applications.

The degree of crystallinity also decides the drug release behavior of the encapsulated drugs, as the amorphous regions of semi-crystalline polymers displays a higher degradation rates due to less organization (Harting et al. 2019). A polymeric chain containing stereo-regular structure with repeating monomeric units results in the crystallization of high molecular weight linear polymers. Crystallinity significantly influences the rate of release of the drugs from polymeric nanoparticle depending on the orientation and size of the polymeric chains. A higher degree of crystallinity results in a slower release of the drug molecules. However, the polymeric nanoparticle with high molecular weight and porosity pose a low impact on the drug release properties (Ehrenstein 2001).

4.3.5 Biocompatibility

Polymeric nanoparticles present potential applications as smart drug delivery systems owing to their biocompatibility, structural variety, bio-imitative properties, and targeted delivery (Liechty et al. 2010; Sung and Kim 2020). The contemporary applications of polymeric nanoparticles include the delivery of vaccines, antibiotics, and anti-cancer chemotherapeutics (Cheow and Hadinoto 2014; Gad et al. 2016). The target specific drug delivery through polymeric nanoparticles overcomes the intricacies associated with the conventional anticancer drug delivery systems such as low water solubility, rapid clearance, lower retention, poor selectivity, and toxicity towards healthy tissues (Calzoni et al. 2019; Sabzaver et al. 2019). The polymeric nanoparticles promote the oral delivery of anticancer therapeutics, the emerging phenomenon in anticancer drug delivery (Ulbrich et al. 2016, Khuroo et al. 2014).

The encapsulation of deliberated pharmaceuticals in the polymeric nanoparticles enables the evasion of efflux transporters, provides defence against the enzymatic degradation and promotes the ingress of cargo drug across the physiological membranes (Singh and Lillard 2009; Rollerova et al. 2011). These factors promote an optimal effective concentration of the drug at the target site. The polymeric nanoparticles enable active and passive targeting of solid tumors, whilst sparing the normal cells (Lee and Feijen 2012; Padhi and Behera 2020). The passive targeting of tumors by polymeric nanoparticles focuses on the enhanced permeability and retention of the therapeutic within the faulty tumor vasculature and lymphatic network (Yan et al. 2019). Whereas, the active targeting involves the receptor mediated endocytosis of the polymeric nanoparticle encapsulated drug inside the tumor vasculature (Attia et al. 2019; Behera and Padhi 2020).

The polymeric nanoparticles for active drug transport bear ligands such as proteins, engineered antibodies, and peptides for the recognition by tumor specific receptors (Luo and Prestwich 2002). Importantly, the polymeric nanoparticles deliberated for anticancer drug delivery bear surface masking ligands fabricated to prevent the recognition by immune cells thereby preventing their elimination, or hyperresponsiveness by innate immune response (Guo et al. 2019). Mainly, the

hydrophilic polymers with blood compatibility such as polysorbate, poly-(ethylene glycol), monomethoxy poly-(ethylene glycol) restrain phagocytosis, and delay the opsonization by plasma proteins thereby promoting a sustained lifetime in systemic circulation (Gutjahr et al. 2016; Rodgers et al. 1997).

The nanoparticles obtained from biocompatible polymers including poly (lactide-co-glycolic acid) and polymethylmethacrylate serve as immunopotentiating antigen-delivery vehicles. The system displayed application for an improved polyclonal antibody production towards clenbuterol and hapten. The delivery of immune adjuvants by nanosystems presents a marked efficacy for the administration of vaccines. The nanoformulation promotes the cross penetration of cargo molecules that plays a vital role in the induction of immune response to counter the various intracellular pathogens and tumor progression. It allows the enhancement of the activity of the molecular adjuvant, prolonging its exposure to immune cells, while reducing its toxicity. Thus, the co-delivery of antigens and immuno-potentiators in polymeric nanoparticles seems to be an efficient way to induce a potent immune response. Similarly, the fabrication of polymeric nanoparticles with flexible, hydrophilic polymers having improved water solubility such as polyethylene oxide promotes a controlled and targeted delivery of the cargo drug molecules mainly by reducing the interactions between polymeric nanoparticles and plasma proteins (Ilinskaya and Dobrovolskaia 2016). This restrains the uptake of drug carrying polymeric nanoparticles by reticulo endothelial system thereby improving the circulation time while maintaining an optimal concentration of the drug in blood (Li and Huang 2009).

4.4 Controlled Drug Release to Solid Tumors by Polymeric Nanoparticles

The polymeric nanoparticles constructed with biodegradable polymers exhibit the unique property of controlled release of the encapsulated drug molecules, which proves highly beneficial for a sustained therapeutic effect of the deliberated molecules (Taghizadeh et al. 2015). The controlled drug delivery of polymeric nanoparticles prevents the adverse effect of the chemotherapeutic on healthy tissues caused by a burst release, which may potentially harm the cells by altering the physicochemical homeostasis (Tang et al. 2018). The rate of drug release by these systems depends on the degradation rate of the encapsulating polymeric nanoparticles in systemic circulation (Zhang et al. 2019).

Particularly, the biodegradable polymers including, but not limited to polysaccharides, polypeptides, cellulose, starch, and amylose represent an ideal drug delivery system for carrying the desired therapeutics to the target tumor cells whereby facilitating their sustained release (Rapoport 2007). Besides, several synthetic polymers with a considerable physiological tolerance and a trivial immunogenicity present a robust candidature as controlled-release drug delivery vectors (Bawa et al.

2009). The biodegradable polymeric nanoparticles promote the drug delivery at various locations in the host organism and hold the prominence of carrying a variety of drugs irrespective of their molecular weight and water solubility (Jin et al. 2015). The macromolecular drugs including proteins, carried by the polymeric nanoparticles release at the target site over an extended period, which lowers the dosage frequency of the cargo drug, while at the same time provides protection to this macromolecular therapeutics against enzymatic degradation (Li et al. 2019).

The controlled release profile of polymeric nanoparticles rests on several critical parameters. These include:

(a) delayed but not sustained release of drug,
(b) Sustained zero-order release of the therapeutic,
(c) Considerably delayed release followed by a constant release of the cargo molecules,
(d) Delayed followed by a tight pulse release of the pharmaceuticals,
(e) Multiple pulse release of the therapeutics at specific period (Cheng et al. 2016).

Besides biodegradation, the diffusion-controlled systems based on polymeric nanoparticles for sustained drug release plays a frontier role in delivering the therapeutics at their target site (Li et al. 2014). These systems comprise the diffusion of the entrapped drugs through the core of polymeric nanoparticles, followed by the biodegradation of the carrier polymer after the drug exhaustion (Sethi et al. 2014). The process necessitates the maintenance of drug in its saturated state within the core of nanocarriers to follow the zero-order release kinetics (Jabbari et al. 2013).

The chemically controlled systems comprise the most extensively utilized nanocarriers for achieving a controlled release of the encapsulated drug. These systems mainly comprises of degradation of chemically labile linkers or unstable bonds that undergoes biodegradation thereby releasing the payload drug molecules (Danyou et al. 2019). Similarly, the bioerodible systems comprises of labile bonds that undergo enzymatic or hydrolytic degradation *in vivo* thereby releasing the appended drug molecules, which link to the polymeric nanoparticles via these labile bonds (Rezk et al. 2019).

Temperature-triggered polymeric drug delivery systems represent a widely applied nanosystem that utilizes polymeric properties such as swelling change of polymeric networks, thermally reversible transition, crystalline melting, and glass transition temperature (Kamaly et al. 2016). The temperature alterations caused by disturbance in homeostasis serves as a stimulus for controlled release of the therapeutics directed for managing the diseases accompanied by fever (Lai et al. 2014).

Overall, the controlled or sustained release drug delivery systems utilize the local conditions in tumor microenvironment by using the polymeric nanoparticles containing stimuli-responsive chemical bonds or morphology (Jabbari et al. 2013). These characteristics alter, undergo degradation or experience a collapse while responding to the physicochemical parameters exclusive to the tumor physiology (Sun et al. 2018). Figure 4.2 represents types of controlled drug delivery systems based on polymeric nanoparticles.

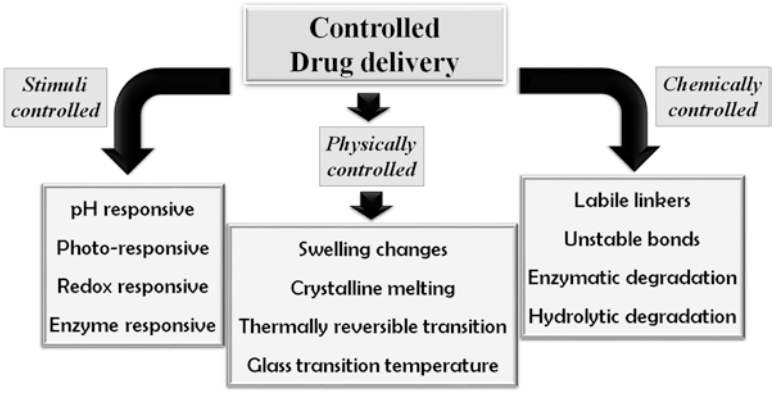

Fig. 4.2 Stimuli-sensitive and chemically/physically controlled drug delivery systems for the realization of effective drug absorption at the target site in order to achieve an optimal therapeutic effect. For controlled drug delivery systems, three approaches can be adopted. Stimuli controlled drug delivery systems can be designed as pH responsive, photo responsive, redox potential responsive and enzyme responsive. Physically controlled drug delivery systems can be designed by considering the physical properties like swelling parameter, crystallinity and temperature. Chemically modified drug delivery systems can be designed by linkage of labile linkers, incorporation of unstable bonds and delivery systems liable to degradation by enzymes or hydrolysis

4.5 Polymeric Nanoparticles for Stimuli-Responsive Targeted Drug Delivery

The polymeric drug delivery systems, especially the vesicular drug delivery systems based on polymeric nanoparticles, the 'polymersomes' utilize the intrinsic physicochemical and biological microenvironment of solid tumors (Wells et al. 2019). The microenvironment acts as a stimulus to promote responsiveness in the drug delivery vehicles, thereby prompting the cargo drug release (Wei et al. 2017). Acidic pH of tumor microenvironment and the intracellular compartments including endosomes and lysosomes serve as a stimulus for causing the responsiveness in polymeric nanoparticles for releasing the entrapped drug molecules (Cheng et al. 2014). These drug delivery systems however exhibit stability at the physiological pH to prevent the loss of encapsulated drug molecules in systemic circulation (Rao et al. 2018).

The pH-responsive polymeric drug delivery systems mainly comprises of functional groups that exhibit proton donor/acceptor properties in response to the pH gradient across the tumor microenvironment that leads to alterations in the polymer structure and its hydrophobicity (Bhattacharya et al. 2016). The polymeric nanoparticles containing ionizable carboxylate or amine groups represent the frontier candidates for designing pH-responsive drug delivery systems. In a recent report, Kong et al. (2020) developed pH-responsive polymeric nanoparticles in the size range 72.9–208 nm and grafted hydrophobic side chains for the delivery of anticancer drug doxorubicin (Kong et al. 2020).

The schiff base linkages between dialdehyde cellulose and amino-containing compounds formed the basic structure of polymeric nanocarriers. The concentration and length of hydrophobic grafts determined the size tenability of polymeric nanoparticles that displayed stability in the alkaline and neutral environment for up to a month. These drug delivery systems decompose in the acidic pH of the tumor microenvironment, thereby selectively releasing the encapsulated drug to the tumor cells, whilst displaying a minimal toxicity towards the surrounding healthy cells. Palanikumar et al. reported pH-responsive, highly stabilized polymeric nanoparticles for the targeted delivery of anticancer drugs doxorubicin and paclitaxel (Palanikumar et al. 2020). The drug delivery nanovehicles comprised of drug loaded poly (lactic-co-glycolic acid) copolymer covalently crosslinked to a bovine serum albumin shell, also minimized their interactions with macrophages and serum proteins that restrict the target recognition. The membrane functionalization with acidity responsive rational membrane peptide facilitated the tumor cell internalization owing to the pH sensitivity of the nanosystem. The reported polymeric drug delivery system displayed a specific targeting of human breast and pancreatic cancers, without potentially harming the healthy cells.

Bobde et al. reported PEGylated N-(2-hydroxypropyl) methacrylamide conjugated to doxorubicin via pH responsive hydrazone linker for a pH sensitive delivery of the latter in metastatic tumors at pH 6.5 (Bobde et al. 2020). The polymeric nanoparticle based drug delivery system demonstrated a heightened uptake of the conjugated anticancer drug in murine mammary carcinoma cell line (4T1) and breast cancer (MCF-7) cell lines while displaying a similar cytotoxicity comparable to the free doxorubicin. Importantly, a rapid drug release occurred from the polymeric nanoparticles at an intra-tumoral pH 6.5 and intracellular pH 5.5, compared to the physiological pH 7.4 thereby suggesting the higher stability of the nanosystem in optimal biological environment.

Zhao et al. reported lignin-histidine conjugation based self-assembled pH-responsive polymeric nanoparticles for the delivery of 10-hydroxycamptothecin (Zhao et al. 2020). The conjugation of amphiphilic lignin with pH-sensitive molecule 'histidine' for obtaining small sized polymeric nanoparticles of diameter 30–40 nm proved highly advantageous due to their ability to surpass the physiological barriers owing to a smaller size. The drug release happened in weak acidic environment, similar to the tumor tissues, which also facilitated accumulations of drug molecules at the target tumor site. This polymeric drug delivery system demonstrated a high drug loading capacity, marked biocompatibility, significant biodegradability and trivial cytotoxicity.

Wang et al. reported carboplatin and paclitaxel loaded lipid-polymer hybrid nanoparticles with average size 169.9 ± 5.6 nm for pH-responsive drug release and a superior cellular uptake efficiency (Wang 2020). The nanoparticles released the anticancer drug at pH 5.5 however, the drug release halted considerably at pH of 7.4. The lipid-polymer nanoparticles maintained high concentration at the target tumor site for 24 h following administration thereby suggesting a controlled release property. The fabrication of folate functionality on the lipid-polymer nanoparticles imparted pH-sensitivity and maintained the compatibility with lipid bilayer of the

target tumor cells. This allowed membrane fusion of the drug delivery system for highly efficient delivery of cargo drug molecules. Gardouh et al. reported the synthesis and antitumor potency of doxycycline loaded polymeric nanoparticles (Gardouh et al. 2020). The biological investigations indicated reduction in the mass of solid tumors for the animal models treated with the nanosystems, mainly at a dose 5 mg/kg, which produced a similar healing effect compared to that of 10 mg/kg doxorubicin. The nanosystem enabled the targeting of solid tumors in particular.

The tumor microenvironment exhibits redox stress owing to the redox imbalance caused by the generation of free radicals and erroneous expression of the enzymes responsible for maintaining this balance. Lu et al. reported redox-sensitive prodrug conjugates based on hyaluronic acid appended anticancer drug doxorubicin, which undergoes a pH-sensitive release from the carrier system (Lu et al. 2020). The nanosystem possessed ability to self-assemble into spherical nanoparticles in aqueous solution, which enabled their internalization to the tumor cells. The nanosystem displayed marked stability at the physiological pH, displayed a high drug loading capacity and triggered drug release at acidic pH. The nanosystem exhibited superior biocompatibility, low toxicity towards healthy cells with enhanced penetration and accumulation inside tumor cells. The *in vivo* biodistribution and pharmacokinetics analysis suggested a prolonged circulation time for the nanosystems in bagg albino mice models and considerable accumulation in 4T1 tumor model.

Santra et al. reported self-immolative nanoassemblies based on amphiphilic polyurethane containing redox-responsive, hydrophobic backbone and hydrophilic triethylene glycol appended to the backbone of tertiary amine substituent (Santra et al. 2020). The pH-sensitivity of the nanosystem resulted in the generation of positively charged nanoassemblies at acidic pH of the tumor microenvironment. Interestingly, the presence of reducing agents such as glutathione resulted in redox-sensitive disassembly of the nanosystem followed by the release of encapsulated therapeutics. Mazzotta et al. developed redox responsive release of anticancer drug methotrexate via folate fabricated chitosan nanoparticles in response to the reducing tumor microenvironment (Mazzotta et al. 2020). The nanosystem exhibited marked physiological stability and promoted a rapid drug release in the reducing environment. The drug release characteristics of the nanosystem depended on the disulfide bonds present in the polymeric structure, which caused a 2.8-fold improvement in the rate of drug release. In addition, the nanosystem displayed a marked tolerance towards biological systems, which further validated its tumor-selective drug delivery applications.

Barve et al. reported enzyme-responsive polymeric micelles of the anticancer drug cabazitaxel for prostate cancer treatment (Barve et al. 2020). Cabazitaxel delivers anticancer performance by acting as tubulin inhibitor, however a poor affinity towards the p-glycoprotein, poor solubility, and a lack of target specificity restrain its wide spread applications. The biodegradable, amphiphilic block-copolymer micelles for cabazitaxel demonstrated enzyme responsive target specific release of the anticancer drug in the presence of matrix metalloproteinase – 2 expressed in tumor microenvironment, which triggered the drug release. The polymeric micelles displayed a superior drug-loading tendency, in addition to high entrapment efficiency.

Battistella et al. reported enzyme-responsive immunotherapeutic polymeric nanoparticles in tumor therapy (Battistella et al. 2019). The nanosystem consisted of amphiphilic diblock copolymers containing hydrophobic toll-like receptor agonist and hydrophilic peptides that act as substrates of matrix metalloproteinases generated in the tumor microenvironment. The exposure of the nanosystem towards matrix metalloproteinases generated microscale immunostimulatory assemblies that accumulate in the tumor cells and caused a significant reduction in the growth of primary tumors. Yao et al. reported matrix metalloproteinase-triggered self-assembled polymeric nanoparticles as efflux pump inhibitors in resistant cancers (Yao et al. 2019). The polymeric nanoparticles and their micelles effectively mitigated the efflux of anticancer chemotherapeutics whilst facilitating their cellular uptake, penetration and retention in the target tumor cells. Figure 4.3 represents drug release by polymeric nanoparticles in response to an external stimulus.

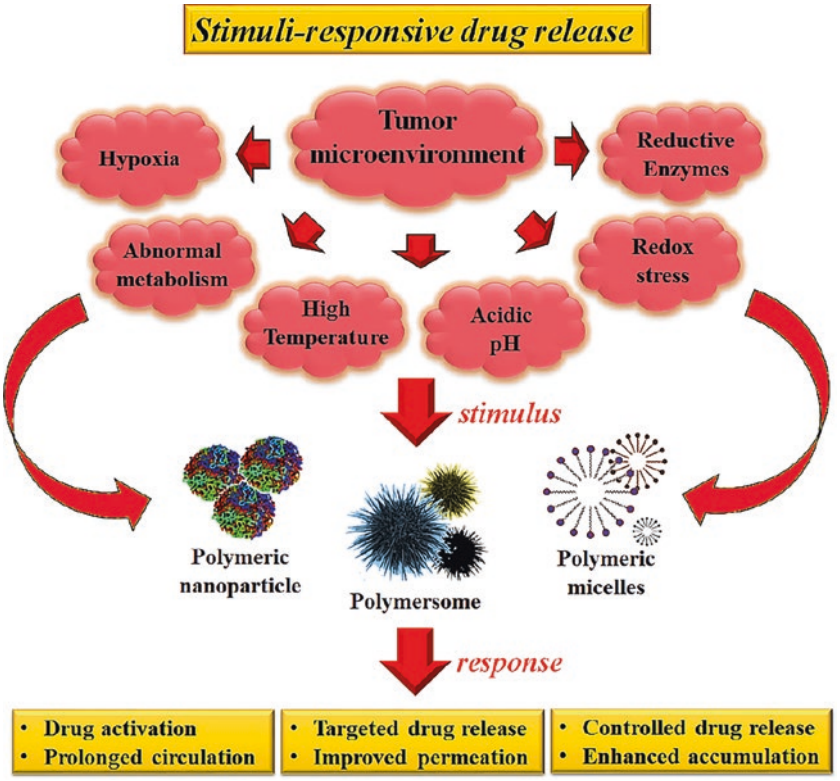

Fig. 4.3 Stimuli responsive drug release by polymeric drug delivery systems. The typical physicochemical properties of a tumor microenvironment serve as stimuli for affecting the drug release from the polymeric delivery systems. These stimuli include redox stress, acidic pH, high temperature, abnormal metabolism, hypoxia, and abnormally functioning enzymes. The delivery systems like polymeric nanoparticles, polymerosomes and polymeric micelles with response to these stimuli activate the drugs, increases their circulation time in the body, helps in targeted drug delivery by improving the permeation through biological barriers, controlled drug release and increase in drug accumulation at the target site

The accumulation and prolonged retention of the polymeric drug delivery system encapsulating the cargo drug molecules effectively improved the therapeutic efficacy of the directed anticancer drugs while sensitizing the target tumor cells towards chemotherapy. Ramezani et al. reported matrix metalloproteinase – 2 responsive polymersomes for colorectal cancer therapy (Ramezani et al. 2020). The tethering of polyethylene glycol to polylactide via synthetic peptide exhibited sensitivity towards the tumor linked matrix metalloproteinase – 2 enzyme. The nanosized polymersomes enabled a controlled release of 7-ethyl – 10 -hydroxycamptothecin in physiological environment, with a significant 7-fold enhancement in the rate of release in the presence of matrix metalloproteinase-2 enzyme, which prompted the targeted drug delivery against the nucleolin positive cells.

4.6 Conclusion

The polymeric nanoparticles provide an essential tool for drug delivery to aggressive solid tumors, instigated by chemical, physical or morphological stimulus. The properties such as physiological tolerance, *in vivo* degradability, trivial toxicology towards healthy cells and ability to carry both hydrophilic/hydrophobic chemotherapeutics make polymeric nanoparticles an attractive choice as drug delivery vectors. The stimuli created by the tumor physiology which triggers the drug release by polymeric nanoparticles presents an exciting feature for developing the advanced, smart nanocarriers without causing any potential harm to the healthy tissues in the vicinity of solid tumors. The benevolence of drug loaded polymeric nanoparticles towards the homeostasis creates precedence over the customary drug delivery systems. However, the immunogenicity presented by several biodegradable polymers poses concerns towards disturbing the physiological harmony of cellular microenvironment, which needs to be addressed prior to developing the polymeric nanoparticle based drug delivery vehicles. In addition, some polymeric architects that fails to auto-degrade *in vivo* need surgical removal from the solid tumors. Nevertheless, the polymeric nanoparticles owing to their unique properties emerge as a widely applied drug delivery system for targeting the solid tumors.

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Chapter 5
Passive and Active Targeting for Solid Tumors

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5.1 Introduction

Cancer is a disease condition where abnormally mutated cells are divided in an uncontrolled way which leads to complex malignancy, if untreated (Padhi et al. 2015). The associated complexities potentially affect every organ of human body. Cancer is the most devastating disease as more than seven million deaths are reported each year. The conventional chemotherapeutic approaches are unable to provide a sufficient recovery rate due to inherent drawbacks. In addition to chemotherapy, solid tumors are also being treated using surgery or laser therapy, or a combination thereof (Thakar et al. 2021).

Conventional approaches have their own limitations in terms of side effects, dosing level, dosing interval, patient safety, etc. There is a constant need to develop an economical approach for the delivery of the drugs to the targeted sites. The current developments in nanotechnology supports better preclinical or laboratory results but did not reach to the clinical or commercial level. The nanotechnology based

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approaches could provide better therapeutic potential than conventional approaches. A number of developed nanocarriers are in the pre-clinical pipeline which are either polymeric, metallic or lipid based (Behera et al. 2020).

In fact few of the commercial nanoformulations like liposomes, polymeric nanoparticles are currently available in the market (Mohanty et al. 2011). Nanotechnology is a solution-oriented technique that helps to interline the desired objectives for the betterment of therapeutic response. Nanomaterials an engineered polymer, inorganic, organic, carbon-based chemical entities use to deliver, identify the biomolecules, releases chemotherapeutic agent at the specific targets. The nanocarriers or nanovesicles are designed from biodegradable, biocompatible materials of origin. The robust physicochemical properties at the nanoscale could potentially illuminate the delivery of the drug to a specific site of action.

The nanocarriers are specially designed for the delivery of drugs across barriers. The nanocarriers deliver an effective concentration of drugs across the barrier membrane and show an effective therapeutic response. Barriers are the natural defense system of humans which protect from the undesired effect. The barriers principally limit the penetration of foreign materials. For target specific approaches, a better understanding of the physiological barriers is necessary to deliver the medicine at the site of action (Barua and Mitragotri 2014).

The targeted nanotechnological approaches utilize homing devices attached to a specific receptor and optimize the therapeutic response. Target-specific nanomedicine delivery could pave the way in the treatment of a variety of diseases. The targeted therapeutics is changing the scenario of treatment by modulating tissue distribution via specific targets and pharmacokinetics of existing chemotherapeutics. The therapeutic modality for the delivery of nanomedicine governs the therapeutic safety and implementation of standard protocols. The pre-clinical and clinical outcomes from the conventional delivery systems in the treated subjects did not improve the survival rate of the patients with additional side effects being reported.

For the delivery of a therapeutic agent, the conventional approach delivers the drug based on the dose and efficacy available. For more specific drug delivery, active and passive targeting approaches were followed upon. This chapter explicitly highlights the concept of interaction between biomolecules and homing devices for targeting to solid tumors. The chapter also highlights the possible targeting molecules, delivery aspects, and challenges associated with drug delivery. The anatomical alterations and microenvironment are two principle sites that are possibly targeted in solid tumors. The anatomical differences between cancer tissues and normal tissues can passively deliver the chemotherapeutic agent.

In addition to this passive targeting approach, the tumor microenvironment like pH, temperature, oxygen content, etc. can be approached for the delivery prospects. The active targeted drug delivery specifically recognizes receptors, surface proteins, amino acids, enzymes that are upregulated or overexpressed into the tumor environment. By selecting a specific ligand-receptor component, active targeting satisfactorily delivers the drug (Behera and Padhi 2020; Mohanty et al. 2011).

5.2 Targeting Methods

5.2.1 Passive Targeting

Nanocarriers entrapping chemotherapeutics are able to deliver the drug through passive targeting approaches. During the process of angiogenesis, a new vascular structure is formed which enables vascular permeability. The new vesicles formed during the angiogenesis process may develop into defective architecture or leaky tumor vasculature with wide fenestrations. The large size tumors (about 1–2 mm in diameter) have the ability to develop independent blood supply (Folkman 1995).

Fenestrated vasculature enables direct transport of nanocarriers in the tumor interstitium via diapedesis. The alignment of endothelial cells is poor which leads to variable pore size between 10 and 1000 nm. The nanocarriers having size below 200 nm are easily transported through these pores without any interruption and the rate of accumulation is also high when the size of the nanocarrier is below the prescribed limit. A study suggests the fact that particle sizes of nanoparticles above 100 nm has a higher retention time in the tumor microenvironment (Dreher et al. 2006). The hypoxic tumor is responsible for the production of vascular endothelial growth factors.

The vascular permeability accelerates the upregulation of the endothelial receptor on the surface of solid tumors. The add-on functionality in vascular permeability was reflected through leakiness, sprouting, and survival of endothelial cells (Verheul and Pinedo 2000). Extravasation of solid tumors increases vascular permeability inside the interstitial fluids. Transportation across the cell through extravasated vessels increases retention time of nanocariers. Due to the small size of nanocarriers, they can easily cross endothelial barriers using extravasated tumor vessels. The transportation across endothelial barriers is majorly due to the enhanced permeability and retention effect (Padhi and Behera 2020).

The fenestrated vessels easily transport biomolecules or nanocarriers across the endothelium and gets accumulated inside the tumor tissue. The transport of molecules from the fenestrated vessels is also termed as permeability effect. An extravasated blood vessel increases permeability across tumor tissues. In addition, the tumor tissues lack the lymphatic function which helps to increase the retention effect that emerges from interstitial fluid pressure. The decrease in tumor perfusion and compression of blood vessels may be a sign of high interstitial fluid pressure in tumor interstitium (Badera et al. 2004; Chamundeeswari et al. 2019).

The stromal compartment is also considered as a contributing factor in the enhanced permeability and retention effect. The density of the extracellular matrix and non-homogeneous distribution contributes to penetration and accumulation effects. The collagen and hyaluronic acid contributes to the development of a strong extracellular matrix but tumor tissues that have lesser density may be responsible for the penetration effect.

The cell surface density interferes with the deep penetration of chemotherapeutic agents at the tumor surface. The other factors such as presence of dendritic cells,

smooth muscle cells, macrophage, myofibroblast, pericytes, endothelial cells, etc. contributes to the enhanced permeability and retention effect as well (Golombek et al. 2018; Venning et al. 2015). The advantage of passive targeting is that low molecular weight therapeutic agents can be easily transported and gets retained at the tumor sites. Maximum types of solid tumor can accumulate nanoparticles utilizing the enhanced permeability and retention effect.

High molecular weight nanocarriers can easily deliver low molecular weight drugs into the tumor using enhanced permeability and retention effect. The second major limitation of the solid tumor is the transient nature of the enhanced permeability and retention effect. High heterogeneity at developmental stages of the tumor may alter the enhanced permeability and retention effect. Few solid tumors like prostate and pancreatic cancer do not show enhanced permeability and retention effect (Maeda et al. 2001).

The drug-loaded nanocarriers possess a long circulation time due to the biocompatibility of selective polymers used during the formulation process. The coated polymeric nanoparticles, liposomes, nanosized metal nanoparticles, micelles, etc. deliver the drug to solid tumors via the enhanced permeability and retention effect. If we compare the rate of drug accumulated or delivered in the extravagated tumor by nanocarrier conjugates, it is usually 10–100 times more than the free drug (Mohanty et al. 2011).

The drug delivery to extravasated tumor tissue as compared to the normal tissue is represented in Fig. 5.1. Direct delivery of the drugs through the extravasated vessel may deliver the drug promptly to tumor tissue, but at the same time drug also gets accumulated in normal tissue (Fig. 5.1a). Based on alternative approaches like stimuli responsive release, pH sensitive release can enhance the accumulation rate in tumor tissues. The engineered nanocarrier specifically delivers actives to the tumor cell without affecting normal cell function as represented in Fig. 5.1b.

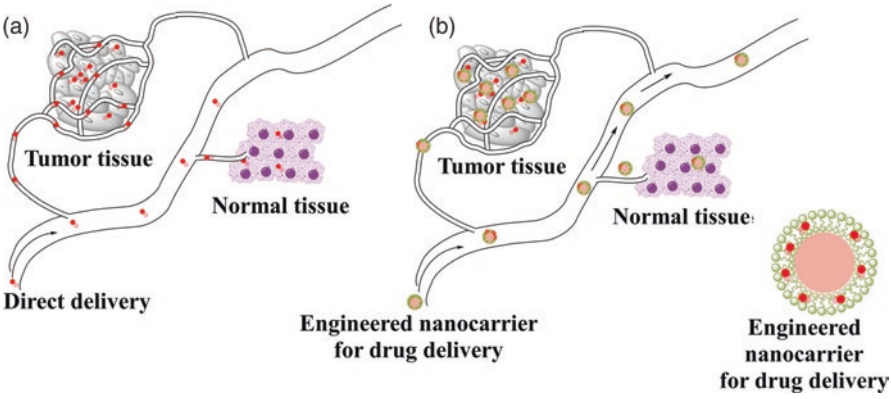

Fig. 5.1 Direct drug delivery (**a**) and engineered nanocarrier for drug delivery to extravasated vessel (**b**). The comparative drug delivery shows higher drug accumulation for engineered nanocarrier in tumor cells without affecting normal cell behavior

5.2.2 Active Targeting

The urge for the development of active targeted therapeutics helps in maximizing the therapeutic efficacy in cancer therapy. The basics behind active targeting are the use of homing devices (ligand) to bind with molecular signatures (receptors). Tissue anatomy has wider importance in active targeting as it utilizes the basic lock and key concept. The directional flow is considered for drug-receptor targeting as well as biomolecule receptor targeting. The desired specificity must be known before interaction or suggestive pharmacological response. The targeted approaches are able to minimize the side effects.

The active targeting can maximize effectiveness through reduction of toxicity, enhancing longer circulation, prognosis using surface grafting, multidrug resistance, restricted biodistribution, reduction in side effects, etc. (Das et al. 2009). Most of the drug molecules are potent with narrow therapeutic window, which needs to be highlighted to design a drug delivery system. For active targeting, ligand binding approaches are used to deliver the bioactives at the site of action. The active targeting approaches can differentiate between tumor cells and normal cells. This is based on simplified lock and key simulated approach where the ligand binds to the over-expressed receptor and selection of ligand is based on affinity towards the receptor.

In recent years of computational approaches, the selection is somehow made easy by simulation mechanisms. Naqvi et al. discussed the detailed computational approach used for active targeting (Naqvi et al. 2018). The uptake of drugs is dependent on the interaction and expression analysis of receptors or antigens. The accumulation rate of ligand-drug complex is much higher as compared to others. The derivatization is of two types, i.e. internalizing ligand and non-internalizing ligand. Drugs are cross-linked to the carrier molecules which protect the drug from enzymatic degradation and enhance the circulation time.

Tactics used for coupling of ligand molecules with the carriers may be after or before the drug loading step. Based on the type of drug to be delivered or site at which the tissue environment or number of barriers to be crossed, the coupling may differ. The activated functional groups from the surface proficiently perform the ligation process during ligand binding. The biomolecules coupled with the nanocarriers may enhance the circulation time. The biomolecules used for active targeting are generally polymers, proteins, amino acids, antigens, antibodies, antibody fragments, peptides, enzymes, fibrinogen, DNA, RNA, nucleotides, etc. (Chamundeeswari et al. 2019).

The homing devices from biomolecules perform stealth function in active targeting. These biomolecules are sometimes used as single agents or in several combinations. Based on the types of binding desirables, the ligands are classified into neutral ligand and active ligand. The neutral ligand only participates in the ligation process and does not have any therapeutic output or interference. Major types of drug delivery prospects are from the neutral ligation category. While in the case of active ligand interaction, in addition to ligation, active ligands show synergistic or additive

activity with the therapeutic agent. A very less number of studies are highlighted for ligands for active ligation (Marcucci and Lefoulon 2004). The selection of appropriate ligand is the most important step in active targeting to tumor cells. The selection of ligands is based on abundant availability of receptors on the cell surface and needs to possess a mechanism by which it delivers the active drugs inside the cell. Simultaneously it must enhance the penetration inside the cell or regulate specific role either by releasing some channels or controlling the expression of biomolecules. The selected interaction must have the ability to recycle its preposition for further promotional interaction and have an active role in regulating cellular functions. The selected antigen or receptor must back onto the surface of the cell for interaction. The ligand-receptor interaction must envisage high avidity, internalization, and control release kinetics from nanocarrier core (Das et al. 2009).

5.2.2.1 Targeting of Cancer Cell

Direct targeting of cell surface receptors is a promising area of research. This module of chemotherapeutic delivery provides effective treatment in cancer therapy. The over-expressed receptors from the surface of the cancer cells improve the internalization and thereby transportation inside the cell. The active targeting of nanocarriers improvises internalization and tumor accumulation. Targeting cell surface ligands leads to direct cell killing. Cells available at the tumor periphery also lead to strong cytotoxic inhibition. Details of surface receptors like transferrin, folate, glycoproteins (lectins), and epidermal growth factor receptor are elaborated in the following sections.

5.2.2.2 Targeting of the Tumoral Endothelium

The shortfalls of active targeting can be overcome by targeting endothelial cells. Anatomically, the number of layers available on the tumor cell and endothelia act as barriers for the transport of chemotherapeutic agents. Endothelium targeting has been highly popularized for delivering chemotherapeutic agents due to potential therapeutic outcomes. Tumor cell density or interstitial fluid pressure may interfere for passive transportation of nanocarriers. The advantageous part of targeting tumor endothelium improves the antitumor potential and direct entry of chemotherapeutic agents inside tumor interstitium.

Endothelial cells reduce the intake of oxygen and nutrients to tumor cells with an additional possibility to deliver the content into tumor vasculature. Generous binding with endothelial receptors on the surface of tumor cells directly transport the drugs across endothelium which increases the concentration of low molecular weight drugs in tumor interstitium (Mitra et al. 2005; Pasqualini et al. 2000).

Targeting tumor endothelium also prevents the formation of new blood vessels. The targeted therapeutics to endothelial domain inhibits the blood supply to tumor cell thereby reducing the metastatic capability of growth of tumors. The designed

nanocarrier specifically targets tumor endothelium and kills the cells supporting tumor blood vessels and tumor core. The extravasation is not necessary for nanocarriers to target tumors. Endothelial cells are genetically stable as compared to tumor cells. Conjugation between ligand-receptor is easily possible and avoids drug resistance compared to metastatic tumor cells. The expression of endothelial markers is similar in most of the tumor types (Gosk et al. 2008).

The endothelial receptors comprise of integrins α5β1, αvβ3, α2bβ3, aminopeptidase –N and so on. The peptides (arginylglycylaspartic acid) have high affinity towards integrins. The αvβ3 is upregulated on the neovascular endothelial cells. The integrins possess calcium-dependent signaling pathways and help in endothelial migration (Desgrosellier and Cheresh 2010).

A class of matrix metalloproteinase is expressed on endothelial cells which performs an important role in angiogenesis and metastasis. The cell invasion and migration of endothelial cells are accelerated by the release of membrane type I matrix metalloproteinase. The new capillary formation starts after the release of type 1 matrix metalloproteinase. The overexpression analysis suggests that matrix metalloproteinase is observed in colon, lung, cervical cancer (Genís et al. 2006).

The aminopeptidase – N is a type of matrix metalloproteinase, is also expressed on the endothelial cell surface. Aminopeptidase-N has a high affinity towards asparagine-glycine-arginine (Asn-Gly-Arg NGR peptides) and removes amino acids from the N-terminal segment of proteins and peptides (unblocked protein type). The aminopeptidase initiates extracellular matrix degradation and tumor cell invasion (Pasqualini et al. 2000; Saiki et al. 1993).

Vascular endothelial growth factors are expressed through endothelial cells expressing angiogenesis and neovascularization (Shadidi and Sioud 2003). The upregulation of two major receptors, vascular endothelial growth factor receptor 1 and vascular endothelial growth factor receptor 2, are potentially available as target receptors on the endothelial surface. Tumor hypoxia and oncogenes upregulate vascular endothelial growth factor receptor and targeting vascular endothelial growth factor inhibits the vascular endothelial growth factor receptor 2 binding (Carmeliet 2005).

Endothelial cells also express transmembrane glycoproteins like vascular cell adhesion molecule – 1. In the process of angiogenesis, cell to cell adhesion is activated due to the expression of immunoglobulins like vascular cell adhesion molecule – 1. Most of the angiogenic tumors like gastric cancer, breast cancer, renal cell carcinoma, etc. expresses vascular cell adhesion molecule – 1 (Dienst et al. 2005).

5.2.2.3 Stimuli-Responsive Nanocarriers

The design of stimuli-responsive nanocarriers aids in triggered drug release at the targeted site. Many scientists are working on endogenous and exogenous stimuli to enhance the release of chemotherapeutic agents at the specified site. The active nanocarriers maintain integrity and do not release the encapsulated content without

responsive interaction. Once the triggering factor comes, the nanocarrier either bursts or releases the encapsulated chemotherapeutic agents at the desired tumor site.

The triggering factor may be any stimuli allowing the release of its loaded content. The interaction is specific only with the nanocarriers and not with the loaded chemotherapeutic agents. Nanotools are more specifically designed to administer the stimuli sensitive drugs at the site of tumor tissues (Bilan et al. 2016; Nikam et al. 2020; Sharma et al. 2015).

The applicability of stimuli-responsive carrier can be enhanced by coupling with bio- imaging agents which provide theranostic modules for the diagnosis and delivery of the chemotherapeutic agents (Juthi et al. 2020; More and Deshmukh 2020; More et al. 2021). The formulation strategy remains the most difficult task during designing stimuli responsive nanocarriers. Starting from selection of host response, response time, material selection, etc. each factor needs to be screened very stringently. Academic scientists and industrial experts have considered the shortcomings and implicated advanced techniques to design tumor-targeted release by active targeting approach.

Internal Stimuli

The change in pH from acidic to neutral or basic to acidic is used as specific internal stimuli for manipulating the drug release pattern. The nanocarriers variably release a drug based on small fluctuations in the pH of the internal environment. The presence of redox gradient or certain metabolic enzymes from tumor interstitium might be helpful for the release of drugs from the nanocarrier compartment.

The change in redox potential creates differentiation at the surface of the nanocarrier and is responsible for the release of the drug. Certain enzymes from tumor microenvironment interacts with engineered nanocarrier surface either by opening the channels or degradation of nanocarrier which provides burst release of the entrapped drug. Finding the accurate release potential at endosomal pH is very tricky (Meng et al. 2012).

Polyhistidine, eudragit, polysulfonamide are known to be suitable polymers for pH-triggered release. Histidine possesses a lone pair of an electron from unsaturated nitrogen, which provides positive charge on its surface. The presence of nitrogen in the imidazole ring acts as a weak base which may acquire a cationic charge at acidic pH 6.0. The destabilization of polyhistidine releases the drug into an acidic compartment. Copolymerized poly-L-lactic acid/polyethylene glycol with polyhistidine/poly (ethylene glycol) forms nanocariers and vesicles which further releases the drugs in acidic pH (Lee et al. 2003).

The drug encapsulated nanocarriers becomes destabilized at pH 6.0 and releases the drugs in intracellular pH when comes in contact with early endosomes (pH 6.0) or lysosomes (pH 5.0). While extracellular pH is neutral, nanocarriers do not release the drug in the extracellular compartment.

A study inferred the fact that mixed micelle entrapping doxorubicin releases the drug based on pH variations. The mixed micelle was conjugated with folic acid to

enhance the stability. The mixed micelle formulation showed a cytotoxic effect against wild-type ovarian cell lines. The mixture of polymer (poly-L-lactic acid and polyhistidine-co-phenylalanine) forms a mixed micelle structure that controls the release of doxorubicin from the inner core (Kim et al. 2008).

Polysulphonamides perpetrates a negative charge at neutral pH. A slight change in pH causes ionic interaction of polysulphonamides and subsequently destruction of polymeric surface leads to the release of drug. Delivery of cell-penetrating peptides using polysulphonamides is the best-suited carrier to transport across the cells (Berry 2008).

Prodrugs are substantially delivered to reduce the degradation of the drug during systemic circulation. In tumor-targeted therapy, specific enzymes can interact with prodrugs or polymeric carriers to convert into actives. Tumor-specific activation of prodrugs in presence of enzymes shows its cytotoxic potential.

Selection of enzyme must be appropriate which converts the prodrug into active moiety. The matrix metalloproteinase-2 and matrix metalloproteinase-9 expressed in tumors, can cleave the linkage between polymer and drug. A study showed that dextran conjugated with methotrexate was delivered at the site of action. The tumor-associated enzyme aided in breaking the peptide linkage between methotrexate and dextran which released the drug within tumor tissues (Gullotti and Yeo 2009).

Thiol-induced dissociation or redox association utilizes the exchange of polymeric nanocariers in the tumor environment. Reversible conversion of thiol from disulphide bridge takes place in presence of a reducing agent. The reducing agent is responsible for exchange of thiols with other molecules and forms a new bond. Disulfide exchange favorably releases the drug in the tumor interstitium. The micellar structure consisted of supramolecular complexes that released the drug-using thiol-induced dissociation (Ghosh et al. 2009).

External Stimuli

External stimuli or exogenous factors are utilized for the triggered release of the entrapped drug. The external factors such as ultrasound, light, temperature, magnetic field, etc. are the defined strategies for the release of drug from nanocarriers. The thermosensitive polymers with low critical solution temperature precipitate at a certain temperature above threshold temperature (i.e. low critical solution temperature). At the specific temperature, the nanocarrier loses its structure or integrity and releases the drug from the core.

In a study, thermosensitive liposomes were fabricated using distearoyl phosphocholine and poly N-isopropyl acrylamide. The thermosensitive liposome showed stability at physiological temperature and did not release the enclosed content. Due to the external temperature effect, the lipids underwent a phase change which resulted the release of the entrapped drug (Kono 2001).

Poly (N-isopropyl acrylamide) is a thermosensitive polymer used for designing nanocarrier for the temperature sensitive trigger. By using external heating devices, tumor surfaces are heated, while some internal chemotactic are also used to increase

the temperature. The physical methods are easy and economical for temperature sensitive drug delivery to the tumor.

Another important facet of external stimuli is the magnetic effect. The external magnet is placed on the surface of the tumor which helps to accumulate superparamagnetic iron oxide nanoparticles and recurrent heating helps in the release of drug at the site of action. In addition to drug delivery, superparamagnetic iron oxide nanoparticles can facilitate imaging capability with contrast agents used in magnetic resonance imaging. For active targeting, superparamagnetic iron oxide nanoparticles is grafted with receptor-specific ligands and delivered at the desired tumor site (Hilger et al. 2002).

Most interesting class of external-stimuli sensitive polymers are based on the photo/light effect. The class of photoresponsive polymers helps in the release of encapsulated drug after irradiation in the presence of light. When light of specific wavelength interacts with a polymer, it loses its structural conformation which releases the encapsulated drug. In a study by Ghosh et al., pyrenyl-methyl ester got cleaved in presence of ultraviolet light and transformed the hydrophobic methacrylate structure to hydrophilic methacrylic units releasing the drug. The effect of light dissociated its micellar structure and released the content (Ghosh et al. 2009).

Ultrasound-based external stimuli release the drugs from nanocarriers. The mechanical or thermal effect produced in presence of ultrasound can induce drug release. The heating effect transforms the polymer phase due to high-intensity focused ultrasound. The high-intensity cavitation induces a mechanical effect that destructs the polymer and releases the drug in the external compartment (Schroeder et al. 2009).

5.3 Surface-Modified Targeted Nanoplatforms

5.3.1 Antibody, Enzymes and Proteins

The differentiation between a normal cell and a cancer cell is based on the presence or absence of surface receptors, antigen, proteins, enzymes, etc. The density or expression may increase or decrease on the surface of cancer cells. For designing target specific release, the targeting moiety is attached to the nanocarrier surfaces which act as a ligand which specifically binds to cell surface receptor or antigen.

Advances in the field of immunotherapy have led to enhancement of therapeutic response in cancer therapy. The hybridoma technique utilizes the structure of antibody or antibody fragments with a strong affinity towards antigen or receptor surface. The advanced techniques utilize either a monoclonal antibody or fragments of antibody as represented in Fig. 5.2.

The variable domain, Fv, and single-chain variable domain scFv have more affinity towards tumor antigens. Tumor-associated antigen consists of protein type biomarkers overexpressed in many types of solid tumors. Tumor-associated antigen has

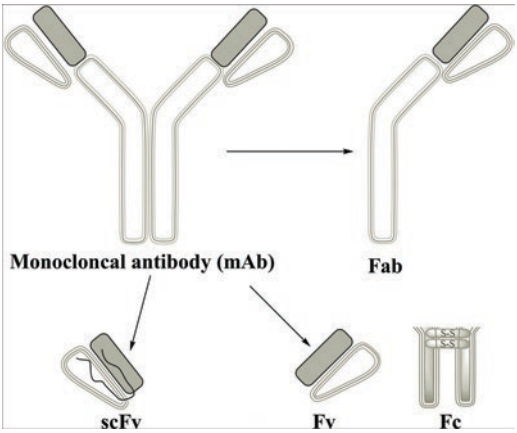

Fig. 5.2 Antibody and its fragments. *mAb* monoclonal antibody, *Fab region* fragment antigen binding, *Fv region* variable domain, *scFv region* single chain variable fragment, *Fc region* crystallizable fragment. The antibody fragments can be used as targeting component in active delivery

a high affinity towards antibodies with high specificity and stability (Chen et al. 2008).

During tumor angiogenesis, vascular endothelial receptors mediate the process and shows high affinity towards antibodies. Vascular endothelial growth factor receptors are one of the most important receptor types in the angiogenesis process. In hematological malignancies, leukemia has overexpression of vascular endothelial growth factor receptors (Bozec et al. 2008). Bevacizumab showed promising results in treatment of prostate cancer which has been approved by the United States food and drug administration (US FDA) for systemic targeting of angiogenesis in tumor cells (Lu et al. 2008).

In breast cancer, over-expression of tyrosine kinase receptor is too high (about 25–30%). The aggressive growth of tumor cells can be inhibited by the trastuzumab antibody (commercially available). The advanced combination therapy (ultrasound and chemotherapy) has shown promising inhibitory potential against breast cancer cell lines and has target specificity towards human epidermal growth factor receptor 2 due to the presence of herceptin. The herceptin has high affinity towards human epidermal growth factor receptor 2 which differentiates human epidermal growth factor receptor 2 positive and negative cells. After incubation with herceptin conjugated nanocarrier, human epidermal growth factor receptor 2 positive cells demonstrated high staining compared to negative cells.

Poly (lactide-co-glycolic acid) nanocarrier delivered paclitaxel with surface modified herceptin for active targeting. The permeability and targetability of herceptin conjugated nanocarrier was enhanced due to ultrasound effect. The frequency of ultrasound increased drug release rate with a maximum up to 33.7% (Liu et al. 2007; Zhong et al. 2020).

The antibody may show cross-reactivity with surface antigens, but sometimes the stronger affinity may get minimized. The cross-reactivity in antibody delivery is sometimes compromised in cancer therapy. The advantageous use of antibody fragments reduces immunogenicity and pharmacokinetics profile. The use of antibody fragments increases the time of circulation and decreases clearance rate after

grafting on the surface of nanocarriers (Fischgrabe and Wulfing 2008). Immunogenicity can be decreased by the use of humanized, fully humanized, or chimeric monoclonal antibody (Gu et al. 2007).

Few more limitations have been considered when monoclonal antibody is used for drug delivery. The heterogeneity of antigens on the surface of the tumor cells may reduce the targeting specificity or the permeability rate. High interstitial pressure opposes the permeability of antigen and passive targeting of monoclonal antibody to surface antigen. Certain overexpressed proteins, antigens, biomarkers are free-flowing within the tissue fluid. Basing on the affinity and molecular structure of antigens, the antibody may bind to cellular intermediates instead of receptors or antigens present on the cell surface and loses its activity (Sanz et al. 2005).

The major challenges associated are not only with the delivery of the drug but also with the penetration of the carrier. The penetrability of antibody grafted nanocarrier is also enhanced and the enhancement in permeability characteristics is related to strong interaction with cell surface receptors which helps to open channels for transportation across membrane. The accumulation rate was enhanced after antibody grafting on the surface of nanocarrier (ElBayoumi and Torchilin 2009).

The ligand-receptor targeting in monoclonal antibody fragments did not show the reticuloendothelial system scavenging effect due to the absence of the crystallizable fragment (Fc region). Fc region interacts with the phagocytic cell through the Fc receptor and responsible for reticuloendothelial system scavenging of particles in presence of the whole monoclonal antibody. Structural part of Fc region was depicted in Fig. 5.2. The advantageous effect of using monoclonal antibody fragment was to establish the good binding affinity towards the specific antigen.

Advancement in the biotechnological processes makes it feasible during the manufacturing of monoclonal antibody fragments from eukaryotic cells. The process was easily verified with a high output rate as monoclonal antibody fragments expressed as a recombinant protein. The monotherapy of monoclonal antibody did not provide a therapeutic outcome. The combination therapy with chemotherapeutic agents provided positive clinical outcomes. The reversible crosslinked chemotherapeutic agent to monoclonal antibody could deliver up to ten chemotherapeutic agents. The manufacturing process of monoclonal antibody was a very tedious job but new biotechnological inventions has made the process more easy (Brennan et al. 2004).

5.3.2 Aptamers

The three-dimensional modification of RNA or DNA oligonucleotides compositely forms aptamer which have a high binding affinity towards antigens. The intramolecular interaction with unique molecular structures shows high specificity and affinity towards antigens. The binding affinity of aptamer is similar to the antibody (Wilson and Szostak 1999).

In nanotechnology, aptamer are grafted on the surface of nanoparticles for recognizing tumor surface antigens. Aptamer can be derived by chemical or biochemical processes. Aptamer structure is similar to an antibody but do not show any immunogenic response. The rapid synthesis methods can easily fabricate the aptamer and analyze checkpoints useful for characterization and storage properties (Pestourie et al. 2005). For the active delivery of the chemotherapeutic agents, aptamer on the surface of nanocarriers should experience smooth penetration (Hicke and Stephens 2000).

Aptamer are exclusively designed for receptor recognition on the plasma membrane. Aptamer have a very short half-life from minutes to hours based on the nucleic acid fragment available. Nucleic acid degrades rapidly and is cleaned from the body by ultrafiltration. The diagnostic methods require rapid clearing substrate and aptamer may be a promising molecule for the development of diagnostics for cancer imaging (Ulrich et al. 2005).

The *in vitro* selection methods like systemic evolution of ligands by exponential enrichment (SELEX) or cell-based SELEX are used for aptamer amplification. The *in vitro* screening method provided a very small size aptamer (15–20 kDa) compared to the antibody (150 kDa) (Ellington and Szostak 1992).

Pegaptanib was the first RNA-based aptamer derived for age-related macular degeneration. Single-stranded PEGylated aptamer has a high affinity towards vascular endothelial growth factor – 165. The affinity for anti-vascular endothelial growth factor receptor enhances permeability and has a major role in angiogenesis. A United States based biotech company developed aptamer AS1411 (previously known as AGRO100). AS1411 acted by binding to cell surface nucleolin and internalization showed antiproliferative effect. The AS1411 is a oligonucleotide which consists of 26-mer unmodified guanosine, which induced growth inhibition in human tumor xenografts when evaluated *in vivo* (Ireson and Kelland 2006).

5.3.3 Folate

Folic acid has a very high affinity towards the folate receptor. Folic acid is a low molecular weight (441 Da), naturally occurring non-peptide moiety. Folic acid plays an active role in many natural processes like the synthesis of purines and pyrimidines. Folic acid is efficiently utilized for targeted delivery approaches due to its non-immunogenic nature compared to antibodies. The affinity of folic acid towards the folate receptor is approximately 1 nM which is very high as compared to other receptor types (Chen et al. 2013; Sudimack and Lee 2000).

In a normal cell, folate receptor is located on the apical stem of endothelium specifically found in lungs, kidneys, choroid plexus and placenta. Overexpression of receptor analysis suggests that a cancer cell has higher expression compared to a normal cell. Many emerging cancer cells like ovarian cancer, osteosarcomas, uterine sarcomas and choriocarcinoma, etc. show overexpression of folate receptor (Sudimack and Lee 2000).

Folate receptor are available in two different (α & β) isoforms based on glycosyl phosphatidylinositol graft. Epithelial cancer expresses α isoform while myeloid leukemia expresses β isoform. Some chronic inflammatory diseases also express β isoform through activated macrophages.

The receptor-mediated endocytosis pathways follow the conjugation of folic acid by the folate receptor in many cancer types. The recent ligand-receptor interaction based on folate expression was analyzed in breast cancer cell line (MCF-7) and glial tumor cell (C-6), which demonstrated optimum uptake efficiency (Sinha et al. 2006). The poly (ethylene glycol)-gemcitabine was evaluated for receptor-mediated endocytosis using folic acid as an anchor. The folic acid anchored carrier can bind effectively in human black cervix carcinoma cell line (KB3-1) also called as HeLa derivative. The Folic acid conjugated nanoparticles showed high inhibition potential than dummy nanoparticles (Pasut et al. 2008).

The intracellular uptake efficiency was enhanced after conjugation of folic acid for the delivery of docetaxel. The folate receptor is overexpressed in ovarian serous cystadenocarcinoma cell line (SKOV3) and hence showed an increased level of the drug at the targeted site (Esmaeili et al. 2008). Molecular ligand-receptor recognition mechanism suggests that folate receptors are available on cell surfaces where the folate conjugate binds efficiently to folate receptor. The folic acid-linked targeted therapeutics may pave the way in exploiting many carcinomas overexpressing folate receptors. The folate based targeting approaches effectively delivers both biomacromolecules and low molecular weight chemotherapeutic agents (Leamon and Reddy 2004; Mohanty et al. 2011).

5.3.4 Transferrin

Plasma membrane receptors have a major function in cellular internalization via iron uptake. Transferrin is a glycoprotein and facilitates the transport of ferric ion (Fe^{3+}) across the membrane by forming a complex. The transposition of two (Fe^{3+}) ions takes place by recurrent conversion of apotransferrin (iron-free) to ferrotransferrin (iron-bound). The transport of iron across the cell membrane occurs via receptor mediated endocytosis. The strong bonding between apotransferrin and two ferric ions is formed at neutral pH while dissociation takes place at pH below 6.0 after internalization.

The dissociated ferric ions are transported into cytosol and apotransferrin returns back to the surface transferrin receptor. The apotransferrin binds to cell surface transferrin receptor at pH 5.0 or 6.0 and the binding is not completed at neutral pH. The recycling vesicles are attached to the plasma membrane after releasing apotransferrin from ferric ions which further creates neutral pH. The pH of the cellular environment becomes suitable for further interaction. The ferric ions are free to bind at neutral pH to the transferrin receptor and the recycling process can be used for targeted delivery approaches. The overexpression analysis suggests that

tumor cell shows 2–10 fold higher expression of transferrin than normal cells (Mohanty et al. 2011; Pun et al. 2004).

The transferrin receptor shows prominent drug delivery targets for tumor expressing transferrin. The transferrin receptor targeting approach successfully delivers small molecules across the blood-brain barrier via transcytosis. The transferrin receptor targeting approach can potentially overcome the multidrug resistance due to p-glycoprotein. Breast cancer also overexpresses transferrin receptor and is targeted for the delivery of many therapeutics. Paclitaxel is delivered to breast cancer cell line (MCF-7), which has transferrin receptor overexpression (Sahoo and Labhasetwar 2005).

It's demonstrates potent therapeutic efficacy at the lowest possible dose of 1 ng/mL as compared to free paclitaxel. The nanocarrier has high cellular uptake due to the presence of Transferrin. In the human prostate cancer cell line (PC3), Transferrin receptor labeled nanocarrier showed 3 times higher efficacy than the unconjugated nanocarrier (Sahoo et al. 2004).

Avidin – biotin pathways efficiently target brain tumors (glioma cells) using transferrin receptor recognition. The biotinylated polyethylene glycol-polylactic acid nanoparticles and nonbiotinylated nanoparticles has shown to deliver paclitaxel efficiently. Transferrin conjugated nanoparticles showed delayed clearance and higher tumor accumulation in the rodent tumor model (Pulkkinen et al. 2008).

The transferrin conjugated nanocarrier has high efficiency as compared to non-conjugated nanocarrier. The rapid exocytosis was observed for non-conjugated nanocarriers. Due to inefficient escape from endosomes, non-conjugated nanocariers are unable to transport across the membrane which leads to rapid clearance. The differentiation in the retention time of conjugated and non-conjugated nanoparticles probably affects the release of chemotherapeutic agents (Das et al. 2009).

5.3.5 Albumin

Albumin has a high affinity towards glycoprotein 60 receptors on the cell surface and is transported across the intracellular spaces. The overexpression of albumin binding protein (BM – 40) is very high in the tumor cells. Once the albumin is transported to tumor interstitium, albumin nanoparticles bind to albumin binding protein BM – 40 which enhances cellular uptake via endocytosis (Frei 2011).

Albumin can increase the circulation time of nanoformulations. The commercial formulation Abraxane® consists of albumin bound paclitaxel which shows a prominent effect in metastatic breast cancer. It lowers the toxicity and the apparent solubility of paclitaxel is increased in presence of albumin. The albumin binds to the glycoprotein 60 receptor domain and enhances circulation time (Desai et al. 2006).

Human serum albumin conjugated with tumor necrosis factor-related apoptosis-induced ligand (TRIAL) of recombinant (Apo2L) nanoparticles showed high selectivity, enhanced solubility with improved pharmacokinetic characteristics. The circulating half-life was increased by 9.2 fold with 2.7-fold increase in

bioavailability. Tumor necrosis factor-related apoptosis-inducing ligand provides potent anticancer activity while conjugation with recombinant ligand like Apo2L enhances therapeutic outcome. The Apo2L/TRAIL-loaded human serum albumin nanoparticles was found to be a long-acting anticancer agent in the tumor microenvironment (Kim et al. 2011).

5.3.6 Biotin

Biotin or vitamin H is highly expressed on the surface of the tumor cells as compared to normal cells. The biotin can target the overexpressed receptors for active targeting. The role of vitamin H is to increase the proliferation rate in the tumor cells. Methotrexate-loaded albumin nanoparticles grafted with biotin increased tumor specificity in breast carcinoma cell lines. Cytotoxicity level was increased after conjugating with biotin with enhanced efficacy in the *in vivo* tumor model (Pérez-Herrero and Fernández-Medardem 2015; Taheri et al. 2011).

Tariquidar and paclitaxel were co-delivered using poly (lactic-co-glycolic acid) nanoparticles grafted with biotin on the tumor surface. The biotin grafting increased the cytotoxic effect as compared to paclitaxel alone. Tariquidar inhibited P-glycoprotein and increased the accumulation rate of paclitaxel in the tumor (Patil et al. 2009).

5.3.7 Hyaluronic Acid

Hyaluronic acid is a potential biomarker for hyaluronidase, overexpressed in many types of tumors. The high affinity of hyaluronic acid can be targeted for overexpression analysis of hyaluronidase and other similar types of receptors. Hyaluronic acid can be derived by chemosynthetic or biosynthetic approaches. Hyaluronic acid has a prominent physicochemical property that enhances its applicability in the biomedical field. Biodegradable, biocompatible, nontoxic hyaluronic acid has a high affinity towards glycoprotein (Non-kinase transmembrane proteoglycan –CD44 receptor) (Choi et al. 2012).

Hyaluronic acid is grafted with a chemotherapeutic agent or engineered on the surface of polymeric nanoparticles for active targeted approaches. In mouse squamous cell carcinoma cell lines (SCC7), overexpression of the CD44 receptor was very high. The intracellular accumulation and cellular uptake efficiency of the engineered nanoparticles Cy5.5 (near-infrared responsive fluorescent dye) – hyaluronic acid nanoparticles were increased due to the presence of hyaluronic acid. The accumulation rate was achieved at maximum level within 2 days in tumor-bearing mice (Choi et al. 2010).

Docetaxel loaded on hyaluronic acid -ceramide and coated with Pluronic 85 showed cytotoxic potential in breast cancer cell line (MCF-7). The targeted delivery of docetaxel overcame drug resistance due to the presence of hyaluronic acid and

Pluronic 85. The CD44 expressing breast cancer cell line (MCF-7) showed enhanced cellular uptake of docetaxel loaded nanoparticles with improved stability (Cho et al. 2011).

5.3.8 Toxins

Leiurus quinquestriatus scorpion venom consists of a small peptide with 36 amino acids conjugated to form a chlorotoxin. A chlorotoxin has a high binding affinity towards surface receptors overexpressed in many types of solid tumors. The chloride ion channels were studied using chlorotoxin. The high affinity of chlorotoxin towards receptors of cancer cell surface showed cytotoxic effect in breast, prostate, lung carcinoma, etc. The chlorotoxin can easily permeate through the blood-brain barriers, hence considered to be a potential ligand for glioma therapy (Yu et al. 2012).

Several probes were synthesized by conjugating ^{125}I, ^{131}I, fluorescent proteins with chlorotoxin for understanding the permeability across blood-brain barrier. The study showed the accumulation of chlorotoxin inside glioma cells with high selectivity (Soroceanu et al. 1998).

Near-infrared responsive fluorescent dye (Cy5.5) was conjugated with chlorotoxin and was termed as tumor paint. The tumor paint was detected during injection and permeation across barriers using the fluorescent effect generated in presence of Cy5.5. The surgical intersection of the tumor by using infrared laser was possible after injection of tumor paint. The chlorotoxin binds to matrix metalloproteinase-2 and showed an affinity towards surface receptor in cancer cells (Veiseh et al. 2007).

Chlorotoxin ligand is also used for the delivery of nucleic acid-based therapeutic agents. The small interfering RNA delivery was done via conjugation with iron oxide nanoparticles encapsulated with polyethylene imine. The amine functionality of polyethylene imine did not detach the ligand in an acidic environment. The chlorotoxin was grafted on the surface of polyethylene imine and delivered small interfering RNA inside the tumor cell by endocytosis. The ligand-receptor interaction facilitated the high internalization potential of chlorotoxin grafted nanocarriers with significant cytotoxic potential (Mok et al. 2010).

Delivery of chemotherapeutic agent using iron oxide nanoparticle delivered methotrexate in presence of surface grafted chlorotoxin. The conjugated system showed high ligand efficiency and cytotoxic effect in rat glioma cell lines (9 L cells) and human medulloblastoma cell lines (D283) (Bharti et al. 2017).

5.3.9 Nucleic Acid

Nucleic acid is a tailor-made therapeutics alternative for synthetic or viral vector delivery. The safety and efficacy are increased in nucleic acid delivery and has been attempted to enhance the therapeutic response. The genetic materials like DNA,

messenger RNA, small interfering RNA and micro RNA can modulate the cell responses via gene expression. The adverse effects or immunogenic responses can be minimized in nucleic acid delivery. Nucleic acid acts as mia ligand as it can easily traverse across the endothelial barrier and reach the site of action for gene silencing. The RNA interference (RNAi) is an emerging technique that involves artificial mediators which can efficiently knockdown target genes.

In cancer therapy, RNA interference therapeutics silences the expressions of oncogenes and improves immune responses and causes anti-angiogenic effect. Safe and efficient delivery to tumor cells can produce the desired gene silencing effect in local and metastatic tumors. The naked small interfering RNA delivery is more complicated due to non-targeted distribution, low transfection, poor endosomal escape, rapid renal clearance, and blood serum instability, etc. To overcome the limitations such as degradation and instability of small interfering RNA, the small interfering RNA is always conjugated with a suitable nanocarrier.

Negatively charged small interfering RNA was encapsulated in mesoporous silica nanoparticles. The mesoporous silica nanoparticles were coated with biocompatible polymers like polyethylene amine and polyethylene glycol via steric interaction. Small interfering RNA was further coupled with trastuzumab, an anti-human epidermal growth factor receptor 2 monoclonal antibody. The *in vitro* study of mesoporous silica nanoparticles showed apoptotic cell death selectively in human epidermal growth factor receptor 2 positive cells only. The *in-vivo* results demonstrated a 60% reduction in human epidermal growth factor receptor 2 protein levels in the HCC1954 xenografts. Repetitive multiple dosing suggested an inhibition in tumor growth within 3 weeks of treatment (Ngamcherdtrakul et al. 2015).

Metal-based nanocarriers also showed a promising response to the delivery of nucleic acid. The nucleic acid therapeutics conjugated with gold nanoparticles reduced enzymatic degradation and improved systemic stability. The gold nanoparticles were grafted with oligonucleotide sequences (POY2T) for active targeting to nucleus of breast cancer cell lines (MCF-7). The ultrasmall gold nanoparticles delivered triplex-forming nucleotide which was noted to bind to c-myc promoter. The gene expression was inhibited in presence of POY2T and showed an antiproliferative effect against breast cancer cell lines (MCF-7). The expression of c-myc promoter was decreased by 40% (Huo et al. 2014).

5.3.10 Virus

In biological therapy, microbes are utilized for targeting cancer cells. Many bacteria or viruses can act against specific cancer cells. Engineered viruses have been proven to have anticancer properties that express therapeutic proteins. The replication of the virus inside the tumor cell blocks apoptosis. The viral-based targets have been approved by the United States food and drug administration to be used in cancer therapy.

The viruses like mumps virus, newcastle disease virus, reovirus, adenovirus, vaccinia virus, and the measles viruses were previously used for cancer therapy. The myxoma or vaccinia strains are a type of poxviruses which were employed to deregulate cell replication, immune evasion in tumor cells (Kirn and Thorne 2009). Engineered pox virus (JX-594) activated epidermal growth factor receptor – Ras – mitogen-activated protein kinases signaling pathways were targeted to the tumor interstitium. A partial increase in immunological response was observed after engineered pox virus insertion due to the expression of granulocyte colony-stimulating factor.

The clinical outcomes of engineered pox virus were very efficient as 6 out of 10 patients were stable and 3 cases showed partial remission. The intra-tumoral injection of engineered pox virus in liver metastatic cancer patients showed minor side effects like flu-like symptoms. The bilirubin level was found to be increased after poxvirus injection (Park et al. 2008).

The advanced melanoma in patients was treated by multiple injections of herpes simplex virus. Another group of patients having non-visceral melanoma received granulocyte macrophage colony stimulating factor expressing viruses using the subcutaneous route. About 16.3% the treated patients showed durable responses. In the control subjects, viruses were seen to spread in the non-injected tumor site also. These viruses among the first modified virus were approved for the treatment of cancer (Andtbacka et al. 2013; Breitbach et al. 2011).

5.3.11 Affibodies

Antibody domain-like affinity derived from *Staphylococcal* protein A has a structure similar to a small peptides called affibody (Nord et al. 1997). The construction of phase libraries was also derived using combinatorial approaches considering protein A as a scaffold. The phase display techniques devised interaction between affibody and targeted biomarker. The protein corona shield technique suggested that an affibody of 6 kilodalton has a selective binding with the human epidermal growth factor receptor (Wikman et al. 2004).

High-affinity proteins are smaller than antibody and can deliver the chemotherapeutic agent at the site of action. Affibody is grafted on the surface of the nanocarriers which acts as a ligand for specific overexpressed cell surface receptors on the tumor cells. The affibody enhances the circulation time of the drugs in blood by avoiding clearance from the mononuclear phagocyte system (Yoo et al. 2019).

Affibody grafted on the surface of mesoporous silica nanoparticles was reported for active delivery of camptothecin. Affibody has a high affinity towards Human epidermal growth factor receptor 2 and binds to the receptor via supramolecular interaction. Due to small size and Human epidermal growth factor receptor 2 specificity, the ligand conjugated mesoporous silica nanoparticles showed high cellular uptake efficiency in Breast cancer cell lines (SK-BR-3 cells).

The conjugated nanocarriers showed high cytotoxic potential as suggested by *in-vitro* studies and possess a high accumulation rate in tumor-bearing mice. The tumor growth was inhibited by 90% which was achieved due to conjugation of affibody. The affibody grafted nanocarriers escaped from the reticuloendothelial pathway with high inhibition potential (Oh et al. 2018).

5.3.12 Peptides and Polypeptides

The peptide-based ligands have a targeting affinity similar to antibodies and less expensive than antibodies. The peptides have a high affinity towards integrins expressed on endothelial cells and play an important role in angiogenesis. Sequence analysis of peptides was analyzed by integrin. The preferred sequence used for integrin targeting was arginylglycylaspartic acid or other several sequences which are designed for targeting in disease conditions. Poly (lactide-co-glycolic acid) nanoparticles grafted with poly (ethylene glycol) and arginylglycylaspartic acid was designed for active targeted delivery of doxorubicin.

The integrin overexpressed receptors on the cancer cells showed very high cytotoxic potential due to the presence of arginylglycylaspartic acid. The poly (ethylene glycol) increased stability and exocytosis during transportation (Wang et al. 2009). Antitumor activity of paclitaxel was increased after conjugation with arginylglycylaspartic acid. The poly (lactic-co-glycolic acid) nanoparticles specifically delivered paclitaxel across tumor endothelium. The arginylglycylaspartic acid peptide had a high affinity for αvβ3 integrins and enhanced the *in vivo* response in mice bearing transplantable liver tumors (Danhier et al. 2009).

The delivery of peptide-based ligand was evaluated in a brain tumor. Low-density lipoprotein receptor-related protein was overexpressed in the blood-brain barrier. Lipoprotein receptor-related protein helped in transportation of the drug for glioblastoma therapy and pituitary gland tumors. Peptidic sequence Angiopep-2 has a high affinity for lipoprotein receptor-related protein in blood-brain barrier.

Poly (ethylene glycol) copolymerized poly epsilon-caprolactone grafted with angiopep-2 was used for active delivery of paclitaxel in glioblastoma cell lines. The nanoparticles showed a high antiproliferative effect in glioblastoma cell lines (U87MG). The accumulation was increased due to the presence of angiopep-2 in UG87MG tumor-bearing mice with increased rate of apoptosis. The lipoprotein receptor-related protein targeting showed prominent potential for blood-brain barrier crosslinking (Xin et al. 2011).

A nuclear localizing signal is a type of peptide sequence that also showed a high affinity towards the surface receptor. The nuclear localizing signal enters the cell and targets the nucleus via cytoplasmic factor. Doxorubicin encapsulated in poly (lactide-co-glycolic acid) nanoparticles was conjugated with nuclear localizing signal and targeted the nucleus in breast cancer cell line (MCF-7 cells). The doxorubicin delivery showed enhanced uptake, accumulation, and cytotoxic effect in the nucleus of MCF–7 cells (Misra and Sahoo 2010).

Cytokeratin is a polypeptide, expressed mostly in cancer cells and used as a biomarker for detection of malignancy. The breast cancer cell lines and other types of solid tumors over-express cytokeratin (Soudy et al. 2017). Acidic and basic heterodimers are two major types of cytokeratin found within the cancer cells. Acidic or type I (cytokeratin 9–23) polypeptides and basic or type II (cytokeratin 1–8) polypeptides are two heterodimers used for selective active targeting (Stroescu et al. 2006).

Monoclonal antibody has high specificity towards cytokeratin. Poly (lactic-co-glycolic acid) nanoparticles grafted with monoclonal antibody for targeting cytokeratin were observed in breast cancer cell lines. The covalent or noncovalent attachment was observed while studying the interaction between monoclonal antibody and cytokeratin (Kocbek et al. 2007).

5.3.13 Miscellaneous

Second-generation bio-adhesives presenting lectin, mucin, mannose, etc. are recognized as surface receptors. Sugar moieties and structurally similar drugs have an affinity towards the lectin receptor. Carbohydrate functional chemotherapeutic agents have high-affinity lectin receptor and shows high cytotoxic, inhibition of tumor growth. Selective lectin-like wheat germ agglutinin has high specificity to brain targeting.

Lectin receptor from the surface of the brain endothelia transports the drugs, proteins, and peptides across the blood-brain barrier without interruption. Lectin opens a novel pathway for targeting brain tumor. The cytoadhesive property of lectin has helped to improve the bioavailability and showed synergistic potential along with the chemotherapeutic agent.

Peptide phase libraries or peptide sequences also have ligand targeting capacities which provides more insights in preparing libraries from bacterial, plasmid, or combinatorial approaches. Due to the small size (10–15 amino acids), the low immunogenic, stable, manufacturing process of peptides were used as an alternative to antibodies for ligand-receptor targeting. The limitation of peptide-based targeting is its non-specific adhesive nature with integrin receptor (Newton et al. 2006).

AdNectins is another class of oligopeptide derived from the human fibronectin domain (10FN3). AdNexux pharmaceuticals isolated AdNectin and named it as angiocept which has a high affinity towards vascular endothelial growth factor receptor 2. In the treatment of solid tumors and non-hodgkins lymphoma, AdNectins showed excellent targeting effects (Getmanova et al. 2006).

Sigma receptors are over-expressed in many solid tumors especially in melanoma. Anisamide has a high affinity towards the sigma receptors available on the surface of tumor cells. Anisamide is chemically a “benzamide” derivative which acts as a ligand for an active targeting approach. Sunitinib was conjugated with anisamide in a polymeric micelle for active delivery. An *in vivo* study conducted on murine melanoma cell lines (B16F10) tumor-bearing mice showed a greater tumor

inhibition rate after the active delivery of sunitinib. Anisamide has a high affinity for sigma 2 receptors and less affinity for sigma 1 receptors (Dasargyri et al. 2017).

5.4 Challenges

The challenges associated with solid tumors, sometimes can be beneficial for a targeted delivery approach. If the formulator selects one specific target, there may be the possibility of change in the activity or expression of alternative pathways. The oral route of administration is a preferred and most convenient route of administration in cancer therapy. Technically, oral route of administration is the most challenging one due to the presence of different pH, microflora and the composition of gastric fluid, residence time, poor absorption, etc.

Several attempts have been made to align nanocarriers with pathological applications and proprietary use for clinical usage. Understanding the pathophysiology of diseases can be implicated to address the challenges for the fabrication of nanocarriers. The biological parameters and physicochemical properties of the nanoformulation affect the permeation, distribution, accumulation, retention, release of chemotherapeutics from nanocarriers. Disease biology is helpful in the clinical translation of nanomedicine which scrutinizes the small pathophysiological changes (Hare et al. 2017).

The entry of nanocarriers into tumor microenvironment is based on the enhanced permeability and retention effect. The distribution of nanocarrier takes place into different compartments like kidney, liver, lung, spleen, bone marrow. Distribution to nonspecific organs shows side effects after the release of the therapeutic agents. The inhaled nanocarriers target macrophages and alter immunologic functions (Khiavi et al. 2020).

5.4.1 Extravasation and the Enhanced Permeability and Retention Effect

The enhanced permeability and retention effect was misinterpreted and many scientists depicted tumor targeting using the enhanced permeability and retention effect, but the enhanced permeability and retention effect is not a gold standard for accumulation of nanocarriers in the tumor. The enhanced permeability and retention effect is observed in only some types of tumors (Maeda 2015). Biological barriers and enhanced permeability and retention effect are not similar in every disease conditions. The enhanced permeability and retention affects the efficiency of nanocarrier, kinetics of drug release, cellular uptake, etc.

The enhanced permeability and retention effect is a heterogeneous phenomenon due to anatomical variability, pathophysiological properties of tumors and stage of

development varies from patient to patient. The permeability rate of nanocarrier varies with types of tumor. Extent of angiogenesis, mutation, vasculature, and tumor maturity are the major accountable factors that determine the size and structure required for transportation of nanocarriers.

The pore size varies between 200 nm to several microns depending on tumor maturity. The differentiating pore size also changes the tumor microenvironment and is held responsible for varying the rate of permeability (Hobbs et al. 1998). The position of the tumor also affects the enhanced permeability and retention due to anatomical sites (Massey and Schnitzer 2010).

The abnormalities of vasculature in tumor tissue can be identified by pericyte density, basement membrane formation along with endothelial cell activation. Many parts within tumor vasculature have a smaller size below 3–4 nm due to the presence of fibroblasts, pericytes, smooth muscle cells, etc. The endothelial linings become more intact due to the increasing cell density at the surfaces. Only small molecules like albumin can pass the tumor interstitium with pore size below 3–4 nm.

Vascular abnormalities are observed in tumor tissues with physiological changes in blood flow. Osmotic pressure within the tumor increases with increasing interstitial fluid pressure. The major challenges associated with extravasations or enhanced permeability and retention in solid tumors is higher interstitial fluid pressure. The generation of high interstitial fluid in tumor tissues may be due to the leaky vasculature and poor functionality of lymphatic vessels (Heldin et al. 2004).

The higher interstitial fluid pressure leads to an outward flow of fluid and is responsible for poor diffusion rate. The average interstitial fluid pressure recorded is about 20 mm Hg which is comparatively very higher than normal (0 or − 1 to −3 mm Hg). This process limits the diffusion of nanocarriers into the tumor environment. The variability in interstitial fluid pressure is observed in different types of tumors. Comparative investigation suggests that a deep-seated tumor has a higher pressure than the peripheral one (Peer et al. 2007).

Transport of drugs into the tumor occurs by convection method not by diffusion. The process of convection channelizes the transport of high molecular weight drugs from the circulatory system to the interstitium due to high interstitial fluid pressure. The transport of drug is decreased as transcapillary action gets decreased due to high interstitial fluid pressure value (Heldin et al. 2004; Jain 1987). The extravasation starts its role in leaky or fenestrated vasculature. The penetration can be enhanced by inflammatory mediators like histamine and tumor necrosis factor – α. High tumor cell density also affects the penetration of nanocarriers (Ernsting et al. 2013).

5.4.2 Tumor Microenvironment and Tumor Interstitium

The surrounding microenvironment around the tumor defines the target for therapeutic molecules. Generally, tumor targeting therapeutics is designed as per the tumor microenvironment.

Differentiation of normal and tumor tissues can be achieved by different mechanisms of vascularisation, oxygenation, perfusion rate and metabolism. Cellular hypoxia begins to develop after the tumor size increases above 2 mm^3. The angiogenesis is initiated once the levels of cellular hypoxia-inducible factor transcription increases, which leads to activation of proangiogenic proteins, integrin and inhibitors. Hypoxia-inducible factor transcription factor upregulates vascular endothelial growth factor, platelet-derived growth factor, or tumor necrosis factor – α (Carmeliet 2000).

The angiogenesis process is completed in 5 major steps with balanced regulation of activators and inhibitors. The first step of angiogenesis starts with the activation of endothelial cells. The angiogenesis initiates the expression of dimeric transmembrane integrins $\alpha_v\beta_3$. The integrins transport nutrients to the tumor interstitium by interacting with extracellular matrix proteins. The second step simultaneously starts with the degradation of the basement membrane and endothelial cell which migrates through the extracellular matrix to form new blood vessels (Avraamides et al. 2008).

At the final stage of angiogenesis, matrix metalloprotease is activated from endothelial cells which start degrading the extracellular matrix and basement membrane. New vessel lumens are formed on the inner side of endothelium. Pericytes and smooth muscle cells stabilizes the vessels and remodeling of the angiogenesis process starts in immature vasculature (Stollman et al. 2009).

The angiogenic switch is responsible for the dissemination of cancer cells and the progression of metastasis in the human body. The vasculature developed near the tumor cells is very complex. Oxygenation potential in the tumor environment gets dampened with pH variability in each compartment. The perfusion rate and metabolic activity also becomes abnormal.

The process of angiogenesis starts forming new blood vessels that requires oxygen and nutrients for development of the malignant state. The energy requirement is provoked through glycolysis pathways in the hypoxic tumors. This decreases the pH (5–6) around the tumor cells as compared to normal cells at neutral pH. The overexpressed proteins, antigens, enzymes are regulated through angiogenesis (Van Sluis et al. 1999).

5.4.3 Physiological Barriers and pH

In the tumor microenvironment, intracellular pH remains constant whereas extracellular pH changes to slightly acidic. In normal tissues, the pH is 7.4 while pH at tumor site is reduced to a lower value of pH 5.0–6.0. High glycolysis levels lowers the pH in the hypoxic cancer cells (Feron 2009). The process of conversion of in situ tumor into an invasive tumor is based on low pH and low oxygen level. Warburg effect suggests that defect in the mitochondrial respiratory chain may be responsible for lowering oxygen content and responsible for the reduction in energy sources. The decrease in energy resources hampers the glucose oxidation pathway (Golias et al. 2019).

Metabolism within the tumor cells needs biosynthetic pathways like glycolysis. Tumor cells requires high glycolytic rate to maintain this requirement. Nicotinamide adenine (NAD^+) is formed by catalytic conversion in presence of glycolytic enzymes which converts pyruvate to lactate. Cytotoxicity is eliminated by removing lactate formed within the tumor cells. Exclusion of the process with the elimination of one proton from carboxylic acid to lactate leads to acidification of extracellular space.

During this conversion, cytosol becomes alkaline due to the sharing of a proton with lactate molecule. Carbonic anhydrase IX also contributes to acidify the pH environment within intra and extracellular spaces. The carbonic anhydrase IX causes conversion of carbon dioxide to bicarbonate and reversion of anion which may acidify the environment by reducing the pH (Brahimi-Horn and Pouysségur 2007; Feron 2009).

The drug is distributed in tumor mass, host tissue, extra and intracellular spaces and other compartments of tumor cells. The partitioning of the drug depends on the variable pH available in each compartment of the tumor cell. In a low pH environment or an extracellular environment, weak acidic drugs are easily distributed and diffuse across cell membranes in presence of a fraction of weak acid. The intracellular environment has slightly basic pH; the weakly acidic drug ionizes in the intracellular compartment and gets accumulated in the cytosol.

The accumulation of the ionized drug in cytosol leads to the development of a multidrug-resistant effect (Simon 1999). Tumor cell clones possibly traps the drug after delivery and reduces the activity in acidic organelles. In exocytosis pathways, the drug may be transported out of the cell (Danhier et al. 2010). Physiological barriers also limit the penetration of the drug. The transport of the drug across the endothelial cell, cell membrane, blood-brain barrier has limited penetration due to tight junctions (Nizzero et al. 2018).

5.4.4 Efficacy Versus Toxicity

From the past years, many nanomedicines have been evaluated clinically and enormous statistical data is available. Many research organizations have prepared databases to access the existing datasets and promote for analysis of the effective level of the chemotherapeutic agent. The clinical studies help to evaluate the systemic toxicity and efficacy of chemotherapeutics. The clinical investigator must align with the studies to find out complex interventions for cancer patients.

The complications for combination therapy must be reported. Two or more chemotherapeutic agents have a variable molecular structure, Log P, dosing interval, dose frequency, pharmacokinetics, pharmacodynamics, etc. which are responsible for interchanging the toxicity and efficacy level of the combination therapy. The two pKa values may create interference during the delivery of the drugs. The interstitial pH of the tumor needs to be analyzed and ionization of the drug must be monitored during release from the polymeric nanocarriers. The N-(2-hydroxypropyl)

methacrylamide polymeric nanoparticles delivered a combination of two drugs doxorubicin and gemcitabine.

The passive targeting of conjugated nanoparticles on N-(2-hydroxypropyl) methacrylamide polymers showed a long circulation time. The *in-vivo* study suggested that polymers conjugated selectively and delivered both doxorubicin and gemcitabine simultaneously without increasing toxicity (Lammers et al. 2009).

5.4.5 Translation from the In Vivo *Model to the Human Model*

Different observations are noted in the preclinical and clinical investigations due to variable tumor types available in the animal as well as human. It is also observed that the enhanced permeability and retention effect has variations in the animal model than in human tumor. Rodents grow at a faster rate and tumor also has similar growth rate. The rate of growth of tumors in rodents is higher as compared to human tumors.

The human tumor takes about a year to grow up to 1–2 kg (20 cm), while in rodents tumor grows 1 cm within 2–4 weeks. A blood vessel in a human tumor develops leaky vasculature while in a rodent, the tumor vessel may not be developed completely and more leaky than a human tumor vessels (Lammers et al. 2012).

During tumor growth, anatomical variations are also noted. Subcutaneous tumors have different growth determinants than native (orthotropic) tumors. The differentiation in the growth rate is observed due to alterations in the surrounding microenvironments (Sutherland 1988). Clinicians screen the tumor position and type of tumor before providing treatment to patients.

The rates of growth of tumors vary in benign tumor and metastasized or submetastasized tumors. While understanding the tumor type, the immune response is a considerable factor that shows a significant effect. The immunogenic effect may be positive or negative based on the growth stage available. The physiological, biological, and immunological differences are unable to show reproducible results in transformation from preclinical to clinical data (Begley and Ellis 2012; He et al. 2019; Prendergast 2008).

Efficiency level can be improved by considering the translational shortfall in the preclinical investigations which improvises the therapeutic response and cost-effectiveness in clinical studies. Clinicians must establish the sequence of studies integrating head-to-head comparison from animals selected for studies with the respective cell lines. Based on the type of tumor treated, at least a minimum of 5 models or a maximum of 10 models are selected for comparison.

The panel model methodology includes a subcutaneous xenograft model, orthotropic model, metastatic model, transgenic model, etc. In panel model methodology, the selection is based on therapeutic outcome. The required therapeutic dose is effective during panel screening and one of the objectives of the selection model. The data validation must be performed through statistical analysis and the animal model must provide additional cross comparison (Lammers et al. 2012).

5.4.6 Metastasis

The nanomaterials are designed or screened in a proximal way to effectively deliver the chemotherapeutic agents to the desired site of action. The increasing death rate in cancer patients is due to metastatic tumor growth which becomes unresponsive to monotherapy. The locally confined tumor or diagnosed at the initial stages may increase the survival rate. Surgery, radiotherapy are alternative ways to treat locally confined tumors. Adjuvant chemotherapy is employed to prevent metastasis after surgery or radiotherapy (Rampling et al. 2004).

New methods or protocols are required for the development of anti-metastatic formulation. After surgery or radiotherapy, patients lose response and immunity to therapy or chemotherapy. The scientists have been working on the development of novel formulations that would enhance the immune response and accelerate therapeutic efficacy. Local delivery to metastasized tumors is another strategy used in the treatment protocol (Wang et al. 2012).

5.4.7 Selection of Material for Designing Nanocarriers

The selection of carrier substrate is an important aspect of target-specific approaches. The binding affinity must be high for specific receptor or cell targets available at the tumor site. The loading efficiency, transportation, permeability, accumulation is based on the nature of the nanocarriers selected. The material properties reflect the functionality of the fabricated nanocarriers. The alternating factors like size of nanocarrier, surface charge, and circulation time in blood define the ability of formulation to withstand the systemic circulation. For cancer delivery, the selected nanocarriers must possess biodegradability, biocompatibility and nontoxicity to a normal cell.

In target-specific approaches, nanocarriers must extravasate and diffuse into tumor interstitium. The vascular permeability coefficients of different nanocarriers are different based on the type of tumors (Biffi et al. 2019). The vasculature size may vary between 10 and 800 nm and small-sized nanocarriers can be transported across vasculature (V. Torchilin 2011).

During the fabrication of nanocarriers, a scientist cannot overlook the size of the nanocarrier used for targeting. If the size of the nanocarrier is below 200 nm, it can be transported into the tumor cells by enhanced permeability and retention effect. Effective size of the nanostructure must be between 10 and 500 nm for cancer-targeted approaches (Fang et al. 2011).

Molecular weight is another important factor for the transport of macromolecular ligand across cell barriers for active targeting approaches. The initial layers can extravasate particles of molecular weight of 3–10 kilodalton with a size of 2–3 nm at a faster rate. The macromolecular ligands or nanocarriers of molecular weight

between 70 and 2000 kilodalton have a slightly slower rate of transportation (Dreher et al. 2006).

Penetration of nanocarrier and cellular association of ligand-based biomolecules is dependent on surface charge. Surface charge significantly affects the retention and interaction property within the human body and at the site of targeting. Highly positive charged nanocarriers are opsonized and eliminated from circulation due to opposite charges on the surfaces of blood vessels (Maeda et al. 2009; Xiao et al. 2011). While negatively charged nanocarriers may be retained but based on charge density the interaction may vary (Arvizo et al. 2011).

The positively charged nanocarriers have an advantageous effect on tumor blood vessels. Positively charged nanocarriers escape by endocytosis to avoid elimination. In addition to this, positively charged nanocarriers enter the cytoplasm via disruptive interaction with the endosomal membrane (Ding and Ma 2013). The major challenge related to nanocarrier delivery is related to retention of nanocarrier in the systemic circulation for a longer time. Two specific properties are required to maintain healthy interaction i.e. haemocompatibility and longevity.

The longer circulation time is beneficial for passive as well as active targeting approaches which specifically provides longer passage to reach the selected target (Torchilin 2010). The reticuloendothelial system and macrophages may remove circulating nanocarriers from blood. Steric protection avoids the nanocarrier from capture and clearance pathways. While considering these parameters, active as well as passive delivery of therapeutic agent can be feasibly enhanced using smart nanocarriers (Hossen et al. 2019).

5.5 Preclinical and Clinical Intervention for Transposing to Market

Translation of chemotherapeutically derived nanocarriers for clinical use is an expensive and time-consuming process. The selection of material for chemotherapeutic delivery of nanocarriers is complex than conventional formulations. The design of nanoformulation is the first step in the pharmaceutical process. The formulation can decide the route of administration, dosage regimen and frequency, biocompatibility and degradability, physicochemical stability of the developed formulation.

Pathophysiological challenges need to be considered while translating nanoformulation to clinical studies. The major hurdle in delivering nanocarriers to the tumor site is the heterogeneity of human tumors as compared with the animal tumor. During the translation of nanocarrier at clinical stage, physicochemical properties of nanomaterial must be accomplished to cross the biological barriers. The property of nanocarrier enhances target specificity and reduces accumulation in non-target organs.

Many developed formulations did not reach commercial scale as the formulator may not have focused on patient biology and heterogeneity of tumor during formulation development process. These considerations restrict the investment made by pharmaceutical industries. To avoid or reduce the associated risks, preclinical data should be exclusively evaluated for safety, efficacy, pharmacokinetics, pharmacodynamics, etc. in animal models (Park 2017).

Reproducibility should be correlated with the disease type and not with animal used during the experimental procedures. Appropriate selection of animal models should be based on disease specificity which can provide accurate data for treating patient subgroups from the preclinical investigations. Anatomy, physiology, internal environment, biomarkers, concentration, overexpression of receptors is differentiated in animal species as compared to humans.

Preliminary differentiation should be considered while selecting a route for delivery of the drug to animals or humans. To reduce the bias during the study, appropriate animal models should be selected at preclinical stages. The selection of gold standard and evaluation of control groups are very helpful in reducing bias (Begley and Ellis 2012).

An active targeting approach has different challenges than a passive targeting approach. Ligand recognition, presence of receptor at the surface, free-flowing enzymes or proteins with similar molecular fingerprint-like receptor, expression variability, optimal ligand density, or few more factors are considered while choosing the targets. The selection of ligand depends on the expression of receptor, location, internalization rate and immunogenicity, etc. Overall these factors majorly affect the clinical translation of actively targeted nanocarriers (Kraft et al. 2014).

Clinical interventions like heterogeneity, inter and intra patient variability, disease staging, physiological complications, and many other factors unlikely affect the translation of the formulation in clinical stages.

Few other factors are likely correlated with clinical studies to signify inter and intra patient variability depending on the immune response received from an individual patient and other related complications. These factors affect the commercialization of nano carrier-based formulations into the market. Limited accessibility of drugs to the tumor tissues, the requirement of high doses, intolerable cytotoxicity, the development of multiple drug resistance, and non-specific targeting are the major obstacles to the clinical use of cancer drugs and cancer therapy.

5.6 Commercialization and Government Regulations

Nanocarriers demonstrate delivery of the chemotherapeutic agent for enhancing many aspects of therapeutic efficiency. Although preclinical results may be effective but do not guarantee to be translated for clinical outcomes. For the delivery of chemotherapeutics from bench to bedside, several challenges need to be addressed by formulation scientist starts from the selection of polymer for nanocarrier to still provide prominent therapeutic potential.

The structural and physicochemical complexities of nanocarriers are the most important contributing factors for large-scale manufacturing processes. The complex synthetic formulations have more hurdles to translate to clinical stages. Commercial products are developed on cost, quality, and process parameters. During the commercialization of nanocarriers, major challenges are associated with the qualitative test and significant quantitative validations. The in-process quality checks scrutinize big hurdles to transpose from laboratory to kilo lab to pilot scale, and further to commercial level (Kakkar et al. 2018).

In nanocarrier developments, by-products concentrations are too high or contaminants are too high which makes the product unsuitable for large-scale manufacturing processes. The nanotechnological advances may enhance the effectiveness but the cost of production is too high with low production yield. The reproducibility rate was lower and showed batch to batch variability during pilot-scale batches. Unavailability of in-house expertise, infrastructure facilities, equipment, and instruments might affect the commercial cost (Hafner et al. 2014; Narang et al. 2013; Singh 2017; Tinkle et al. 2014).

Industrial-scale manufacturing of liposomes delighted the industry over successful fabrication methods without the use of solvents and multiple steps (Jaafar-Maalej et al. 2012). Delivery of multiple therapeutic agents and ligand coating on the surface of nanocarriers might be a challenging environment for large-scale manufacturing. The process flow might go through multiple steps and have inevitable errors for good manufacturing of nanocarriers (Hua et al. 2018; Tinkle et al. 2014).

The batch-to-batch variability needs to reduce while submitting data to regulatory bodies. The investigator must access the data for consecutive batches and need to evaluate physicochemical properties of drug products. For composition of nanocarriers, an investigational new drug requires chemistry, manufacturing, and control information. The characteristics of nanocarriers and reproducibility allow the initiation of clinical trials. In addition to this, the properties of drug substances are well defined with their analysis criteria. The strength, potency, quality, purity of drug substance was clearly mentioned for generating a report for the submission to regulatory bodies (Food and Administration 2003).

United States food and drug administration seeks the data for the product under investigation stage or clinical stages. Regulatory factors depend on validation parameters of production batches and manufacturing practices, quantitative measurement, safety, and efficacy level of developed product (Sainz et al. 2015). Regulatory authorities like food and drug administration, therapeutic goods administration, european medicine agency, central drugs standard control organization etc. are responsible for implicating guidelines for the conventional framework.

First approved clinical nanoformulation has standards that meet the general framework of regulatory authorities. Investigation or clear memorandum is needed to make use of nanoformulations for clinical use (Tinkle et al. 2014). The fabrication process of nanocarriers consists of multiple ingredients which makes the final composite structure more complex. For manufacturing of nanoparticles on a large scale, regulatory processes, protocols, guidelines, requirements, regulations, must be redefined to make the smoother entry into commercial markets.

During the development of regulation, the complexity of nanocarriers, route of administration, pharmacokinetic, pharmacodynamics, clinical trials data, characterization data, evaluation aspects, etc. must be taken into account. The cost of regulatory approval, time for submission to the regulatory body must be overlooked to negatively affect the progress and development of nanoformulations.

In the future global regulatory standards must be established while considering inputs from industry, academia, research, and regulatory body. The specialized infrastructure facilities must be developed individually for each type of nanoformulation, such as liposome, solid-lipid nanoparticles, polymeric nanoparticles, dendrimers, protein conjugates, peptide conjugates, etc. Regulatory bodies should develop stringent and uniform guidelines for testing protocols for the evaluation of nanocarriers and standard protocols for toxicity studies (Patnaik et al. 2021). It should also provide a validated documentation list to be submitted for approval of formulation which reflects safety, efficacy and stability of nanoformulations.

5.7 Conclusion

Identification of tumor types is a very difficult task. Several reports suggest that anatomical position also changes the vascular structure and tumor environment. The results obtained from the preclinical investigations do not match with clinical data which may be due to differentiation in tumor types. The nanoformulation strategies offer prominent therapeutic outcomes with an enhanced biological response.

The new approach of synthesis produces uniform-sized nanoparticles which has efficient physicochemical properties for biomedical applications. Engineering of the nanoparticulate system is possible with new advanced techniques of manufacturing for finding active targets. The multifunctional nanoparticles have high clinical translation and can be used for diagnosis as well as therapeutic applications.

Many nano-based formulations have obtained food and drug administration or other regulatory approval for clinical or commercial use. Over interpretation or misconceptualization may fail many clinical trials at early stages. Complex fabrication approaches may not transfer the product to commercial batches; although the nano pharmaceuticals may be multifunctional, multiple-step manufacturing might be tedious for large-scale manufacturing.

Nano pharmaceutical formulations may not provide optimum efficacy rate in monotherapy and fail to clear the preclinical stage. The image-guided instrumental diagnostic facilities are not available for every patient undergoing cancer treatment. Nanomedicine based therapy is not feasible for low-income countries. To overcome these shortfalls, young researchers must focus on biological, pathological, targeted ligand, and the development of innovative instrumental facilities in the near future.

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Chapter 6
Polymeric Nanoparticles to Entrap Natural Drugs for Cancer Therapy

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Abbreviations

EPR	Enhanced permeability and retention
MOLT-4	cell line derived from lymphoblastic leukaemia
SKOV-3	ovarian cancer cell line derived from the ascites

6.1 Introduction

Humankind has been dealing with cancer for as long as we know. Humans have been plagued by this disease for centuries now, having a long history and its prevalence curve is ever-rising. Hippocrates (460–370 BC), the Greek physician known as the father of medicine, coined the word 'cancer' when he used terms like 'carcinos' and 'carcinoma' to describe certain tumors. The ancient egyptians concluded way back after diagnosing the first-ever documented case that 'there is no treatment' for this disease (Faguet 2015). Cancer is now considered to be a modern disease since its prevalence and its impact is more substantial on our society than on our ancestors.

Scientifically, cancer itself is a vast group of diseases that arises from transforming normal cells into tumor cells via a multistage mutative process that can spread to any part of the body (Cooper and Hausman 2000). These mutative changes may occur due to the interaction of an individual's genomic factors with either physical

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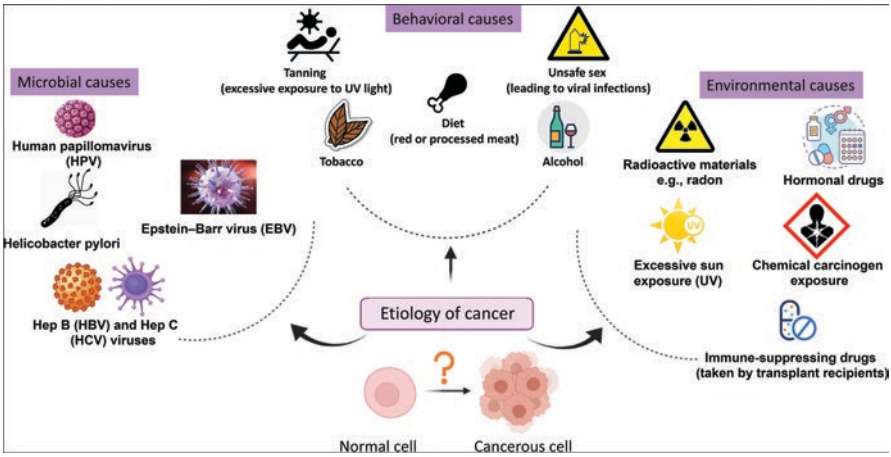


Fig. 6.1 Etiology of cancer highlighting the microbial, behavioral and environmental causes. The associated risk factors causing genetic changes leading to cancer prognosis and tumor formation are discussed. *EBV* Epstein-Barr virus, *HBV* Hepatitis B virus, *HCV* Hepatitis C virus, *UV* Ultraviolet

carcinogens (ultraviolet radiations, ionizing radiations comprising of alpha particles, beta particles, gamma rays, and neutrons) that have sufficient energy to cause direct cell damage, chemical carcinogens (asbestos, components of cigarette, aflatoxin, and arsenic) or biological carcinogens (human papillomavirus, hepatitis B, hepatitis C, *Helicobacter pylori* that are responsible for causing chronic infections) (https://www.who.int/news-room/fact-sheets/detail/cancer).

Tobacco utilization, frequent alcohol consumption, lack of exercise and physical inactivity, unhealthy and processed diet, and chronic inflammation or infections caused by bacteria, viruses, or parasites are the major relevant risk factors (Fig. 6.1) (https://www.who.int/cancer/prevention/en/).

6.2 Cancer

Few defining features of cancer is the rapid creation of abnormal cells having loose and feeble margins that grow beyond their usual boundaries, mild to extreme hypoxic conditions, high tendency to divide and multiply, increased glucose demand, infinite replication potential, ability to defy cell death and potentiality to invade different parts of the body nearby and spread to other organs, through the process of 'metastasis'. Metastasis can be repeated in cycles or multiple sequences and survive long enough due to the cascade effect in affected patients (Weiss 2000). During the progression of this disease, the abnormal mass of cells that is formed becomes heterogeneous. It creates a diverse population of cells and varied responsiveness to different kinds of treatments, thus displaying pleiotropic effects.

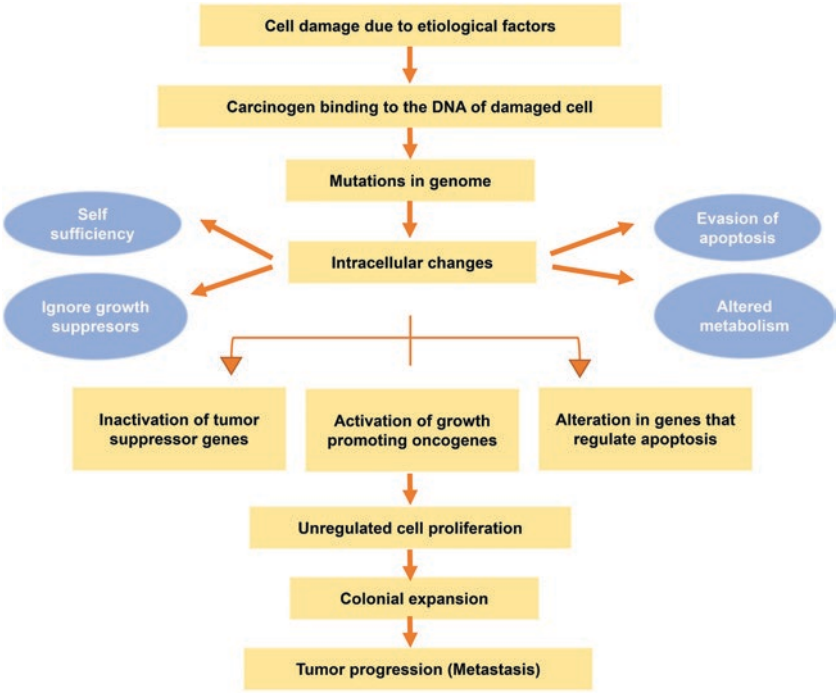


Fig. 6.2 Pathogenesis of cancer. The initiation of metastasis beginning from cell damage due to etiological factors followed by tumor progression by mutations in genome leading to intracellular changes. The intracellular changes inactivate tumor suppressor genes, activate growth promoting oncogenes and alter the genes regulating the apoptosis and the final formation of a malignant neoplasm occur by uncontrolled cell proliferation and metastasis

Cancer is a disease driven by genetic mutations, and recent advances have given deep insights into the biology of cancer. A cascade-like process that begins with the effect of etiological factors that possess the mutative potential and ends with the production of a malignant and deadly tumor is explained in the following flowchart (Fig. 6.2) (Griffiths et al. 2000). Etiological factors of chemical, viral, radioactive origin, and their intricate interplay that is capable of causing DNA damage enables the development of mutations in the cell. Failure of DNA repair occurs via inherited mutations due to genes that affect DNA repair or the genes that affect cell growth or apoptosis. This happens when either the immune system or checkpoints of cell try to repair the cell damage caused but cannot do so (Bernstein et al. 2013). The pronounced effect of this inability is the mutation in the genome of somatic cells during intracellular changes. The mutation caused leads to the activation of growth-promoting oncogenes, alterations of the gene that regulate apoptosis which indirectly decreases apoptosis, and inactivation of cancer suppressor genes.

Genetic level mutations are capable of causing drastic and life-threatening situations. This leads to altered gene products and loss of regulatory gene products,

which hamper the entire host genomic functioning. Such a change at the genomic level and the takeover of the host machinery by the carcinogen leads to an expansion of their population via clonal expansion and additional mutations that cause the progression of cancer (Wang et al. 2018a, b). Through metastasis, the tumor growth spreads, and the heterogeneous nature of the cancer is observed. The outcome of their entire process is forming a malignant neoplasm, that is deadly and fatal (Birner et al. 2016).

Today, cancer is the second leading cause of death globally after cardiovascular diseases, accounting for an estimated 9.6 million deaths as cited by the world health organization data (Siegel et al. 2020). Approximately 1 in 6 deaths today across the globe, account for cancer. The most leading type of cancer in men is lung cancer, whereas, among women, breast and ovarian cancer are more prevalent. The likelihood of being diagnosed with invasive cancer in terms of estimated probability is slightly more for men (40.1%) than for women (38.7%). The higher risk in men is still unknown, but it maybe due to differences in environmental exposures and the effect of endogenous hormonal interactions.

Recent research suggests that sex differences in immune function and response may also play a vital role (Klein and Flanagan 2016). Among children and adolescents, cancer is the second most prevalent cause of death in the united states of america for children aged 1–14. Leukemia is reported to be the most common childhood cancer accounting for 28% of the cases (Siegel et al. 2020). In middle-aged and geriatric patients, chronic inflammation and co-morbidity become the primary factor that ultimately causes cancer. There is a clear correlation between chronic inflammation and genetic factors in cancer evolution and its indirect correlation to one's innate immunity (Coussens and Werb 2002). Early detection and diagnosis seem the most plausible way through which we can stack the odds of avoiding cancer in our favor (Crosby et al. 2020).

The current century comprises many exciting times in cancer research. Upcoming technologies are providing deep insights into understanding cancer initiation, progression, and resistance. Also, an endless array of identified cancer drug targets and promising drug combinations are giving hope to clinicians and patients (Akkari et al. 2020). Due to the complex labyrinth of cancer, a deep understanding of its biology and chemistry at the corelevel is of utmost importance to devise treatment strategies and develop preventive medicine. The treatment approaches depend on the type of cancer, the progression stage of the disease, the availability of treatment options, and the treatment goals. Methodologies for cancer treatment may be provided in combination or individually, depending upon the severity and spread in the host machinery. Globally many procedures are available for cancer treatment, with even more being studied. Conventional methods for cancer treatment include several techniques that are discussed below (https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types.html).

- Surgical treatment was the standard and the most preferred treatment method before 1950 until the evolution of modern techniques. Types of surgical treatments are minimally invasive surgery, open surgery, cryosurgery, photodynamic therapy, and laser surgery (Benjamin 2014).

- Chemotherapy comprises of drug administration procedures to curb the abnormal and rapid growth of cells (Huang et al. 2017).
- Radiation therapy utilizes an external beam of high-energy radiations or internal radiations (brachytherapy) by using radioactive implants. This emerged after 1960 to treat local diseases (Bawaskar and Bawaskar 2012).
- Targeted therapy aims to target a specific site and to cause no disturbances in the surroundings, such as the abnormal cell vasculature or its intracellular organelles (Talwar et al. 2017).
- Immunotherapy includes immune checkpoint inhibitors, cancer vaccines, and monoclonal antibodies targeting compromised immune systems. A combination of immunotherapy and targeted therapy may also see the light of the day (Vanneman and Dranoff 2012).
- Hormone therapy or endocrine therapy targets explicitly tumors that use hormones to grow and palliatively treat cancer (Abraham and Staffurth 2016).
- Stem cell therapy utilizes embryonic or somatic cells for cancer treatment. These cells express growth factors and cytokines to regulate the cellular immune pathways of the host. These pathways can be modified to escape the host immune response and act as cellular delivery agents (Zhang et al. 2017).

Extensive and exclusive research on cancer has enabled scientists to come up with multifaceted and more effective treatment regimens. Extracellular vesicles are responsible for cancer development, modification of tumor microenvironment, and rapid cell proliferation. Extracellular vesicles have been widely explored as efficient drug delivery vehicles. Another promising opportunity to explore is gene therapy that includes direct in situ administration of exogenous genes into the host's tumors (Abbas and Rehman 2018). Thermal ablation methods and magnetic hyperthermia have paved new ways and opened up arenas for precision medicine offering localized treatment (Pucci et al. 2019). Radiomics and pathomics significantly contribute to the current scenario for the development of innovative approaches for data management and analysis to serve as a diagnostic and prognostic indicator in cancer (Shakir et al. 2019).

At the same time, this growth in research and scientific progress has a significant impact on countries' economy since new methods of diagnosis, treatment, and prevention are directly correlated to the direct and indirect costs of healthcare in cancer treatment (Jönsson 2019). According to a data published, the expenditure for healthcare that the united states government bore in 2010, accounted for almost US dollar 137.4 billion for cancer survivors, and the forecast for the national economic burden of cancer care for the current 2020, along with the upcoming years was drastic (Bradley et al. 2008). This burden continues to grow even today, exerting tremendous physical, emotional, mental, and physical strain on individuals, families, communities, health systems, and countries at large. Various poverty-stricken and middle-income countries that possess limited resources, patchy government systems, grossly under-funded healthcare systems, and drowning economies face major repercussions. A large number of patients have no access to timely and quality diagnosis and treatment facilities (Shah et al. 2020). In just a matter of time,

researchers would find various ways to impede cancer and strike a balance between affordable yet effective healthcare, similar to the scientists who have found ways to identify death's major causes throughout history.

Ongoing research on this disease has surfaced innovative platforms to explore multiple other treatment options. Nanomedicine offers a versatile platform of biocompatible and biodegradable systems that can deliver conventional chemotherapeutic drugs easily *in vivo*, improved pharmacokinetic profile, providing increased bioavailability and concentration around tumor tissues. Nanoparticles can be explored for different applications, beginning from scratch as a diagnosis to the very end as therapy. Polymeric nanoparticles that maybe of natural or synthetic origin and are biodegradable are highly resourceful (Jain 2012). Natural antioxidants and many phytoconstituents have been recently introduced as anti-cancer adjuvant therapies due to their anti-proliferative and pro-apoptotic properties.

6.3 Drugs of Natural Origin in Cancer Chemotherapy

Nature has been a bountiful source of therapeutic agents since time immemorial. Nature is brimming with a vast repertoire of life forms (Demain and Vaishnav 2011) that contain molecules of immense pharmaceutical importance. It has never failed to provide unique and exclusive medicinal aid comprising of plant, marine, and animal-derived compounds that have better patient acceptance and compliance. From the very discovery of penicillin in 1928 as an anti-bacterial to the association of quinine and salicylates with cinchona and willow, respectively, it has revolutionized medicine. Medicinal plants, marine species, and terrestrial animals have multiple ethnopharmacological uses that have also been explored to combat cancer. Researchers in every niche of the world are bioprospecting natural origin drugs for cancer chemotherapy due to their wide spectrum of unmatched chemical diversity. More than 60% of the drugs approved today for cancer chemoprevention contain natural scaffolds or pharmacophores directly or indirectly. Almost three-quarters of the drugs used today for cancer prevention have been derived from natural sources and mainly include taxols, polyketide macrolides, anthracyclines, isoprenoids, alkaloids, indolocarbazoles, and glycopeptides. These aforementioned structural classes contain drugs that act by several unique mechanisms, including inhibition of key enzymes involved in cellular metabolism, inducing apoptosis or programmed cell death, causing cell membrane damage, inhibition of tumor-induced angiogenesis and DNA cleavage by topoisomerase inhibition (Demain and Vaishnav 2011).

The first class of natural anti-cancer compounds is plant-based, derived by extraction and isolation of its phytoconstituents (Table 6.1) (Cragg and Pezzuto 2016).

Another important class of naturally derived anti-cancer compounds is micro based antibiotics or their derivatives. The following table (Table 6.2) lists some of these compounds:

Table 6.1 Plant-based anticancer compounds that are produced by extraction and isolation of phytoconstituents, their mechanism of action and indications

Anti-cancer drugs from plant origin	Mechanism of action	Indication	References
Vinblastine (Vinca alkaloid isolated from *Vinca Rosea*)	Inhibition of mitosis at metaphase through its interaction with tubulin	Vinblastine is commonly used to treat cancers such as Hodgkin's lymphoma	Roussi et al. (2012)
Vincristine (Vinca alkaloid isolated from *Vinca Rosea*)	Inhibition of microtubule formation in the mitotic spindle, resulting in an arrest of dividing cells at the metaphase stage	Chemotherapeutic agent for acute leukemia, Hodgkins and non-Hodgkins lymphoma	Roussi et al. (2012)
Vinorelbine (semisynthetic derivative of vinca alkaloid prepared from vindoline and cathrantine)	Inhibition of mitosis at metaphase through its interaction with tubulin	It is used for breast and ovarian cancer and acts by blocking the depolymerization of microtubules	Roussi et al. (2012)
Etoposide (semisynthetic derivative of podophyllotoxin from the rhizome of *Podophyllum Peltatum*)	Inhibition of Topoisomerase-II enzyme and stabilization of DNA-enzyme complex	Etoposide is used for lung cancer, choriocarcinoma, ovarian and testicular cancer, lymphoma, and acute myeloid leukemia	Lee and Xiao (2012)
Teniposide (semisynthetic derivative of podophyllotoxin from the rhizome of *Podophyllum Peltatum*)	Inhibition of topoisomerase-II enzyme and prevention of cell mitosis by causing single and double-stranded DNA breaks	Teniposide was approved for tumors of the central nervous system, malignant lymphoma, and bladder cancer	Lee and Xiao (2012)
Paclitaxel (derived from the bark of Pacific yew tree, *Taxus brevifolia*)	Tubulin targeting drug that stabilizes the microtubule polymer and protects it from disassembly	First-line treatment for locally advanced or metastatic non-small cell lung cancer	Hartwell (1982)
Docetaxel (semisynthetic derivative of paclitaxel)	Suppression of microtubule dynamic assembly and disassembly	Treatment of breast cancer	Hartwell (1982)
Camptothecin (alkaloid obtained from bark and stem of *Camptotheca acuminata*)	Binds to topoisomerase I and DNA complex resulting in a ternary complex, causes stabilization, and prevents DNA re-ligation	Camptothecin is used for recurrent colon cancer and has unusual activity against lung, ovarian, and uterine cancer	Rahier et al. (2012)
Camptothecin (alkaloid obtained from bark and stem of *Camptotheca acuminata*)	Binds to topoisomerase I-DNA complex and prevents re-ligation of these single-strand breaks	Treatment of ovarian cancer and certain types of small cell lung cancer	Rahier et al. (2012)

(continued)

Table 6.1 (continued)

Anti-cancer drugs from plant origin	Mechanism of action	Indication	References
Topotecan (semisynthetic derivative of Camptothecin)	Binds to topoisomerase I-DNA complex and prevents re-ligation of these single-strand breaks	Used to treat colon and rectal cancer	Rahier et al. (2012)
Irinotecan (semisynthetic derivative of Camptothecin)			

Table 6.2 Naturally derived anti-cancer compounds that are produced from microbes, examples of the compounds belonging to that particular chemical class and their indications

Anti-cancer drugs from microbial origin	Examples	Indications	References
Aromatic polyketides (anthracyclines)	Daunorubicin, doxorubicin (adriamycin), epirubicin, piririubicin, idarubicin, valrubicin, amrubicin	Doxorubicin produces regression in neoplastic conditions such as Wilm's tumor, neuroblastoma, and leukemia	Strohl et al. (1998)
Glycopeptides, non-ribosomal peptides, anthracenones	Bleomycin (and its derivatives such as pingyangmycin and blenoxane), phleomycin, Actinomycin D (dactinomycin), mithramycin, streptozotocin	Bleomycin is utilized for squamous cell carcinomas, testis tumors, and Hodgkins lymphoma Actinomycin D is used for Wilm's tumor in children	Zhen and Li (2009)
Quinones, polyketides, indolocarbazoles, polyketides	Mitosanes (mitomycin C), enediynes (calicheamicin) glycosides (rebeccamycin) macrolide lactones (epothilones, ixabepilone)	Mitomycin C is used for disseminated adenocarcinoma of the stomach and pancreas	Rawls (1998) and Xue et al. (1999)
Nucleosides, halogenated compounds	2″-deoxycoformycin (pentostatin) Salinosporamide A	Pentostatin is utilized for the treatment of hairy cell leukemia Salinosporamide A used for the treatment of multiple myeloma	Neumann et al. (2008)

Few recently discovered anti-cancer compounds under this category are elaborated below:

- The monoclonal antibody Avastin® (bevacizumab), an angiogenesis inhibitor, is a first-line treatment for metastatic colorectal cancer. United States food and drug administration approved antiangiogenic agents pegaptanib (macugen) and ranibizumab (lucentis) as anti-cancer agents in which macugen is an aptamer of

the vascular endothelial growth factor and lucentis is an anti- vascular endothelial growth factor antibody (Wagner et al. 2006; Guba et al. 2002).

- Fumagillin, produced by *Aspergillus fumigatis*, was found to act as an anti-angiogenesis compound (Ingber et al. 1990).
- Rapamycin (sirolimus) derivatives, a narrow spectrum polyketide antifungal agent used as a potent immunosuppressive agent for organ transplantation inhibits mammalian target of rapamycin phosphatidylinositol lipid kinase and shows anti-tumor activity (Brown et al. 2003).
- Temsirolimus, a mammalian target of rapamycin protein kinase inhibitor, has been approved by United States food and drug administration for renal cell carcinoma (Rini et al. 2007).
- Lovastatin has anti-tumor activity against Lewis lung carcinoma cells (Alberts et al. 1980).

The third class of natural anticancer compounds is abundantly obtained from marine sources (Table 6.3).

Despite an array of natural anticancer agents, a vast majority of natural habitat is still underexplored by biotechnologists and pharmacists due to multiple problems. Due to this reason, out of innumerable products formulated, very few hit the real market (Cox 1994). The major limitations that are associated with the drugs extracted from natural sources possess are enumerated below:

- Difficulty in accessing the source of the natural constituent
- Lack of sufficient amount of the natural component extracted
- Troubles in synthesizing the required amount of the compound
- Complex isolation and purification processes
- The high toxicity of active component
- Ecological considerations

Plant extracts obtained during the drug discovery process have various phytoconstituents from a single part of the part belonging to a single source, thus creating hindrances in isolation and purification during synthesis. In the case of marine biodiversity, the situation is different. Marine natural products have large molecular sizes, multiple chiral centers, and intricate structures. Other specific issues for marine drug research includes lack of funding and resources for infrastructure, apprehension in the minds of big pharmaceutical companies to invest time and money in marine drug research due to lack of certainty about the market potential, and their reluctance in performing late-stage clinical development procedures that are mandatory for regulatory approval. For microbially derived compounds, the difficulty faced during cell culturing procedures, standardized culture conditions, lack of literature on isolation methodologies and production of specific metabolites at particular phases of cell growth poses a setback to the drugs that originate from the above source. Thus, it becomes imperative to develop extensive and collaborative endeavors and some expansions in bioengineering, novel formulation techniques, and bioengineering to explicate the discovery of natural products in the anti-tumor drug development domain. One such

Table 6.3 Marine-based anti-cancer compounds, their respective mechanism of action and their indications

Anti-cancer drugs from marine origin	Mechanism of action	Indications	References
Cytarabine (Cytostar) (synthetic agent derived from the caribbean sponge, *Cryptotheca Crypta*)	Antimetabolite antineoplastic agent that inhibits synthesis of DNA	Used for non-hodgkin's lymphoma	Rayl (1999)
Trabectedin (Yondelis) (synthetic agent derived from sea squirt species, *Ecteinascidia turbinate*)	Binds to the minor groove of DNA and interferes with cell division, DNA repair, and transcription process	Treatment of liposarcoma and leiomyosarcoma	Bailly (2009)
Aplidine (extracted from marine ascidian, *Aplidium albicans*)	Induces rapid p53-independent apoptosis in different cancer cell lines *in vitro* it also induces a cell cycle perturbation with a block of human leukemic MOLT-4 cells (derived from the leukemic cells of a patient with acute lymphoblastic leukaemia whilst in relapse) mainly in G1 phase	Treatment of multiple myeloma and T-cell lymphoma	Broggini et al. (2003)
Bryostatins, dolastatins, cephalostatins, dolastin (a group of macrolide lactones obtained from the marine organism, *Bugula neritina*)	The bryostatins modulate protein kinase C. Dolastatins' activity cause inhibition of tubulin polymerization, tubulin-dependent guanosine triphosphate hydrolysis, and nucleotide exchange, and it is a potent noncompetitive inhibitor of vincristine binding to tubulin. Cephalostatins increase the stress level in the endoplasmic reticulum, thus initiating a cascade of the pathway that ultimately leads to apoptosis. Dolastatin is a potent, tubulin-targeted, vinca-site binding, anticancer agent that induces mitotic arrest and inhibits cell proliferation in various cell types	Treatment of leukemia	Trindade-Silva et al. (2010)
Soblidotin, Synthadotin (a synthetic derivative of Dolastatin, derived from marine bacterium; synthadotin is a bacterial peptide)	Soblidotin acts as a potent tubulin inhibitor. Synthadotin is a novel tubulin polymerization inhibitor	Treatment of Sarcoma and lung cancer.	Kobayashi et al. (1997)

technology is utilizing a novel drug delivery system strategy to formulate nanoparticles incorporating these natural origin drugs, to aim for targeted therapy, and overcome the limitations mentioned above (Bhatnagar and Kim 2010).

6.4 Role of Nanoparticles in Cancer Therapy

The various thorough check has evidenced that tissue, and cell dispersion profiles of anticancer moiety can be reserved by entanglement in submicronic colloidal systems. Nanoparticles play major roles in the site-specific delivery of drugs to the metastatic cells and their site. For example, gold can be synthesized into different forms like rods, cubes, cages, spheres, and wires this can be used in diverse applications like drug delivery. Quantum confinement levels of nanoparticles can be used to determine accurate tissue imaging (Biju et al. 2008). Polymeric nanoparticles have varied and distinctive characteristics that catapult them towards cancer therapeutics. Metal-based nanoparticles, possess unique features such as non-immunogenicity, less toxicity, optical property, and ease of surface conjugation (Mocanu et al. 2009). In contrast, liposomes have exclusive properties like high biocompatibility, non-toxicity and have the ability to protect awide range of compounds that are of hydrophilic and lipophilic nature (Kondratowicz et al. 2019).

6.4.1 Concept and Recent Development of Polymeric Nanoparticles Conjugated with target-specific ligands

The mechanism of drug targeting by polymeric nanoparticles is widely divided into two types, active and passive targeting (Fig. 6.3). In cancer treatment, biologically active anticancer molecules enter the tumor site with inadequate selectivity and reduce dose toxicity, causing adverse effects to normal tissues. Targeting cancer cells using nanoparticles attached with anticancer agents is an anticipatory plan that aids in overcoming these challenges. Drug targeting can be accomplished by the specific pathophysiological appearance of the tumoror by actively targeting drug carriers, using some target-specific ligands.

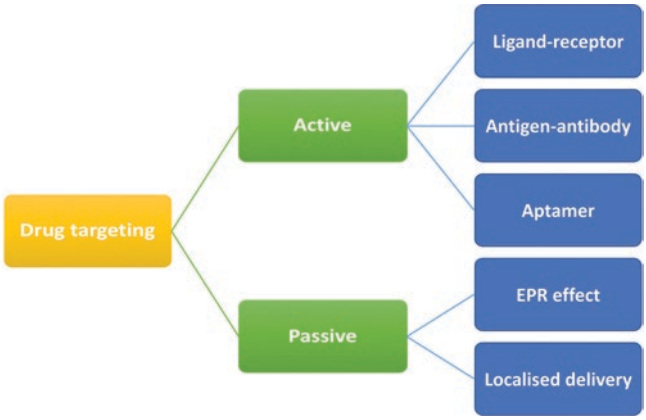


Fig. 6.3 Types of drug targeting by nanoparticles including active and passive drug targeting. Active drug targeting includes ligand-receptor interactions, antigen-antibody complex formation and aptamer. Passive targeting involves localized delivery approach and enhanced permeability and retention (EPR) effect

6.4.1.1 Active Targeting

Target ligands are attached on nanoparticles' surface, showing increased cellular uptake by receptor-initiated endocytosis and thus increased drug build-up in a cancer cell. Active targeting for cancer isanticipated to reach the specific site of action by molecular identification of cancer cells through antigen-antibody and ligand-receptor interaction. Active targeting can be met by conjugating the therapeutic agent or the carrier with a specific ligand, thus letting a preferential build-up of the drug in the tumor site, in individual cancer cells, or specific molecules in cancer tissue (Lamprecht et al. 2001; Scherer et al. 2002). Favorable outcomes of drug targeting depend upon the selection of the targeting agent, which should be plenty, have increased linking and specificity of confining to surface receptor of the cell, and must be adequately adapted to functionalization by conjugation. Receptors and antigens may be articulate, especially in infected cells only. Therefore the cancer endothelium specifies various sites for cancer therapy (Parveen and Sahoo 2008).

6.4.1.2 Passive Targeting

Passive targeting shows the worthy accumulation of nanoparticles within the tumor microenvironment. Due to the changes in the anatomy and physiology of the tumor which contain leaky vasculature, there is an accumulation of the drug at the site. This is coupled with the improper lymphatic drainage of biomolecules in solid

tumors, allows an increased localization and retention of high molecular weight drugs in solid tumors. This is called enhanced permeability and retention. Some of the polymer-based nanoformulations are Genexol-PM (i.e. active component is paclitaxel and polymer used is poly (D, L-lactide)-poly (ethylene glycol)-methyl ether is an amphiphilic block copolymer, which can self-assemble into polymeric micelles), micellar nanocarriers like NK 911, NK 105, NC6004 are used in passive tumor targeting which has several advantages like patient compliance, increased drug tolerance, reduce side effects (Matsumura and Maeda 1986).

6.4.1.3 Advances of Polymeric Nanoparticles in Conjugation with target-specific ligands

Almost 25% of the pharmaceutical drugs and their derivatives are obtained from natural sources (Swamy and Sinniah 2016). The current natural product-based drug discovery scenario has been invoking eagerness in routing synthetically manageable starting material, which imitates their similar sections in chemistry (Rodrigues et al. 2016). Natural compounds can be helpful in several major disorders like cancer because natural compounds have many advantages over synthetic which includes reduced toxicity, lowered side effects, cost-effectiveness, high therapeutic potential but also has some limitations like biocompatibility, toxicity, *in vivo* instability, poor absorption, and poor solubility. To tackle all the above problems nanotechnology came into the picture for targeting, drug control, and efficient release patterns (Martinho et al. 2011; Jahangirian et al. 2017). As nanoparticles have a small size, they can travel easily in the body compared to the larger molecules. Nano-medicine is well recognized today as drug carriers and drug encapsulating agents as well since it enables controlled and site-specific release (Martinho et al. 2011; Patra et al. 2018; Saka and Chella 2021).

Nano-drug showcases high bio-availability as they show uptake by endocytosis. Nowadays, various approaches are being used to develop polymeric nanoparticles. The green chemistry approach for nanoparticle synthesis reduces the hazardous products in the biosynthetic process, therefore ultimately diminishing the burden of side effects of medication. For instance, nanoparticles having diversity in the diameter of hyaluronic acid can be produced by altering the estate of hydrophobic swap of hyaluronic acid. The nanoparticles were systemically administered in the mice with cancer, and then, its effect was evaluated. They developed a multifaceted thermostatic system using poly(ethylene glycol) and conjugate hyaluronic acid nanoparticles for the early diagnosis of colon cancer and in targeted therapy (Martinho et al. 2011; Patra et al. 2018).

6.5 Polymeric Nanocarriers in Guided Cancer Therapy

Since the inception of nanotechnology in the past decade, nanomaterials' development and design have become a foremost field of the research endeavor. An emerging module in this field is nanomedicine, wherein nanoscale materials are blossoming for use as imaging agents or drug delivery applications. Polymeric nanoparticles are sub-micron (1–1000 nm) colloidal particles that embrace active pharmaceutical ingredients encapsulated within or adsorbed to macromolecular substances i.e., polymer. Recently, the polymeric nanoparticle has a promising role in guided cancer therapy due to its advanced feature like targeting and controlled drug release. With the growing demand for the development of alternative approaches to combat cancer, the natural product (drug) reinforced polymeric nanoparticles will be the promising candidates (Ahuja et al. 2020). The polymeric nanoparticle exhibits some fascinating advantages, such as enhancing bioavailability by altering the biophysiochemical parameter (Vanti 2021). Most of the natural product-derived molecules have compromised solubility, however, incorporation in the polymeric nanoparticle shows the scientifically enhanced solubility and permeability.

Also, the polymeric nanoparticle has great importance such as low immunogenicity, biocompatibility, easily biodegradable, and easy elimination and excretion through renal or liver clearance. The natural molecules like curcumin, vincristine, vinblastine, paclitaxel, etc. show poor intrinsic bioavailability, however, when they get incorporated in the biopolymer or polymeric nanoparticle, such as poly (vinyl alcohol), polyvinylpyrrolidone, poly(lactic-co-glycolic acid), poly lactic acid, poly (ethylene glycol), and polyglycolic acid. They show targeted as well guided cancer therapy (Ravichandran 2013; Grottkau et al. 2013; Aggarwal et al. 2003).

6.6 Polymeric Nanoparticles Entrapping Drugs of Natural Origin

6.6.1 Polymers and Drugs of Natural Origin

Polymers are either natural or synthetic substances composed of many repeating subunits known as monomers and forming the macromolecule. These polymers are gaining utmost importance due to their use in day-to-day life, biodegradability, and biocompatibility (Liechty et al. 2010). Some of the natural polymers, including proteins, are amino acid polymers, and in a similar manner, the nucleic acids, which are nucleotides polymers, are found in humans. (https://www.britannica.com/science/polymer). These natural polymers in humans make them more interesting to develop certain formulations based on these polymers to ensure site-specific delivery and achieve therapeutic efficacy. Incorporating certain therapeutically active agents inside the polymer matrix could lead to the development of the formulations achieving the target specificity and higher efficacy with reduced toxicity.

The polymeric nanoparticles are one such formulation where the nanoparticles ranging from the size of 10–100 nm are prepared from the biocompatible and biodegradable polymers and incorporate the therapeutic agent in either entrapped form, encapsulated form, dissolved form, or attached to a matrix of nanoparticles (Jeevanandam et al. 2018). Polymeric entrapment or encapsulation of the drugs particularly from the natural origin possibly leads to the controlled release of the drug, enhanced therapeutic efficacy, decreased metabolism, site-specificity, and might potentiate the drug's overall activity towards the targeted disease. Thus considering the advantages offered by this type of polymers, their use in the formulation would certainly lead to the betterment of humankind. Several preparation methds of polymeric nanoparticles include solvent evaporation, supercritical fluid technology, nanoprecipitation, ion gelation, salting out, coacervation method (Sagar et al. 2018).

6.6.1.1 Natural and Synthetic Polymers for the Preparation of Polymeric Nanoparticles

There is an array of natural and synthetic polymers that are used in pharmaceuticals; out of that, a handful of them are utilized for the preparation of polymeric nanoparticles, which are listed below in Table 6.4 (Coelho et al. 2010).

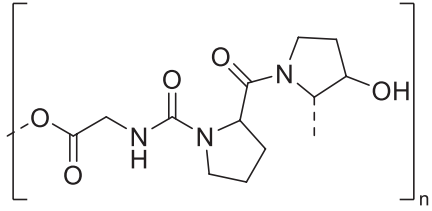
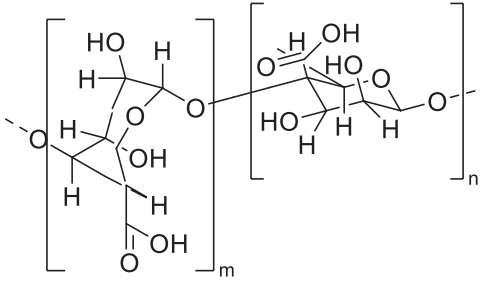
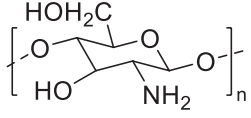
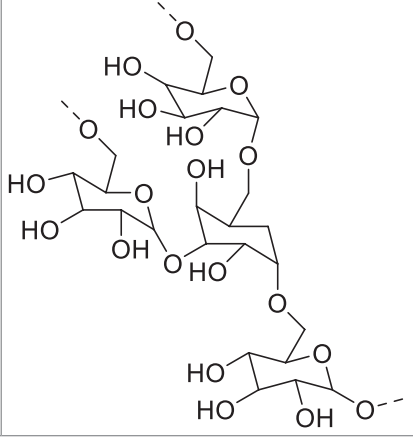
Chitosan

It is the most abundant natural amino – polysaccharide polymer obtained by the chitin's deacetylation (Divya and Jisha 2018). It is the principal constituent of the exoskeleton of the cell wall of fungi and arthropods. It is the linear polymer of N-acetylglucosamine, and removing the acetyl group from this leads to the development of the chitosan. Chitosan is the linkage of β-(1-4)-linked glucosamine and N-acetyl-D-glucosamine. It possesses certain characteristics like good biocompatibility, biodegradability, lowered toxicity, and allergenicity which makes it of more interest to develop the polymeric nanoparticles entrapping the drugs for the targeted therapy (Parhi 2020; Coelho et al. 2010; Ibrahim and El-Zairy 2015; Sinha et al. 2004; Cheung et al. 2015; Elieh-Ali-Komi and Hamblin 2016).

Poly(Lactic-Co-Glycolic Acid)

Poly(lactic-co-glycolic acid) is the United States Food and Drug Administration approved co-polymer for its versatile applications in the biomedical and tissue engineering field. It is serving the purpose with the presence of essential characteristics, including biocompatibility and biodegradability. These properties are important for the drug delivery system. It has large clinical exposure, favorable degradation, and sustained drug release can be achieved with the entrapment of the desired drug. The biodegradation of the poly(lactic-co-glycolic acid) polymeroccurs in four steps: hydration followed by initial degradation, further followed by bulk degradation, and lastly resulting in solubilization. It, in general, undergoes hydrolytic degradation, and the degraded products are non-toxic, so it is used as a biomaterial for the preparation of the nanoparticles and the development of medical devices. Several factors

Table 6.4 Natural and synthetic polymers that are widely used for preparing the polymeric nanoparticles along with their chemical structures

Natural polymers for drug delivery		Synthetic polymers for drug delivery	
Name of the polymer	Structure of the polymer	Name of the polymer	Structure of the polymer
Collagen		Poly(lactic acid)	
Alginic acid		Poly(lactic-co-glycolic acid)	

Chitosan		Poly(Ɛ-caprolactone)	
Dextran		Poly(ethylene glycol)	
Cellulose		Poly(alkyl cyanoacrylates)	
		Poly(methyl methacrylate)	

affect the degradation process of poly(lactic-co-glycolic acid), like the effect of composition, the effect of crystallinity, the effect of molecular weight, the effect of pH, the effect of the drug and drug loading, the effect of shape and size of the matrix (Coelho et al. 2010; Makadia and Siegel 2011; Jain 2000; Middleton and Tipton 1998; Wu and Wang 2001; Kapoor et al. 2015).

Polylactide

Polylactide or polylactic acidis the synthetic aliphatic polyester obtained from the polymerized lactic acid. It is formed from lactic acid which is found in the environment as the metabolic intermediate or the by-product in several plants and animals. Thus, the degradation products of the polylactic acid are non-toxic and offer good biocompatibility with the human body. It is generally prepared by the ring-opening polymerization method in industries. The several other techniques like direct polycondensation and direct polymerization can be used, but the widely used and scalable approach is ring-opening polymerization. Poly (DL-lactide) has lower tensile strength and higher elongation capability and rapid degradation time makes it suitable for use in the drug delivery system (Coelho et al. 2010; Hagen 2016; Middleton and Tipton 1998).

Polyglycolide

Polyglycolide, commonly known as polyglycolic acid is the linear polyester obtained by the ring-opening polymerization of glycolic acid. It has been known as the tough fiber-forming polymer along with the bottleneck as the hydrolytic instability. So, keeping this limitation in mind and exploring its biocompatibility, its use in the biomedical field is enhanced while combining with the other copolymers for the sustained drug release and tissue engineering fields. It is highly crystalline and having a high melting point (220–225 °C). Fibers from the polyglycolide exhibit high strength, modulus and they are not readily soluble in most of the organic solvents due to their higher crystallinity and soluble in higher fluorinated organics like hexafluoro-isopropanol (Coelho et al. 2010; Gilding and Reed 1979; Middleton and Tipton 1998).

Polycaprolactone

It is considered as tissue compatible and used as biodegradable sutures in the biomedical field, composed of the repeated hexanoate units. It is used as a copolymer that enhances the other polymers' properties and makes them more compatible with the use. It is having better miscibility as well as biodegradability when used with the other polymers. It is prepared by the hydroxycarboxylic acid's poly condensation 6-hydrohexanoic acid and the ring-opening polymerization of the Ɛ-caprolactone. Its degradation is catalyzed by the carboxylic acids liberation during hydrolysis and also by the enzymes, which result in the faster decomposition of the polymer (Coelho et al. 2010; Labet and Thielemans 2009; Middleton and Tipton 1998).

6.6.1.2 Drugs of Natural Origin for Polymeric Nanoparticles in Cancer Therapeutics

Nature has blessed humanity with several compounds for treating different kinds of diseases, and our reliance is much higher on the natural origin drugs for the treatment of diseases like cancer. Several natural compounds are identified showing the anticancer activity, including the catharanthus alkaloids, taxanes, camptothecin, combretastatin, podophyllotoxins, geniposides, colchicine, artesunate, salvicine, and many others (Iqbal et al. 2017; Sinha et al. 2009; Kinghorn et al. 2016). Also, several lead molecules of marine-based anticancer compounds are identified, including the psammaplin A, psammaplin F, psammaplin G, biprasin, didemnin B, plitidepsin, dolastatin, ecteinascidin, cyanosafracin B, halichondrin B, etc. (Simmons et al. 2005; Jimenez et al. 2009; Kinghorn et al. 2009).

Vinca alkaloids

Vinca alkaloids are the phytochemicals isolated from the *Catharanthus roseus* (*C. roseus*) belonging to the family Apocynaceae are the diverse group of phytochemicals being used in several types of cancers like lung, breast, liver, leukemia (Fig. 6.4). The major vinca alkaloids being used as the anticancer agents are vincristine, vinblastine, vinorelbine, and vindesine. These are binding specifically to the

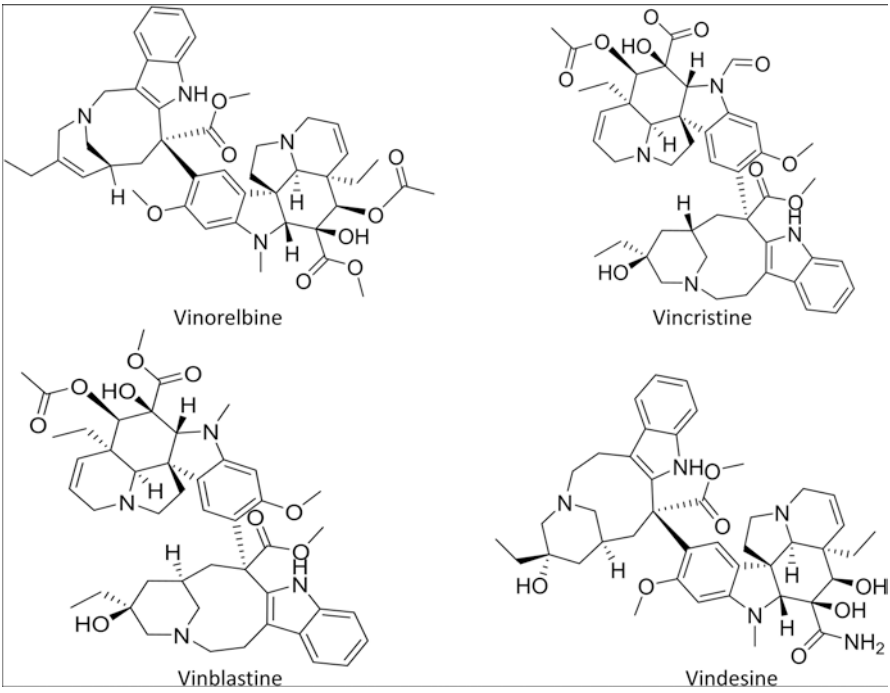


Fig. 6.4 Chemical structures of the vinca alkaloids, mainly vinorelbine, vincristine, vinblastine and vindesine

tubulin heterodimers and results in the disruption of the microtubules by arresting the cell cycle at M-phase and commonly known as the antimitotic agents (Sinha et al. 2009; Kinghorn et al. 2016; Gordaliza 2007).

Taxanes

The most widely explored taxanes, docetaxel, and paclitaxel, are known for their promising anticancer activities and are obtained from *Taxus baccata* (*T. baccata*) and *T. canadensis* and is used to treat a wide range of malignancies, including lung, ovarian, and breast cancers (Fig. 6.5). It induces apoptotic cell death, and cell arrest occurs at M-phase and results in cell cycle disruption (Gordaliza 2007; Sinha et al. 2009).

Camptothecin

They are commonly known as the topoisomerase poisons and are obtained from the *Camptotheca acuminata* belonging to the Nyssaceae family (Fig. 6.6). Topotecan and irinotecan are used widely for treating ovarian, lung, and colorectal cancer. They inhibit the topoisomerase-I in many cancers and serves as the anticancer agent (Kinghorn et al. 2016; Gordaliza 2007).

Ellipticine

It is a natural anticancer compound obtained from the *Bleekeria vitensis* and *Ochrosia elliptica* belonging to the family of Apocynaceae (Fig. 6.7). It exhibits anticancer activity by inhibiting the topoisomerase-II in several cancers. It also inhibits the phosphorylation of the p53 and also inhibiting the cyclin-dependent kinase-2 in human lung and colon cancer (Sinha et al. 2009; Gordaliza 2007).

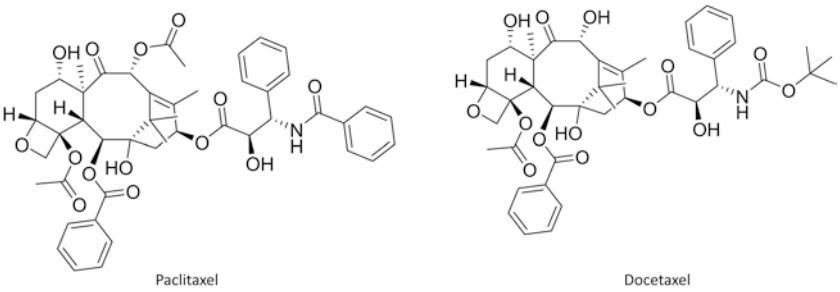


Fig. 6.5 Chemical structures of the taxane derivatives mainly paclitaxel and docetaxel

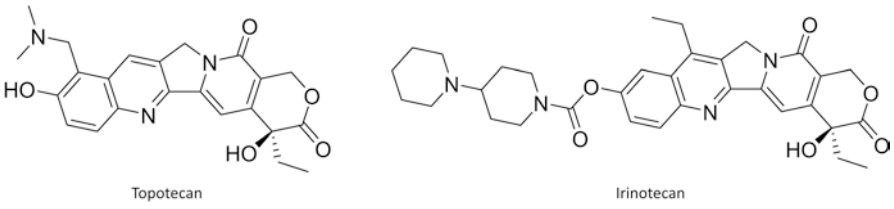
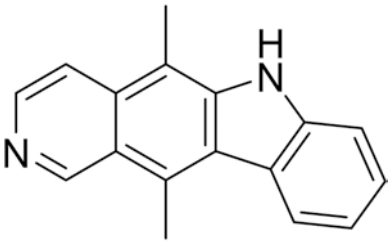
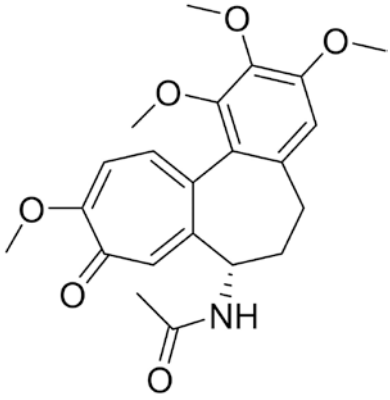


Fig. 6.6 Chemical structures of camptothecin derivatives mainly topotecan and irinotecan



Ellipticine

Fig. 6.7 Chemical structure of ellipticine



Colchicine

Fig. 6.8 Chemical structure of colchicine

Colchicine

It is obtained from the *Colchicum autumnale,* which exhibits potential anticancer activity and arrests the cell cycle by stopping the cell division at different cell cycle intervals (Fig. 6.8). It shows the aggressive tumor-killing and the normal cell cycle arrest and thus it shows the toxicity and therapeutic effects. So, the polymeric entrapment of these natural compounds can lead to a potential anticancer agent with lesser toxicity and side effects (Sinha et al. 2009; Gordaliza 2007).

Apart from the drugs that are mentioned above, there are other agents used very often in the treatment of cancer are enlisted as follows (Table 6.5).

6.6.2 Mechanism of Drug Release for Polymeric Nanoparticles

The drug release mechanism is mainly of four different types, and these are considered as the major pathways for the targeted drug delivery and drug release from the polymeric nanoparticles (Fig. 6.9) (Kamaly et al. 2016; Fredenberg et al. 2011; Son et al. 2017).

Table 6.5 Drugs of natural origin used in cancer therapeutic agents, their biological source, the target that they act upon and the mechanism of action

Natural compound	Source	Specific cancer target	Mechanism of action	References
Vinca alkaloids Vincristine Vinblastine Vindesine Vinorelbine	*Catharanthus roseus*	Bladder cancer, non-small cell lung cancer, acute lymphocytic leukemia	Disintegrates the microtubule and damages the mitotic spindle ultimately leading to mitosis and apoptosis	Khazir et al. (2014) and Wei et al. (2017)
Taxanes Docetaxel Paclitaxel	*Taxus baccata, Taxus brevifolia*	Adenocarcinoma, breast cancer cell lines, cervical carcinoma, colon adenocarcinoma	Antimitotic agents	Isah (2015), Mody et al. (2016), Rafiei and Haddadi (2017), Gradishar (2012), Habib et al. (2013), Mäenpää (2003), Liebmann et al. (1993) and Yin et al. (2012)
Camptothecins Topotecan Irinotecan	*Camptotheca acuminata*	Colon cancer, breast cancer, leukemia, prostate cancer	Topoisomerase-I inhibitor	Patankar and Waterhous (2010), Takagi et al. (2007), Pommier (2006), Stewart (2004), Benedetti et al. (1997) and Adams et al. (2006)
Combretastatins	*Combretum caffrum*	Human thyroid papillary carcinoma	The destabilization of the microtubules	Liang et al. (2016); Yeung et al. (2007)
Podophyllotoxins etoposide teniposide	*Podophyllum peltatum, Podophyllum emodi*	Small cell lung cancer	Topoisomerase-II inhibitor	Pang et al. (2018), Hande (2008), Rao et al. (2005) and Liu et al. (2014)
Elipticine	*Ochrosia elliptica*	Leukemia	Disruption of the cell cycle by cyclin B1 and cell division control 2	Kuo et al. (2005, 2006) and Stiborová et al. (2011)
Colchicine	*Colchicum autumnale*	Hepatocellular carcinoma	Depolymerizes the microtubules	Lin et al. (2013) and Wu et al. (2015)

(continued)

Table 6.5 (continued)

Natural compound	Source	Specific cancer target	Mechanism of action	References
Artesunate	*Artemisia annua*	Chronic myeloid leukemia	Vascular endothelial growth factor inhibitor and antiangiogenic	Zhou et al. (2007) and Zhao et al. (2013)
Roscovitine	*Raphanus sativus*	Breast cancer	Cyclin dependent kinase inhibitor	MacCallum et al. (2005), Goodyear and Sharma (2007), Hahntow et al. (2004) and Whittaker et al. (2004)
Salvicine	*Salvia pronitis*	Leukemia, stomach cancer	DNA splitting and inhibiting re-ligation	Meng and Ding (2007) and Hu et al. (2006)
Psammaplin	*Poecillastra sp., Jaspis sp., Psammaplin aplysilla*	Human endometrial cancer cells	Histone deacetylase inhibition along with the farnesyl protein transferase inhibition, aminopeptidase inhibition	Shim et al. (2004), García et al. (2011) and Ahn et al. (2008)
Halichondrin B	*Halichondria okadai, Lissodendoryx sp*	Leukemia	Tubulin inhibitor and cell cycle arrest at G2 phase	Bai et al. (1991)
Dolastatin	*Dolabella auricularia, Symploca hydnoides*	Breast cancer	Disrupts mitotic cell division and stopping cell cycle	Miyazaki et al. (1995) and Tan (2007)
Didemnin	*Tridemnum solidum, Aplidium albicans*	Acute lymphoblastic leukemia	RNA, DNA, and protein synthesis inhibitor	Vera and Joullie (2002) and Baker et al. (2002)
Ecteinascidin	*Ecteinascidia turbinata*	Leukaemia	Induces cell death by interfering with cellular transcription-coupled nucleotide excision repair inducing cytotoxicity	Zewail-Foote and Hurley (1999)

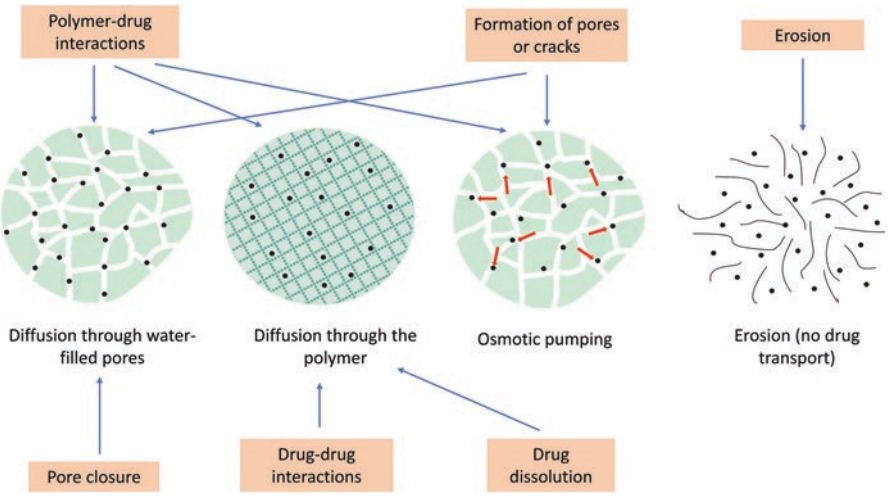


Fig. 6.9 Types of drug release mechanisms from polymeric nanoparticles. The mechanism proceeds by diffusion through water-filled pores, diffusion through the polymer, osmotic pumping mechanism and erosion involving no drug transport

6.6.2.1 Diffusion Through Water-Filled Pores

Polymeric nanoparticles absorb the water immediately. The water occupying the polymeric matrix leads to the formation of the water-filled pores over a certain period. The pores become more in number and lead to the release of the drug from them.

6.6.2.2 Diffusion Through the Polymeric Matrix

In general, non-degradable drug delivery diffusion is the primary driving force for the release of the drug through the polymeric matrix. It is depending upon the permeability and the thickness of the polymeric matrix rather than the concentration of the drug entrapped or encapsulated inside.

6.6.2.3 Osmotic Pumping

Entering of water inside the non-swelling system is occurred by the osmotic pressure, and the drug release based on this mechanism is termed osmotic pumping. For the osmotically driven drug delivery system, osmogenes and semipermeable membranes are required, making the phenomenon easier and possible.

6.6.2.4 Erosion

There are two types of erosion mechanisms for drug delivery:

- **Surface erosion** refers to the degradation of the matrix from the outer matrix surface, leading to reducing its size from the outer surface towards the inner surface. The process is reproducible, and controlled drug release is obtained.
- **Bulk erosion:** When water penetrates more inside the polymeric matrix and major portion of the bulk polymer, the hydrolysis of matrix, and controlled drug release is obtained.

The polymer-drug conjugate complexes are pharmacologically active macromolecular structures that comprise of polymer attached to the linker via chemical bonding, linker further attached to the drug molecule, and the targeting ligand (Fig. 6.10).

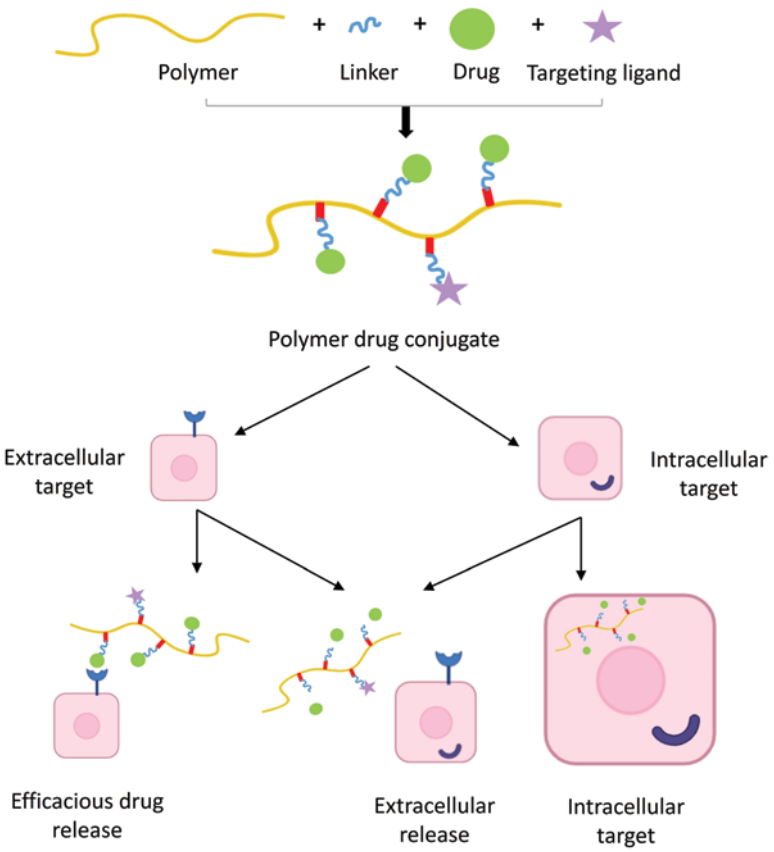


Fig. 6.10 Polymer-drug conjugate formation phenomena followed by the release of the drug from the conjugate via action upon an intracellularly or extracellularly located target

This complex assembly is now capable of attaching to the intracellular or extracellular targets for entry into the tumor microenvironment. The rationale behind the concept of conjugating the drug to a polymer is to achieve:

- Prolonged circulation time of the drug
- Restricted body distribution
- Selective drug release

The drug to be conjugated to the polymer can include small molecules, peptides, proteins, or aptamers that today have proven to be the mainstay of novel drug delivery field to be translated into clinical practice (Ekladious et al. 2019).

6.6.3 Clinical Applicability

Several polymeric nanoparticles developed that are clinically applicable for cancer amelioration and treatment are thereby in clinical trials for different types of cancers. Thorough exploration led to their revelation, as tabulated below in Table 6.6.

The above table is intended to deliver a piece of very brief yet significant information about the polymeric nanoparticles' whereabouts entrapping drugs of natural origin. Detailed information has been provided for the ones that came out as distinctively impactful over this decade. These are as follows:

Polyglutamate conjugated paclitaxel has been investigated to treat ovarian cancer and non-small cell lung cancer. The formulation had 37% of paclitaxel which was covalently linked to the long-chain polymer of L-glutamic acid. This entire drug conjugate had an average molecular weight of 80,000 Da and a prolonged tissue half-life. Results of pharmacokinetic studies on preclinical models of polyglutamate conjugated paclitaxel displayed enhanced drug uptake by tumor cells with a drastic drop in the concentration of free paclitaxel in plasma compared to the standard paclitaxel. The polymeric drug conjugates had the following benefits:

1. It showcased enhanced solubility of lipophilic drugs, indirectly avoiding the usage of toxic solubilizing agents. This further resulted in shorter infusion times and decreased the propensity of patients to hypersensitivity reactions;
2. It attained the required concentration in tumor tissues because of increased permeability of tumor vasculature for macromolecules' entry and their retention in the tumor tissues. This was also mainly due to the dearth of excess lymphatic drainage.
3. It was least toxic to normal tissues because of the absence of large macromolecules that make their way into the normal tissue interstitium.
4. It possessed the capability to decrease multidrug resistance1 gene-encoded resistance by releasing paclitaxel at less accessible intracellular sites.

Females with recurring epithelial ovarian, primary peritoneal, or fallopian tube carcinoma were treated with polyglutamate conjugated paclitaxel. A phase II study was conducted to evaluate the toxicity, response rate of patients and time taken to

Table 6.6 Therapeutic agents entrapped in polymeric nanoparticles, utilizing market available polymers of synthetic or natural origin, the indication for which the nanoparticles could be used for, along with their clinical / *in vitro* / *in vivo* applicability

Polymer	Therapeutically active agent	Indication	Status	References
Polyglutamate	Paclitaxel	Non-small cell lung cancer, ovarian cancer	Phase-III	Sabbatini et al. (2004)
Polyglutamate	Camptothecin	Colorectal and ovarian cancer	Phase-I/II	Bhatt et al. (2003)
N-(2-Hydroxypropyl) methacrylamide copolymer	Paclitaxel	Various cancers	Phase-I	Meerum Terwogt et al. (2001)
	Camptothecin			Bissett et al. (2004) and Wachters et al. (2004)
Modified-dextran	Camptothecin	Various cancers	Phase-I	Kumazawa and Ochi (2004)
Poly (ethylene glycol)	Camptothecin	Various cancers	Phase-II	Rowinsky et al. (2003)
2-Hydroxypropyl methacrylate copolymer	Doxorubicin	Lung cancer, Breast cancer	Phase-II	Vasey et al. (1999)
	Doxorubicin-galactosamine	Hepatocellular carcinoma	Phase-I/II	Seymour et al. (2002)
Dextran	Doxorubicin	Various cancers	Phase-I	Danhauser-Riedl et al. (1993)
Cyclodextrin	Camptothecin	Non-small cell lung cancer	Phase-II	Rana and Sharma (2019)
Poly (alkyl cyanoacrylate)	Doxorubicin	Hepatocellular carcinoma	Phase-III	Rana and Sharma (2019)
Poly (lactic-co-glycolic acid)	Paclitaxel	Human cervical carcinoma cells	*In vitro* and *in vivo* studies	Prabhu et al. (2015) and Danhier et al. (2009)
Poly (lactic-co-glycolic acid)	Doxorubicin	Breast cancer cells, human cervical carcinoma cells	*In vitro* studies	Prabhu et al. (2015), Danhier et al. (2009) and Park et al. (2009)
Poly (lactic-co-glycolic acid)–human serum albumin	Doxorubicin	Glioblastoma	*In vivo* studies	Prabhu et al. (2015) and Wohlfart et al. (2011)
Poly (ethylene oxide)-poly(ε-caprolactone)	Paclitaxel-tamoxifen	Ovarian adenocarcinoma	*In vitro* and *in vivo* studies	Devalapally et al. (2008)

(continued)

Table 6.6 (continued)

Polymer	Therapeutically active agent	Indication	Status	References
Poly (butyl cyanoacrylate)	Epirubicin	Human cervical carcinoma	*In vitro* studies	Yordanov et al. (2012)
Poly(lactic-co-glycolic acid)	Doxorubicin	Lung epithelial cancer	*In vitro* studies	Chittasupho et al. (2009)
Poly (lactic-co-glycolic acid)-b-poly (ethylene glycol)	Docetaxel	Prostate cancer	*In vitro* and *in vivo* studies	Cheng et al. (2007)
Poly(lactic-co-glycolic acid)-poly (ethylene glycol)	Doxorubicin	Human ovarian cancer cells	*In vitro* and *in vivo* studies	Liu and Lin (2012)
Methoxy-poly (ethylene glycol)	Mitomycin C	Human cervical cell carcinoma	*In vivo* studies	Hou et al. (2011)
Pullulan acetate	Epirubicin	Nasopharyngeal epidermal carcinoma	*In vitro* studies	Zhang et al. (2010)
Poly (ethylene glycol)	Irinotecan	Ovarian, breast, and colorectal cancer	Phase-II/III	Prabhu et al. (2015)
Poly (iso-hexyl-cyanoacrylate)	Doxorubicin	Hepatocellular carcinoma	Phase-I/II	Prabhu et al. (2015)
Poly cyclodextrin	Camptothecin	Metastatic solid tumors	Phase-I	Prabhu et al. (2015)
Poly (D, L-lactic acid)	Paclitaxel	Various cancers	*In vitro* and *in vivo* studies	Sutradhar and Amin (2014) and Cirstoiu-Hapca et al. (2009)
Poly(lactic-co-glycolic acid)-poly (ethylene glycol)	Paclitaxel	Aggressive tumours	*In vitro* and *in vivo* studies	Sutradhar and Amin (2014) and Zhao et al. (2015)
Poly (ethylene glycol), poly (ethylene glycol)-b-poly aspartic acid	Docetaxel	Aggressive tumors	*In vivo* studies	Choudhury et al. (2019) and Tran et al. (2015)
Albumin	Paclitaxel	Various cancers	*In vitro* and *in vivo* studies	Choudhury et al. (2019) and Gradishar (2017)
Poly (L-glutamic acid)	Doxorubicin	Various cancers	*In vitro* and *in vivo* studies	Choudhury et al. (2019) and Xu et al. (2016)

(continued)

Table 6.6 (continued)

Polymer	Therapeutically active agent	Indication	Status	References
Poly (oligo (ethylene glycol) methyl ether acrylate)-poly(ε-caprolactone), albumin	Curcumin	Solid tumors	*In vivo* studies	Jiang et al. (2017)
Cholic acid, poly (lactic-co-glycolic acid)-D-α-tocopherol poly (ethylene glycol) succinate	Paclitaxel	Melanoma, resistant tumors	*In vivo* studies	Su et al. (2017)
Monomethoxy-poly (ethylene glycol)-b-poly(lactic-co-glycolic acid)-b-poly (L-glutamic acid)	Curcumin and doxorubicin	Various cancers	*In vitro* and *in vivo* studies	Yuan et al. (2018)
Poly (juglanin dithiodipropionic acid)-b-poly (ethylene glycol)	Doxorubicin and small interfering RNA	Lung cancer	*In vivo* studies	Wen et al. (2017)
N-hydroxysuccinimide ester, folic acid and poly (ethylene glycol)	Docetaxel and resveratrol	Prostate cancer	*In vitro* and *in vivo* studies	Singh et al. (2018)
Poly (n-isopropylacrylamide-co-butyl acrylate), chitosan	Doxorubicin	Ovarian cancer	*In vitro* and *in vivo* studies	Wang et al. (2018a, b)

disease (thrombotic thrombocytopenic purpura) progression (Sabbatini et al. 2004). In another study, there were some significant results. Poly-R-(L-glutamic acid) has two important hallmarks; its biodegradability and efficient solubility. Simultaneously it provides multiple sites for drug conjugation. Being a polypeptide, it biodegrades-readily, and as a polyelectrolyte it possesses one free carboxylate group per monomer. It even aids the solubilization of lipophilic molecules in aqueous media. This polymer is highly explored in the phaseI/II clinical trial for the treatment of colorectal and ovarian cancer, on conjugation with 20-(*S*) camptothecin.

Poly-R-(L-glutamic acid) conjugates of 20-(*S*) camptothecin showed enhanced aqueous solubility as compared to 20-(*S*) camptothecin alone. These conjugates remained stable in aqueous media at neutral pH, yielding efficient prodrugs with favorable pharmacokinetics, and proved to be potent antitumor agents *in vivo*. Xyotax was evaluated as a model to study this polymer efficacy for oncological drug delivery. Enhanced perviousness of tumor vessels and the confinement of macromolecules resulted in potent *in vivo* antitumor efficacy in the case of xyotax. This study thus validated poly-R-(L-glutamic acid)'s utilization as a macromolecule for oncologic drug delivery (Bhatt et al. 2003). Paclitaxel has been used in conjugation to form polymeric nanoparticles; for example, PNU166945 is a novel conjugate containing paclitaxel entrapped in its polymer matrix.

It is currently in phase I clinical trial to ascertain itspotential on diverse cancers. This novel conjugate consisted of a hydroxypropyl-methacrylamide polymer, linked through an amino acid chain at the 2′ position of paclitaxel. The dose-finding phase I study of PNU166945 was performed to document toxicity and pharmacokinetics in the first course to observe any drug behavior alterations. Twelve patients in total with intractable solid tumors signed up for the study and were subjected to a 1 h infusion every 3 weeks with a starting dose of 80 mg/m^2, equivalent to paclitaxel. The dose level was escalated to 196 mg/m^2 to ensure no dose-limiting toxicities. The result showcased a considerable controlled release of paclitaxel and the observed hematologic toxicity of PNU166945 was minimal and only dose-independent. An exception to this study involved two patients, where one developed grade 3 neurotoxicity and another patient with late stage metastatic breast cancer developed a partial response to the polymer-drug conjugate.

Additional *in vivo* studies were performed to establish a linear pharmacokinetic response of the bound andreleased paclitaxel but, this study had to be discontinued prematurely due to severe neurotoxicity. Overall, this concept of polymer-drug conjugation in the case of paclitaxel may be a boon in cancer treatment shortly (Meerum Terwogt et al. 2001).

Targeting chemotherapeutic agents at the solid tumor site is facilitated by polymeric drug conjugates *in vivo* animal models and even in clinical subjects. Camptothecins belong to a highly explored and booming class of anticancer agents employed against a wide spectrum of malignancies and can inhibit topoisomerase enzyme. MAG-camptothecin conjugate contains camptothecin covalently linked to a water-soluble polymer, MAG. MAG is a copolymer of N-(hydroxypropyl) methacryl amide, (20–O-(N–methacryloyl–glycyl–aminohexanoyl-glycyl) camptothecin) and N-(2-hydroxypropyl) methacryloyl-glycinamide. In several solid tumors, this conjugate showcased enhanced permeability, better accumulation at tumor sites, retention effect property, and decreased toxicity compared to camptothecin alone. Initial phase I trials guided the regimen used in this study (Bissett et al. 2004; Wachters et al. 2004). Studies have been carried out using camptothecin as a model drug. PEGylated camptothecin consists of two subclasses; monosubstituted and disubstituted camptothecin. Single functionalized poly (ethylene glycol)s has an unreactive hydroxyl or carboxyl functional group at distal end. It is a convenient vehicle for administering the prototypical camptothecins compared to the ones in which excess sodium salt is used.

The major objectives of this experimental study include the assurance of the efficacy, that is, the preliminary evidence for the anticancer activity, and at the same time to ascertain the maximum-tolerated dose of the pegylated camptothecin complex administered as 1-h intravenous infusion every 3 weeks and to recommend the dose for the phase II clinical trials to evaluate its toxicity. The main objective of this conjugated complex of PEGylated camptothecin was to enhance the aqueous solubility and site-specific targeting of the prepared formulation. A single dose treatment with the conjugated formulation resulted in substantially lower, although biologically relevant, C_{max} values than the one obtained through sodium camptothecins (Rowinsky et al. 2003).

There are other instances where paclitaxel has been used in many countries as a prescribed treatment for ovarian and breast cancers. It works by disrupting the dynamic equilibrium inside the microtubules and arresting the cells in the late G2/M phase of the cell cycle, proving its potential as a cell replication inhibitor. In order to increase its solubility and achieve intravenous administration, it is currently formulated (Taxol®) at 6 mg/mL in a vehicle comprising of 1:1 ratio of Cremophor® EL and ethanol. Cremophor® EL causes adverse side effects like hypersensitivity, nephrotoxicity, neurotoxicity along with the effects caused on the endothelial and vascular muscles. It results in vasodilatation, lethargy, hypotension, and difficulty in breathing.

Nanoparticles have a property to accumulate in solid tumors and can evade the vasculature from endothelial tissues surrounding the tumor mass. It can accumulate in solid tumors by the enhanced permeation and retention effect. This experimental study aimed to design and develop a nanoparticulate formulation based on a polymeric drug delivery system for paclitaxel, free from Cremophor® EL, which was to be administered intravenously. To achieve this objective, paclitaxel-loaded poly (ethylene glycol)-poly(lactic-co-glycolic acid) based nanoparticles were prepared by the nanoprecipitation method. Poly(lactic-co-glycolic acid) was selected as the choice of polymer as it is a united states food and drug administration approved polymer and exhibits the properties like biocompatibility and biodegradability. Along with the poly(lactic-co-glycolic acid), poly (Ɛ-caprolactone-co-ethylene glycol) was added as a copolymer to provide nanoparticles' higher stability. The *in vitro* drug release, cellular uptake, apoptosis were studied, and the *in vitro* anticancer potential of paclitaxel loaded nanoparticles were performed using human cervix carcinoma cells (HeLa). *In vivo* tumor growth inhibition was checked by the formulated nanoparticles in tumor-bearing mice. The formulation developed in this study can be considered as an efficacious and specific anti-tumor drug delivery for chemotherapy (Prabhu et al. 2015; Danhier et al. 2009).

Antibiotics like doxorubicin have also been evaluated for efficient drug delivery by forming polymeric nanoparticles.To facilitate the combined therapy of doxorubicin and photothermal strategies as treatment regimen, doxorubicin-loaded poly (lactic-co-glycolic acid)-gold half-shell nanoparticles were formulated by applying gold films on doxorubicin loaded poly(lactic-co-glycolic acid) nanoparticles. Because of the near-infrared region resonance of doxorubicin-loaded poly(lactic-co-glycolic acid) nanoparticles, when the drug was released from the polymeric nanoparticles, the heat was generated locally in near-infrared region. In comparison with the chemotherapy or photothermal treatment alone, the combination of therapy showed a synergistic effect, proving its efficacious results in shorter treatment time. It possibly could lower the adverse effects and enhance the treatment effectiveness (Park et al. 2009).

Treatment of glioblastoma requires the site-specific delivery of anti-cancer agents, as it needs to penetrate the blood-brain barrier. Poly(lactic-co-glycolic acid) based nanoparticles, when applied with the coat of poloxamer 188 serves as effective drug carriers for delivery to the brain following intravenous administration. This experimental study was based on one such formulation and the anticancer

activity of the surfactant-coated doxorubicin loaded poly(lactic-co-glycolic acid) nanoparticles was determined against glioblastoma in rat using immunohistochemical and histological methods. Results showed considerable anti-tumor potential of the conjugated formulation. In similar kind of screening, best results were observed in doxorubicin-lecithin–poly (lactic-co-glycolic acid)/human serum albumin formulation. Interpretation of the experimental data revealed that the delivery of the drug with poloxamer 188 poly(lactic-co-glycolic acid) nanoparticles was done effectively across the blood brain barrier with desired therapeutically effective concentrations (Prabhu et al. 2015; Wohlfart et al. 2011).

Paclitaxel is probably the most widely used drug for the formation of polymeric nanoparticles against cancer. In the cancers of the reproductive system, ovarian cancer is the most common one among women. To evaluate anticancer efficiency and safety of drug xenografts, a model was established in female athymic (nude) mice. Paclitaxel (20 mg/kg) and tamoxifen (70 mg/kg) were intravenously administered either alone or in combination with poly(ethylene oxide)-poly(ε-caprolactone) nanoparticles. It exhibited great anticancer efficiency with poly(ethylene oxide)-poly(ε-caprolactone) nanoparticles. When the experiment was performed with paclitaxel and tamoxifen as a combination therapy, it revealed the high efficacy of formulation as an antitumor agent. It was not exhibiting any acute toxicity confirmed by body weight changes, hepatotoxicity, and blood cell counts. The conclusion of this study shows that combining tamoxifen and paclitaxel drugs along with bio-degradable polymeric poly(ethylene oxide)-poly(ε-caprolactone) nanoparticles could serve as an excellent strategy to tackle multidrug resistance in ovarian cancer (Devalapally et al. 2008). Co-polymerization has long been used as a tool for the preparation of nanoparticles.

To develop an amino active end group for peptide conjugation, a study was carried out to functionalize poly (ethylene glycol)-poly(lactic-co-glycolic) acid. Copolymerization with poly (ethylene glycol) diamine polymer was done for the pre-activation of poly(lactic-co-glycolic) acid. The pre-activated amphipathic poly(lactic-co-glycolic) acid-poly (ethylene glycol) co-polymer included 97% amino end groups with critical micelle concentration of 3×10^{-9} mol/L. IC_{50} (50% inhibition concentration) of the synthesized poly (ethylene glycol)-poly(lactic-co-glycolic) acid nanoparticles was found to be 100 mg/mL which was much higher than poly(lactic-co-glycolic) acid nanoparticles (1.02 + 0.37 mg/mL). The poly (ethylene glycol)-poly(lactic-co-glycolic) acidhas amphipathic properties by virtue of which in an aqueous solution it immediately forms the shell core structure. This unique feature imparts better peptide conjugation on amino group in polyehtylene glycol chain which is dispersed on the poly(lactic-co-glycolic) acid-poly (ethylene glycol) surface of nanoparticles.

When a study of this peptide conjugated nanoparticle was done on an ovarian cancer cell line derived from the ascites (SKOV3) of a 64-year-old caucasian female with an ovarian serous cystadenocarcinoma), it exhibited three-fold higher uptake than the peptide free nanoparticles. For doxorubicin delivery, when this nanocarrier was used it was found that in both types of nanocarriers (with or without peptide conjugation) system, it showed thatthe drug release at pH 7.4 was slower than at

pH 4.0 (500 U lipase). The IC_{50} value in SKOV3 cells was (3.12 ± 1.44 μg/mL) for peptide free poly(lactic-co-glycolic) acid-poly (ethylene glycol) nanosystem and (0.05 ± 0.03 μg/mL) for peptide conjugated nanosystem. IC_{50} value of doxorubicin loaded peptide conjugated nanoparticles in SKOV3 cells was found to be 62.4-fold lower than that of doxorubicin loaded peptide free nanoparticles (Liu and Lin 2012). To achieve targeted drug delivery, conjugation of folic acid and m-poly (ethylene glycol) on the surface of chitosan nanosystem was done.

To prepare mitomycin-C loaded chitosan nanoparticles with combination of ionic gelation method and chemical cross linking method for coupling folic acid and methoxy -poly (ethylene glycol) respectively. Mitomycin C was selected as a model drug and was loaded to methoxy-poly (ethylene glycol)-folic acid nanoparticles. By using a nude mice xenograft model, *in vivo* study was performed. The studyillustrated that a raising amount of methoxy – poly (ethylene glycol)-folic acid nanoparticles or folic acid nanoparticles were accumulated in the cancerous cells relative to methoxy -poly (ethylene glycol) nanoparticles alone. Outcome proposes that both folic acid and methoxy-poly (ethylene glycol) coupled chitosan nanosystem are attainably extending systemic drug delivery to cancerous tissue. This furtheropens-up a new window for selective targeting of cancer through nanoparticles (Hou et al. 2011).

6.6.4 Recent Patents and Marketed Products Approved for Cancer Treatment

There have been many developments in the field of nanotechnology furnishing polymeric nanoparticles that have led to the inventions procuring patents and marketed products to treat different types of cancers. Since the last two decades, natural origin drugs are being used quite often for preparing these nano-systems to treat cancer, and these products have proved their worth with confidence (Dang and Guan 2020; Prabhu et al. 2015; Bobo et al. 2016; Ventola 2017; Lombardo et al. 2019; Rezvantalab et al. 2018). The list of these products ars listed below in Table 6.7.

6.7 Regulatory Compliance

Nanotechnology has emerged as a new technological revolution providing many opportunities and possibilities, mainly focusing on solutions for unmet needs. Baring in mind a high demand to supply ratio of nanomedicines and a strong need to foster them, their properties have raised several concerns and essential challenges for the industry and regulatory agencies (Kakkar et al. 2018). A lack of general protocol for characterization and preclinical development of this nanotechnology domain and a shortage of specific protocols for each of the nanotechnology

Table 6.7 Recent patents and marketed products approved for cancer treatment including the main therapeutically active agent, the polymer used for entrapment, its indication for use, the tradename of the conjugate, the company manufacturing the formulation and the year of market approval

Polymer	Drug	Indication	Tradename	Company	Year of approval	References
Albumin	Paclitaxel	Metastatic breast cancer	Abraxane	Celgene	2005	Miele et al. (2009)
Poly (ethylene glycol)	Doxorubicin	Metastatic breast and metastatic ovarian cancer	Doxil	Janssen	1995	Tejada-Berges et al. (2002)
Methoxy-poly (ethylene glycol)–polylactic acid	Paclitaxel	Metastatic breast cancer	Genexol PM	Samyang corporation	2007	Kim and Shin (2010)
Poly (lactic-co-glycolic acid)	Leuprolide acetate	Prostate cancer	Eligard	Tolmar	2002	Sartor (2003)
Polylactic acid	Irinotecan	Pancreatic cancer	Onivyde	Merrimack	2015	Wang et al. (2021)

products poses serious provocations, ultimately leading to them failing in late-stage clinical trials. Obstacles in the clinical transformation of the nanomedicines depend mainly on the particular characteristics of the nanoproduct, the size range and physicochemical properties that ultimately determine their interaction with immune cells and plasma proteins, manufacturing processes involved, and the nature of data that needs to be mandatorily provided during the product life cycle which requires *in vivo* and clinical studies (Sainz et al. 2015).

Stringent and specific global regulatory trends are yet to be established, despite the several attempts already performed. As an alternative, the strategies used for the development of conventional medicinal products and new chemical entities have been frequently adapted to evaluate the safety/toxicity and perform compatibility studies of nanomedicines. From the regulator's perspective, the active pharmaceutical ingredient entrapped or incorporated in the nanomedicines dictates the specifications to be analyzed. In the case of polymeric nanoparticles specifically, the API along with the polymer used determines the regulatory procedure (Gaspar 2007). For biological entities, including proteins, peptides, or antibodies, the innovative product developed would follow the regulations stipulated for biological medicinal products and new chemical entities. A critical role needs to be played by the regulatory authorities such as european medicines agency, United States food and drug administration and pharmaceuticals and medical devices agency, academic institutions focused on innovations and industrial stakeholders to provide a refined

scientific platform along with co-operative contributions to achieve common perspectives in nanomedicine development (Sainz et al. 2015).

6.8 Perspective

Polymeric nanoparticles are evolving as a significant gadget for entrapping drugs and are predicted to be a leading tool in pharmaceuticals. They are being used for the delivery of drugs in various diseases with immense success. The same concept of polymeric nanoparticles has also been explored for the entrapment of drugs that are of natural origin to treat cancer. There are polymers of both natural and synthetic genres that can successfully entrap and deliver drugs. This genre is traversing through different generations of advancement and is currently in only the first or second generation of innovation, where the first and foremost generation is all about the enhancements in properties in the drug-polymer conjugates. This is followed by the second generation where active nanostructures are utilized viz. rendering the drug-polymer conjugate bioactive to catapult a drug to a specific target organ or cell that can be achieved. Further advancement in this field will lead to the third and fourth generation with advanced nanosystems such as nanorobotics and molecular nanosystems to control the drug delivery with utmost specificity, thereby furnishing nanoparticles that are suited for guided cancer therapeutics sparing the normal cells and acting on only the cancerous ones.

6.9 Conclusion

Cancer is a disease that has hooked itself with humankind since the dawn of civilization, causing deaths in mammoth numbers worldwide. Since then, the human race has been scouting for the cure for this disease. Fortunately, we have successfully found ways to treat it and extend the life expectancy of the affected individuals, but a complete treatment still seems quite ambitious. On the other side, there are rays of hope in fighting cancer by amalgamating scientific techniques such as nanotechnology with the compounds capable of fighting against cancer that can be naturally procured. There are certain natural and synthetic polymers capable of drug entrapment. These polymers are utilized to produce nanoparticles, i.e., the polymeric nanoparticles that can be successfully employed for drug entrapment. Certain drugs of natural origin are not so effective and specific on their own. They can cause serious adverse effects in the body by rendering non-specific cytotoxicity that kills healthy cells too, besides killing the cancerous ones.

With the entrapment of these drugs in polymeric nanocarriers, the resultant nanosystem has shown far better results in effectivity and tumor cell specificity, sparing the healthy cells and causing significantly less non-specific cytotoxicity. These also have some drawbacks like the variability of effectiveness depending on parameters

like particle shape, size, amount of surface area exposed and surface functionalization, rate of dissolution, the quality of agglomeration of nanoparticles, and the charge present on the nanoparticle's surface. Thus, these parameters have a very narrow error window and demand a high level of cautiousness. Apart from these, inflammation, DNA damage, generation of reactive oxygen species that may lead to oxidative stress causing damage to cellular membranes are some of the ill effects of these nanosystems, while on a brighter note, these systems allow a great deal of specificity in guided cancer therapy. Among the polymers that have been explored, it was witnessed that chitosan and poly (lactic-co-glycolic acid) can show promising effects in terms of biodegradability and biocompatibility when they are administered. Poly(lactic-co-glycolic acid), when used with poly(ethylene glycol) as the copolymer, shows significant cancer therapeutics effects.

On the other hand, polycaprolactone, when used along with other polymers, enhances biodegradability and biocompatibility of the copolymeric system, although it is not a viable candidate when employed without co-polymerization. On exploring the drugs suitable for entrapment with polymeric nanoparticles, it was revealed that doxorubicin, paclitaxel, and docetaxel were more common than the others in terms of usage. The future of nanotherapy in cancer amelioration is promising, with significant tumor targeting, site-specificity, and vector functionality. Although there have been some hiccups in delivering genes and drugs, the sophistication of nanotherapy has mitigated them in no small extent addressing issues associated with aggressive cancers like heterogeneity and biological diversity. New techniques targeting cancer stem cells are being developed that could combat the hurdle of resistance. In particular, new areas are also being explored to utilize polymeric nanoparticles due to their unique ability to deliver multimodal therapeutic agents. In cancer therapeutics, they are being used in versatile roles such as carriers for therapeutic vaccines, targeted monoclonal antibodies, and cell-based immune therapies that can lower the dose and toxicities.

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Chapter 7
Polymeric Nanoparticles that Entrap Drug Combinations Targeted to Solid Tumors

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Abbreviations

ADR	Adriamycin resistant cell line
DNA	Deoxyribonucleic acid
MCF-7	Michigan Cancer Foundation-7
$N_3^{\bullet}$	azidyl radicals
RNA	Ribonucleic acid

7.1 Introduction

Nanotechnology is a field of research involving the design, synthesis and manipulation of size and structure of an engineered particle that is one billionth in size or at least one of its dimensions are considered in nanometre. Nanotechnology simply combines various physiochemical methods with principles to synthesize functional nanoparticles. These nano-sized particles exhibit entirely novel and enhanced properties based on their attributes such as size, form and orientation (Sadowski 2010; Padhi et al. 2020). Interest in developing nanomaterials is increased in recent years because of the simplicity in which they can be produced in numerous shapes. These particles have an answer to solve various scientific problems in the fields of

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agriculture (Lai et al. 2006; Speiser 2008), fibre and garments (Perelshtein et al. 2008), electronics (Huang et al. 2003), space (Liu et al. 2007), forensic science (Choi et al. 2008) and medicine (Bonduelle et al. 1991; Bender et al. 1996; Kawashima et al. 2000; des Rieux et al. 2006; Jahanshahi and Babaei 2008).

Currently, extensive research has been going on to discover new drugs from natural substances due to the development of drug-resistant microorganisms which have evolved resistance to the currently available conventional drugs (Savithramma et al. 2011). Integration of natural substances (alginate, gelatin, chitosan, etc.) of biological origin with nanomaterials resulted in the advancement of various cancer biology tools and diagnostic devices. Recent efforts in improving the efficiency of nanoparticle synthesis and exploring their biomedical applications will hopefully lead to the implementation of these approaches on a large scale and their commercial applications in health care and medicine will take place in future (Kumar et al. 2020).

Nanoencapsulation involves the encapsulation of different drugs to improve their viability, specificity, tolerability and therapeutic index (Safra et al. 2000; Raghuvanshi et al. 2002; Fassas et al. 2003; Kumari et al. 2010). Nanoencapsulation also prevents the drug from early degradation and non-targeted environmental interaction while improving absorption into the target tissue, retention time, bioavailability and intracellular penetration efficiency (Alexis et al. 2008; Padhi et al. 2018). The selection of the proper polymeric system exhibiting high encapsulation efficiency greatly influences various properties of the nanoencapsulation system (Kumari et al. 2010). There is no specific rule to achieve the desired combination, and are usually attained by trial-and-error method (Saranya and Radha 2014).

Polymeric nanoparticle systems are more advanced than other nanoparticle systems (van Vlerken et al. 2007). These nano-drug formulations possess targeted delivery, controlled release, and therapeutic impact which are superior to conventional medicine (Kumari et al. 2010; Khuroo et al. 2014). Size and charge of the particle, surface modification, and hydrophobicity greatly influence nanoparticle targeting capabilities (Bisht and Jha 2017). Among these, the nanoparticle size plays an important role in dictating their synergy with the cell membrane and their infiltration over the physiological drug barriers (Kumari et al. 2010; Padhi et al. 2015). The target tissue, circulation and other biological barriers are considered to select a suitable nanoparticle size (Brannon-Peppas et al. 2004). The surface charge also plays a critical role in nanoparticle penetration and cellular internalization. It also aids in the determination of nanoparticle aggregation in the bloodstream, interaction or sticking of the nanoparticles with the oppositely charged cell membrane (Feng 2004).

Targeted drug delivery requires the persistence of nanoparticles in the body's systemic circulation. However, conventional hydrophobic nanoparticles are swiftly opsonised and greatly cleared by macrophages of the phagocytic system (Mohanraj and Chen 2006). Therefore, the exterior surface of nanoparticles can be altered to enhance their persistence in the blood (Kumari et al. 2010). The surface of nanoparticle plays a major role in their performance. These particles possess anti-adhesive surface properties which act as a steric barrier reducing liver macrophage clearance

and enhanced cellular permeation (Shenoy and Amiji 2005). Similarly, the coating of nanoparticles using hydrophilic polymers will conceive a cloud of chains at the surface which will repel plasma proteins (Kumari et al. 2010; Brigger et al. 2012). Coating the nanoparticles with biomimetic materials such as membrane proteins which are universally recognised as self to protect immune clearance is another distinct approach to avoid immune response (Abeylath et al. 2011).

Morphological parameters and molecular weight greatly influence the performance of nanoparticles. The molecular weight of polymer also affects the release mechanism of the drug, the higher molecular weight slower the drug release into the cells (Zambaux et al. 1999; Kumari et al. 2010). Taking into consideration the aforementioned factors, there appears a high need to design a proper drug delivery system for the target site.

7.2 Types of Polymeric Nanoparticles

Polymeric nanoparticles can be of many forms such as nanocapsules and nanospheres. Nanocapsules are vesicular structures that act as drug reservoirs in which they retain pharmaceutical ingredients/drugs. They contain an aqueous or non-aqueous liquid core in the vesicle cavity which is enclosed by a solid polymeric shell (Khalid and El-Sawy 2017), whereas nanospheres are spherical solid mass of polymers. It may be considered as a spherical polymeric mass in which drug molecules are either impregnated inside the core or adsorbed on its surface (Counreur et al. 1995; Rao and Geckeler 2011). Various natural polymers (polysaccharides, polypeptides, etc.) and biodegradable synthetic polymers (poly(lactic-co-glycolide, polycaprolactone etc.) can be used to synthesise nanoparticles (Takakura and Hashida 1995; Kratz et al. 1999; Liu et al. 2009). Polymeric nanoparticles are synthesised by the self-assembly of amphiphilic diblock copolymers (Hu and Zhang 2012). Some commonly used methods for its preparation are evaporation and diffusion of solvent, salting out and supercritical fluid technology (Soppimath et al. 2001, Walvekar et al. 2019).

Polymeric nanoparticles are promising platforms for drug delivery due to their high stability, biocompatibility, high activity duration and increased bioavailability than other administration routes. These nanoparticles can effectively cross the blood-brain barrier, deliver drugs to genes (gene delivery) and treat cancer with high specificity (Shukla et al. 2019; Padhi and Behera 2020). Some of the vital features of polymeric nanoparticles are:

- Exist with high stability in blood till it reaches the target site.
- Escape capture by the phagocytic system.
- Possess hydrophilic surface to delay the recognition by immune cells.
- Possess penetrating properties to easily penetrate irregular blood vessels of tumour cells.
- Efficiently reach the binding site and release the drug to target cells.

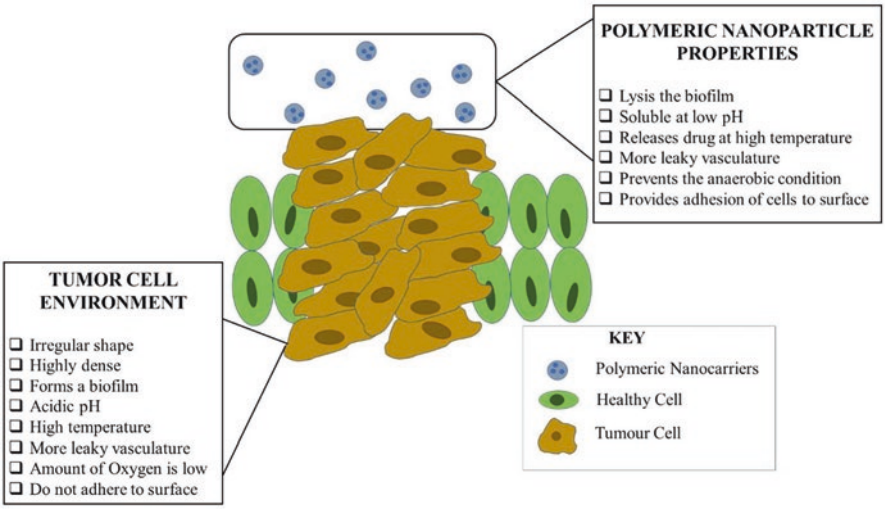

Fig. 7.1 Functional properties of polymeric nanoparticle versus environmental conditions in tumour cells. Polymeric nanoparticles are considered to be the most efficient vehicles for the drug delivery especially into the tumor cell environment due to its excellent pharmacokinetic properties like solubility at very low pH, drug releasing capacity at high temperatures, leaky vasculature, surpassing anaerobic conditions, and easy adhering to the tumor cell surfaces

Polymeric nanoparticles are formulated involving different combinations of chemotherapeutic drug materials which help to a large extent towards the betterment of the cancer treatment due to the various functional properties of polymeric nanoparticles which are described in the Fig. 7.1.

Polymers used in nanoparticle formulations are classified into natural and synthetic polymers based on the source of progenitors. Frequently applied natural polymers are, chitosan, sodium alginate, albumin, gelatin, etc. (Khalid and El-Sawy 2017; Shukla et al. 2019) and synthetic polymers are poly (lactic-co-glycolic acid), polycaprolactone etc. Some of the commonly used polymers and their advantages are discussed below.

7.2.1 Chitosan

Chitosan is a non-toxic, biodegradable polymer. Derivatives of chitosan have been widely employed as nanocarriers in cancer therapy. These nanocarriers exhibit high efficacy and tumour targeting ability with low toxic side effects (Shukla et al. 2019). They can be modified into nano vehicles that allow prolonged and sustained drug release at tumour sites, thus increasing the efficiency of treatment (Agnihotri et al. 2004; Rampino et al. 2013; Tiyaboonchai 2003).

7.2.2 Gelatin

Gelatin is one of the most commonly used natural polymer (isolated from animal bones) for the formulation of nanoparticles due to its cross-linking nature. It forms cross-links with drugs which enables efficient targeting and delivery to the tumour sites (Wei et al. 2014). Biodegradability and biocompatibility are the major advantages of gelatin nanoparticles over other polymeric materials (Shukla et al. 2019). These nanoparticles follow a diffusion-controlled release mechanism to achieve differential and sustained release of encapsulated drug formulations. They can also be used in combination with photolytic enzymes that disrupt the matrix to instantly release of 10–15% drug followed by a timely specific sustained release of polymeric nanoparticles at the tumor site (Shukla et al. 2019).

7.2.3 Polyethylene Glycol

Poly (ethylene glycol) can act by both active and passive mechanisms (targeting approach of drug delivery systems). Poly (ethylene glycol) surface conjugation can be used to effectively deliver numerous drugs. It improves the circulation and retention time by preventing the drug nanocarrier from reticuloendothelial system metabolism and excretion (Owens III and Peppas 2006; Khurana et al. 2018; Pandey et al. 2018; Shukla et al. 2019). Poly (ethylene glycol) allows active ligand targeting which can effectively localise intracellular target which in turn increases the drug efficacy. Thus, poly (ethylene glycol) is a key excipient in novel drug delivery for tumour or cancer therapy.

7.2.4 Polyalkyl Cyanoacrylate

Cellular resistance of widely applied lipophilic drugs is a major problem in cancer therapy (Shukla et al. 2019). Emerging cellular resistance may be due to problems in tumour mechanisms or drug distribution (Krishna and Mayer 2000). Polyalkyl cyanoacrylate nanocarriers can be used to effectively overcome multidrug resistance and deliver the drug to the targeted cells with high sensitivity than other polymeric nanocarriers. As conventional single-drug therapy was not able to completely overcome drug resistance, thus combinational therapy was employed (Shukla et al. 2019).

7.2.5 Polyesters

Polyesters are synthetic polymers comprising various chemically engineered polymers like polylactide, polyglycolide, polycaprolactone, poly (lactic–co–glycolic acid). Different polymers possess unique properties. Polyester can carry different drugs in their hydrophobic core and are coated with poly (ethylene glycol). They are known as sheath polymers and possess enhanced bioavailability, shelf-life and flexibility (Shukla et al. 2019).

7.2.6 Polylactic Acid

Polylactic acid is employed for drug delivery and tissue engineering due to its slow degradation and easy processing ability. Polylactic acid can be combined with other polymers such as poly (ethylene glycol) to form polymeric micelles with a hydrophobic core and hydrophilic shell. Polylactic acid has various isomers which can be used to create novel nanocarriers for cancer therapy. (Xiao et al. 2010).

7.2.7 Polyglycolide

Polyglycolide is a biodegradable polymer with high tensile strength and a high melting point. It is widely used as absorbable sutures and as scaffolds for tissue regeneration. Owing to its rapid degradation property and non-flexibility, polyglycolide is unfavourable for cancer therapy. Polyglycolide is generally used in combination with other polymers to complement the degradation properties and to express synergistic properties (Shukla et al. 2019).

7.2.8 Poly(Lactic-Co-Glycolic Acid)

Poly(lactic-co-glycolic acid) is composed of polylactic acid and polyglycolide and its properties can be varied by altering their weight-by-weight ratio (Parveen and Sahoo 2011). Poly(lactic-co-glycolic acid) is being explored in cancer therapy due to its favourable properties such as sol to gel. Thus, poly (lactic-co-glycolic acid) when combined with therapeutic drugs may provide a breakthrough in cancer treatment (Joshi et al. 2010).

7.2.9 Polycaprolactone

Polycaprolactone is a commercially available synthetic polymer known for its biodegradable and mechanical properties (Guarino et al. 2002). It possesses slow degradation time unto several months and can be used in the controlled release of cancer drugs. Also, polycaprolactone can be used to deliver drugs for treating drug-resistant cancers.

7.2.10 Polyvinyl Alcohol

Polyvinyl alcohol is a colourless, odourless and water-soluble synthetic polymer widely used in film-forming, emulsification and adhesion. It is non-toxic and can be used in combination with other polymers to form nanoparticles for cancer therapy. These nanoparticles are very stable and can be stored for a long period without aggregation or precipitation (Siddharth et al. 2017).

7.3 Advantages of Combinational Therapy Versus Single Drug in Nanosystems

During the early twentieth century, single-drug therapy was the most commonly used treatment regimen. However, conventional dosage forms had various problems such as short half-life, poor patient compliance, poor bioavailability and sub-optimal therapeutic effects (Aungst 1993; Walvekar et al. 2019). These shortcomings are due to the poor pharmacokinetic profile which often ends up in drug delivery to non-target sites, which is one of the significant explanations behind developing resistance (Walvekar et al. 2019). Therefore, single-drug therapy faced a gradual downfall which led to the development of combination therapy for treatment.

Combination drug therapy involves the use of multiple active ingredients (drugs) simultaneously to treat disease conditions (Hu and Zhang 2012). Combination drug therapy involves the use of a ‘drug cocktail’ to treat various diseases. It induces proper collaborative effects, broadens antitumor efficacy, overcomes drug resistance and decreases side effects (Woodcock et al. 2011). However, varied membrane transport properties, pharmacokinetic profiles, dosing and optimization are challenges with combination drug therapy (Hu et al. 2010; Walvekar et al. 2019).

Nanosystems such as liposomes, polymeric micelles, and polymer-drug conjugates are widely employed to carry two or more types of therapeutic drugs (Hu and Zhang 2012; Behera et al. 2020; Hassan et al. 2021). These systems provide controlled combinational drug delivery with unique strength and characteristics which are achieved by the various surface modifications which are illustrated in the Fig. 7.2 (Hu and Zhang 2012).

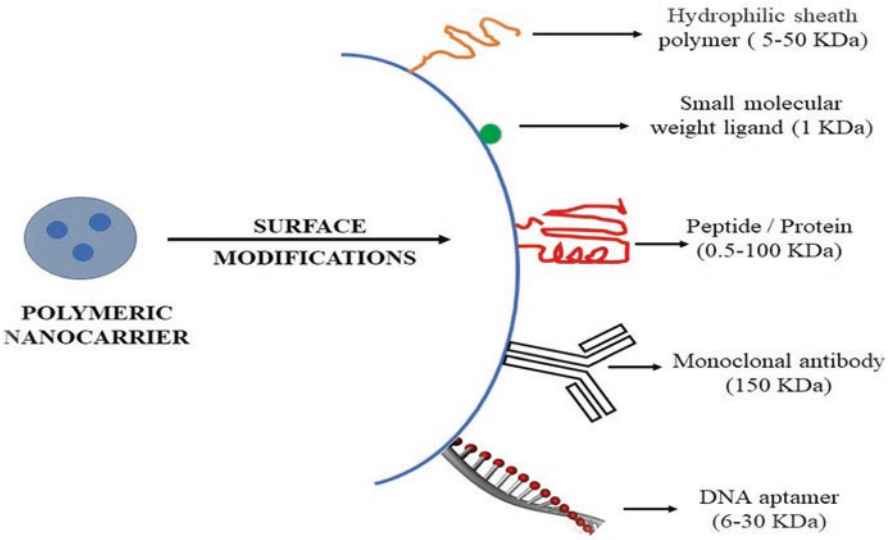

Fig. 7.2 Surface modifications of polymeric nanocarriers with their molecular weights. Surface modifications of the polymeric nanocarrier like hydrophilic sheath polymer, small molecular weight ligand, peptide/protein conjugation, monoclonal antibody, DNA aptamer generally helps in the enhancement of the efficacy, selective targeting, and bio distribution of the anti-cancer drug at the tumor site

Polymeric nanoparticles are compact arrangement/aggregation of polymeric colloidal molecules in an aqueous system (Walvekar et al. 2019). They are composed of a solid structure that gives higher sustainability, more stability, uniform size distribution and a highly controlled drug release profile in comparison to other nanosystems. The solid polymer filled core is used to carry drug cocktails and is more suitable for water-insoluble drugs (Hu and Zhang 2012).

For drug encapsulation, the drugs are mixed with a suitable polymer. The polymers arrange themselves into particles and encapsulate the drug compounds. Different hydrophobic therapeutic compounds have been stacked simultaneously through this physical entrapment approach (Hu and Zhang 2012). Drug–polymer conjugations lead to the development of combinatorial drug encapsulation schemes which have extended their compatibility to hydrophilic drugs (Zhang et al. 2007; Aryal et al. 2010; Kolishetti et al. 2010), precisely controlled drug loading ratios (Aryal et al. 2011), and fine-tuned drug release sequence and kinetics (Sengupta et al. 2005; Wang and Ho 2010).

7.4 The Targeting Approach

The recent surge in technology and research avenues has led to various developments in the field of medicine which in consequence has increased the interest to develop novel and effective cancer treatments. This is because cancer is a disease of various types and caused due to different sources that kill the majority of people across the globe. For treating cancer, there are several treatments among which chemotherapy is a vital one. It is a class of treatment that treats cancer patients using chemicals. Drugs used in this treatment method are highly toxic to normal cells and does not possess much specificity towards the target cells causing various side effects to the patient. To overcome these disadvantages in chemotherapy, effective drug delivery methods were designed (Hu and Zhang 2012).

One of the effective drug delivery methods is the impregnation of active drugs within the nanoparticles. Here, a nanoparticle is used to encapsulate the chemical drug and acts as a drug carrier. This incorporation of the nanoparticle to the chemicals prevents degradation of chemicals, improves the kinetics of drug, increases drug specificity to cells, and improves penetration and image processing modulators. The efficiency of the drug against specific cancer types can be improved by selecting a suitable nanoparticle and manipulating its properties. In general, drug release can be classified into two types based on the mechanism: passive targeting and active targeting mechanisms which are illustrated in the Fig. 7.3 (Milane et al. 2011).

7.4.1 Passive Targeting of Polymeric Nanoparticles Entrapping Combination of Drugs

The passive targeting method exploits the unique feature of angiogenesis in tumour cells. As there is a rapid proliferation of tumour cells their blood vessels formed are loosely packed or leaky. Since nanoparticles are small-sized, the encapsulated chemotherapeutic drug can pass through the loosely packed tumour cells and slowly accumulate over time. This process of penetration and accumulation of nanoparticles are known as the enhanced permeability and retention effect (Clemons et al. 2018). The enhanced permeability and retention effect depends on the formation of blood and lymph vessels, tumour growth, the density of the stromal response and intratumour pressure (Brannon-Peppas and Blanchette 2004). These nanoparticles get accumulated in the tumour cells and the drugs are released in the tumour microenvironment which inhibits the growth of a tumour.

This mechanism of passive targeting method has its limitations. In few cases, after the retention of nanoparticle inside the tumour environment, a new blood leak may remove the accumulated nanoparticles from that region. The uncontrolled

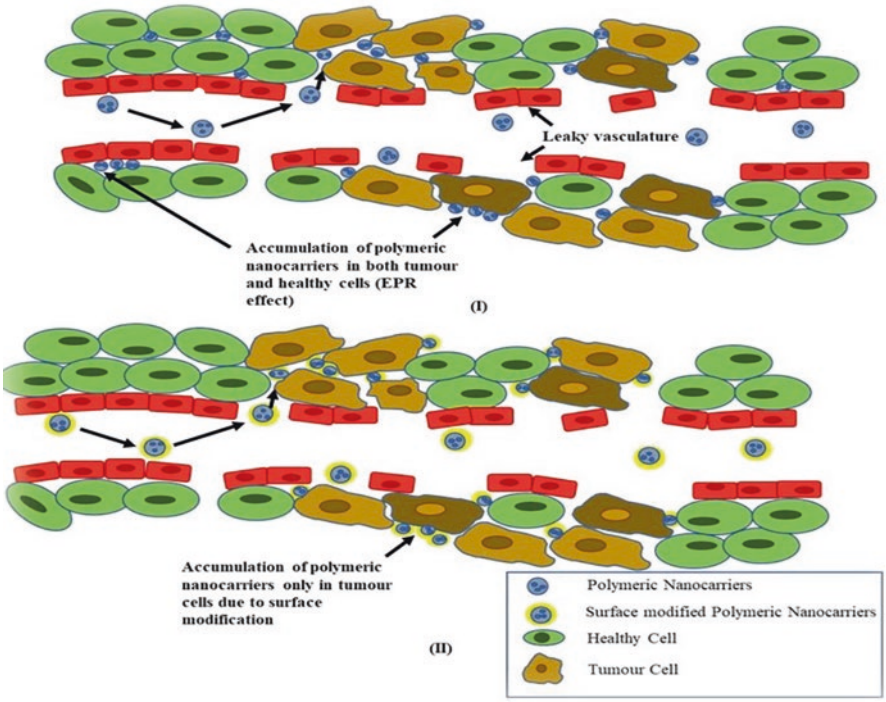

Fig. 7.3 (I) Passive targeting of the nanocarriers happens due to the leaky vasculature of the cell and its environment which is due to the process of angiogenesis. It also helps the easy permeation of the nanoparticles to into the tumor environment and normal cells thus increasing the drug bioavailability at all the cells. (II) Active targeting of the surface modified polymeric nanoparticles not only increases the bioavailability by better cell permeation and retention but also targets the specific tumor cell thus bringing about the changes at the appropriate environment

release of chemicals from the nanoparticle may result in off-target delivery or drug resistance which eventually leads to failure in chemotherapy treatment. Another drawback is that all tumours do not possess enhanced permeability and retention effect and the permeability of nanoparticle is variable.

Successful applications of polymeric nanoparticles entrapping combination of drugs enacting passive targeting approach are enlisted as follows.

- Poly(lactic-co-glycolic acid) nanoparticles entrapped with vincristine and verapamil with moderate multi drug resistance reversal activity on breast cancer cells (MCF-7/ADR cells) resistant to vincristine. A novel method is strategized in order to optimize the effectiveness of the combined cancer therapy where a combination of poly (lactic-co-glycolic acid), vincristine (a cytotoxic drug) and verapamil (chemosensitizer) is synthesized by emulsion solvent evaporation and salting out method. These novel poly (lactic-co-glycolic acid) nanoparticles

entrapping vincristine and verapamil demonstrated moderate multi drug resistance reversal activity against drug-resistant breast tumours *in vitro* (MCF-7/ADR cells) resistant to vincristine (Song et al. 2009; Parhi et al. 2012).

- Poly(lactic-co-glycolic acid) nanoparticles entrapped with paclitaxel and tariquidar demonstrated better inhibition of tumour growth in a mouse model by reversing multi drug resistance. These novel encapsulated nanoparticles showed significant higher toxicity in the drug resistant tumor cells. They also demonstrated *in vitro* and *in vivo* studies which reflected greater inhibition of tumor growth thus showing that combinational dual agent nanoparticles with a modulator and anticancer drug is a promising approach for tumor drug resistance (Brannon-Peppas and Blanchette 2004; Patil et al. 2009; Parhi et al. 2012).
- Calcium alginate-beads entrapping doxorubicin conjugated with N-acetyl histidine and arginine demonstrated enhanced cellular uptake and great cytotoxicity in breast cancer cells (MCF-7). These encapsulated nanoparticles showed a sustained drug release with increasing substitution degree of N-acetyl histidine. These nanoparticles suppressed both the sensitive and resistant human breast cancer cell lines efficiently in a time dependent and dose dependent pattern (Raja et al. 2017).
- Calcium alginate-beads entrapping doxorubicin conjugated with silver sulphide (quantum dot) exhibiting high antitumor efficacy against cervical cancer (Hela) cells and ability to monitor the distribution of nanoparticles in the body (Tan et al. 2017).
- Acid degradable dextran nanopolymer is synthesized with cleavable amine groups as the small interfering RNA carrier. This nanoparticles downregulated the cyclooxygenase-2 which is the major mediator of inflammation in cancer cells and tumor. They accumulate rapidly which in-turn cleaved the amine functional groups in the tumor and cancer cells thus showing great biocompatibility and synthesis reproducibility (Chen et al. 2018).
- Dextran nanoparticles entrapped with paclitaxel which in turn conjugated with albumin significantly inhibited cell proliferation, growth of the tumour, induces apoptosis and prolong circulation time of paclitaxel in colorectal cell lines (CT-26). These nanoparticles are synthesized using aldehyde containing dextran, bovine serum albumin and paclitaxel through desolvation and cross-linking method. These nanoparticles showed a good tumor-targeted uptake, controlled drug release and excellent anti-tumor abilities (Zhang et al. 2019).
- Pullulan is conjugated with polyethyleneimine and mercaptosuccinic acid thus synthesizing thiolated cationic pullalan which exhibits simultaneous gene and drug delivery using redox sensitive cationic polymers resulting in a promising potential in cancer therapy. Entrapping doxorubicin conjugated with polyethyleneimine and mercaptosuccinic acid with Superior uptake, enhance transfection efficiency and retention drug in glioma (C6) (Priya and Rekha 2017). Some of the polymeric nanoparticles and their targeting approach by active method are given in Table 7.1.

Table 7.1 Applications of combinatorial polymeric nanodrug formulations following passive targeting

Polymeric nanoparticle	Targeting approach	Significance	References
Poly (ethylene glycol) nanoparticles	Passive	Doxorubicin drug delivery and theranostics	Wang et al. (2013)
Poly(lactic-co-glycolic acid) nanoparticles	Passive	Entrapped with vincristine and verapamil demonstrating moderate reversal of multidrug resistance	Patil et al. (2010)
		Entrapped with paclitaxel and tariquidar demonstrated better inhibition of tumour growth	Soma et al. (2000)
Calcium alginate encapsulated nanoparticles	Passive	Entrapped with doxorubicin, N-acetyl histidine demonstrating great cytotoxicity over breast cancer cell line (MCF-7)	Raja et al. (2017)
Calcium alginate entrapped nanoparticles	Passive	Entrapped with doxorubicin along with silver sulphide (quantum dot) showing antitumor activity against cervical cancer cell line	Tan et al. (2017)
Acid degradable dextran nanoparticles	Passive	Dextran nanopolymer entrapping cleavable amine group small interfering RNA downregulating cyclooxygenase-2 in cancerous cells and tumors	Chen et al. (2018)
Thiolated cationic pullulan polymer	Passive	Conjugated with polyethyleneimine and mercaptosuccinic acid exhibiting simultaneous gene and drug delivery	Priya and Rekha (2017)

7.4.2 Active Targeting of Polymeric Nanoparticles Entrapping Combination of Drugs

The active targeting method involves the introduction of a targeting molecule like antibody, ligand or protein into a nanoparticle system to facilitate specific binding to the tumour cells. This type of targeting system has high specificity towards the tumour cells as compared to the passive targeting system. In this method, the nanoparticle recognizes and binds to tumour cells by molecule-receptor interaction between the cells and nanoparticle. These nanoparticles that are bound to cells release the drug directly into the cell, which gives an advantage in reducing the chances of off-target drug release.

To improve the efficiency of the drug release component, several approaches were prescribed to improve the retention time of nanoparticle on cell membrane receptors such as epidermal growth factor receptor. This receptor is overexpressed in several cancer types such as breast, renal, ovarian, colon and lung cancer (Clemons et al. 2018) which can be utilised to detect and release the drugs to specific tumour cells. However, the major drawback of this method is that it primarily depends on size, shape, the surface area of the cell and the number of receptors present in the

cell membrane (Abeylath et al. 2011). Epidermal growth factor receptor targeted polymeric nanoparticles loaded with ionidamine and paclitaxel showing targeted antitumor activity by downregulating multidrug resistance in human breast and ovarian tumour cells is an example of active targeting polymeric nanoparticle (Milane et al. 2011; Parhi et al. 2012). However, the intracellular trafficking and intracellular destination of the nanoparticle is a poorly understood process that affects the overall therapeutic outcome (Clemons et al. 2018).

Successful applications of polymeric nanoparticles entrapping combination of drugs enacting active targeting are enlisted as follows.

- Polymeric nanoparticles coated with Hyaluronic acid is used to deliver docetaxel and photosensitizer meso-tetraphenyl chlorine disulfonate simultaneously in order to target and kill CD44 overexpressing breast cancer cells. This therapeutic strategy had superior efficacy over monotherapy by killing proliferative cancerous cells and by reducing their self-renual capacity thus demonstrating the potential of treating cancerous cell lines (Gaio et al. 2020).
- Polymeric nanomaterials are prepared from PEGylated poly (lactic acid-co-glycolic acid-co-hydroxymethyl glycolic acid) for the selective delivery of saporin into the cytosol of HER2 positive breast cancer cells. The uptake of saporin was achieved by coating the surface of the nanoparticles by a nanobody (11A4) which are specific for the HER2 receptor. The PEGylated poly (lactic acid-co-glycolic acid-co-hydroxymethyl glycolic acid) nanoparticles were combined with photochemical internalization which selectively induces cell death of HER2 positive breast cancer cells. This therapeutic approach is a result of receptor-mediated endocytosis of the nanoparticles (Martínez-Jothar et al. 2019).
- A combinational therapeutic system was developed using the photoactivable platinumt (IV) prodrug backboned nanoparticles for the light controlled delivery and synergistic photoactivated chemotherapy along with RNA interference over platinum resistant ovarian cancer cells. Over blue-light irradiation, the polymeric nanoparticles produces oxygen independent azidyl radicals ($N_3^{\bullet}$) with mild oxidation energy helps in the lysosomal escape with less gene inactivation. This effectively reverses the drug resistance and shows an excellent synergistic therapeutic effect against the platinum resistant ovarian cancer cells. Hence this combination of chemotherapy and gene therapy acts as an effective strategy for cancer treatments (Zhang et al. 2020).
- Polymeric nanoparticles entrapping phosphoinositide 3-kinase inhibitors (i.e., wortmannin and PX866) suppressed colorectal cancer lung metastasis growth. These polymeric nanoparticles are composed of poly (ethylene glycol)-polycaprolactone which are highly biocompatible and well characterized degradation kinetics. These nanoparticles deliver a high dose of phosphoinositide 3-kinase inhibitors into the lungs thus suppressing lung metastasis growth selectively and avoid side effects to normal tissues and organs (Rychahou et al. 2018).
- Poly (lactide-co-glycolic acid)-poly (ethylene glycol)-poly (lactide-co-glycolic acid) triblock copolymers entrapping doxorubicin were constructed in order to

efficiently deliver the drug system for combined chemotherapy and immunotherapy. These nanoparticles produce cytotoxic substances like nitric oxide thus inducing tumor cell destruction through immunotherapy. This system works efficiently at physiological temperature and helps in the sustainable drug release (Jiang et al. 2018).

- Colorectal cancer is the most common contributor to the cancer malignancy and cancer mortalities which express five different types of somatostatin receptors. These somatostatin receptors are targeted in the cancer therapy by binding it with the somatostatin analogue namely ostreotide which shows proliferative inhibition of colon cancer cell lines. This particular polymeric nanocarrier is prepared by the conjugation of cetuximab (an oral drug for colorectal cancer) and ostreotide by solvent evaporation method and then its loaded on to the calcium alginate beads which acts as a polymeric drug delivery vehicle. This type of formulation has showed excellent anti-proliferative activity thus proving to be a promising platform in order to treat colorectal cancer. (Abdellatif et al. 2020). Some of the polymeric nanoparticles and their targeting approach by passive method are given in Table 7.2.

Table 7.2 Applications of combinatorial polymeric nanodrug formulations following active targeting approach

Polymeric nanoparticle	Targeting approach	Significance	References
Chitosan-silica nanoparticle	Active	pH sensitive nano carrier with tumor necrosis factor alpha for breast cancer therapy	Deng et al. (2011)
Poly (ethylene glycol) nanoparticle	Active	Targeting human squamous cell carcinoma head and neck cancer	Reuveni et al. (2011)
Poly (lactic-co-glycolic acid)-poly (ethylene glycol) nanoparticle	Active	Prostate and breast cancer	Graf et al. (2012)
Poly (lactic-co-glycolic acid)-polyvinyl alcohol nanoparticle	Active	Induction of DNA damage in doxorubicin resistant breast cancer	Siddharth et al. (2017)
Polyethyleneimine-poly (ethylene glycol) nanoparticle	Active	Targeting tumour	Zhou et al. (2016)
Polyethyleneimine nanoparticle	Active	Lung cancer	Attia et al. (2019)
Poly-glycidyl methacrylate nanoparticle	Active	Effective against epithelial, human breast cancer	Clemons et al. (2018)
Polymeric micelle nanoparticles (poly (ethylene glycol)-polyethyleneimine coated)	Active	Ovarian cancer	Sawant et al. (2014)

7.5 Conclusion

Advances in tumour management therapies are increasing day by day which leads to the exploration of polymers for tumour and cancer therapy. Polymeric nanoparticles have shown the ability to overcome various problems faced by many conventional methods. Polymeric multifunctional Nano therapeutics majorly helps in the overcoming of various disadvantages of conventional methods by prolonging the systemic circulation, targeted drug delivery, minimizing the systemic toxicity, etc. This allowed the emergence of various novel drug formulations for the diagnosis and treatment of different types of cancer which has the capability to potentially eradicate cancer cells selectively and efficiently without damaging the native normal cells and tissues.

Apart from these advantages, combinatorial polymeric nanoparticle drug delivery systems also have an improved pharmacokinetic profile and therapeutic index along with the overcoming of the major obstacle of drug-induced cancer resistance to the cancer treatment. The employment of the nanotechnology in the treatment of cancer is widely studied and used due to its crucial role in the therapeutic process. Different Nano carriers such as polymer-drug conjugates, liposomes, carbon nanotubes, dendrimers, micelles, etc. increases the drug availability at the tumor cells, protecting the drug from degradation, and high blood circulation profile, etc.

However, polymeric nanoparticles require more focus and research to develop new formulations and novel targeting approaches. Even though there are tremendous applications and potential for the combinational drug delivery system, the constant exposure of the nanoparticles to the normal living cells pose serious threat. The intensity of the adversity is based on the size, shape, agglomeration; chemistry, electromagnetic properties, etc. The constant administration of the nanoparticle to the human body might affect the health of the individual at the cellular as well as in the genetic level. This emerging approach of combinatorial polymeric Nano carrier for the treatment of cancer should be used widely with an improved therapeutic outcome and reduced costs. Also more studies should be focussed on the precise dosage of the combinational drugs that should be administered to the patient and systemic release of the drugs at the specific site of action must be studied more intensely before reaching to the clinical trials. These researches will enhance the clinical and economic exploitation of polymeric nanoparticles and is considered as an inevitable step for the future therapeutics.

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Chapter 8
Ligands Specific to Over-expressed Receptors in Solid Tumors

Pratap Kumar Sahu

8.1 Introduction

New growths or neoplasms or neoplastic cells that form an abnormal mass of tissue are called as tumors. Tumors can be benign or malignant (Padhi et al. 2020, 2021). Benign tumors remain localized and usually not harmful. They are slow growing and resemble normal cells. They usually end with 'oma', for example, papilloma (surface epithelium), adenoma (glandular epithelium), melanoma (pigment cells), myoma (muscle tissue), fibroma (fibrous tissue), neurofibroma (nerve sheath) etc. Malignant tumors are also called as cancer. Lung cancer is the most common cancer prevalent throughout the world followed by breast cancer and colorectal cancer (Ramzy et al. 2017).

Malignant tumors show uncontrolled proliferation and rapidly spread to other parts of the body. They manifest de-differentiation, invasiveness and metastasis. They are harmful, if untreated. Malignant tumors are either solid tumors or liquid tumors. Solid tumors (Fig. 8.1) are localized mass of abnormal tissue that occur in surface epithelial tissue (carcinoma), glandular epithelial tissue (adenocarcinoma), connective tissue (sarcoma), hematopoietic tissue of bone marrow (myeloma) and lymphatic tissue (lymphoma). Solid tumors do not contain cysts or liquid areas. The term solid tumor is usually used to distinguish between localized mass of tissue and leukemia. Leukemia are examples of liquid tumors or blood cancers resulting from disorganized proliferation of abnormal leukocytes (DeVita 1997).

More than 85% of all cancers in human beings are solid tumors. Some of the common solid tumors (Zhuge et al. 2009; Mohan 2002) are:

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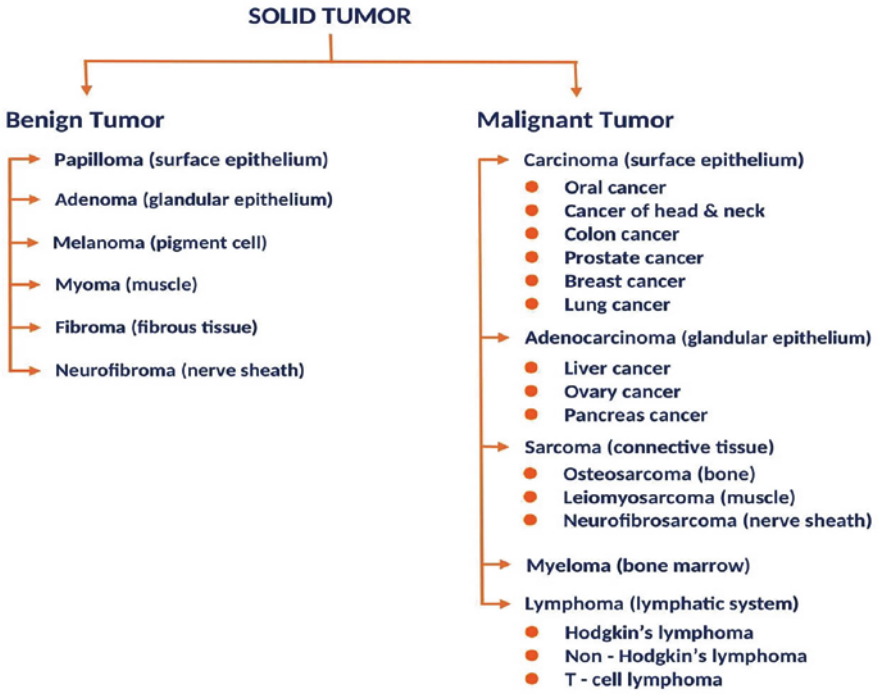

Fig. 8.1 Types of solid tumor (localized mass of abnormal tissue). Solid tumors can be benign or malignant. Based on location, benign tumors are known as papilloma (surface epithelium), adenoma (glandular epithelium), melanoma (pigment cells), myoma (muscle tissue), fibroma (fibrous tissue), neurofibroma (nerve sheath). Similarly, malignant tumors are known as carcinoma (surface epithelial tissue), adenocarcinoma (glandular epithelial tissue), sarcoma (connective tissue), myeloma (hematopoietic tissue of bone marrow) and lymphoma (lymphatic tissue)

1. Hodgkin's lymphoma: It is of B-cell origin involving lymph nodes at the surface of the body. It starts with a painless swelling in the neck, armpit or groin. It mostly occurs in patients in their 20s and 30s.
2. Non hodgkin's lymphoma: It involves extra nodal tissue. It occurs mainly in the bowel (adjacent to appendix), and upper chest. It also occurs in liver, spleen, bone marrow, bones and central nervous system.
3. Brain tumors: They occur in children. Glioma and glioblastoma are some of the examples of brain tumors.
4. Wilm's tumor: It originates in the cells of kidney. It occurs in children and is very different from adult kidney cancers. Mostly it affects one kidney. Very rarely it affects both kidneys.
5. Neuroblastoma: It affects the nerve cells and most of them are located in adrenal glands.
6. Retinoblastoma: It is cancer of the eye.
7. Rhabdomyosarcoma or Rhabdosarcoma: It is located in the muscle tissue and is generally found in head and neck region, and the pelvis.

8. Osteogenic sarcoma: It involves ends of large bones of the leg (femur, tibia) and upper arm (humerus).
9. Ewing's sarcoma: It affects other than long bones of arm and leg such as ribs.

8.2 Interventions for the Treatment of Solid Tumors

Surgery, radiation therapy and chemotherapy are the three types of interventions used for treatment of solid tumors. Surgery combined with chemotherapy is the most commonly used intervention (Ramzy et al. 2017). However, treatment of solid tumor is associated with many limitations (Byrne et al. 2008):

(i) More than 40% of molecules developed as anticancer drugs are poorly soluble in water.
(ii) Most of the formulations of anticancer drugs show poor and inconsistent bioavailability.
(iii) They lack target specific (cancer tissue) selectivity and are equally toxic to normal tissues. The toxicity is serious and sometimes life threatening.
(iv) Measures taken to reduce the toxicity like reducing dose and reducing frequency may result in reduced killing of cancer cells.
(v) Some drugs reach at target site but do not get accumulated at the tumor site which results in decreased efficacy.
(vi) Malignant cells have a tendency to mutate so that there is resistance to anticancer drugs.
(vii) Malignant cells also have a tendency to cause efflux of drugs resulting in their resistance.
(viii) Many solid tumors are poorly vascularised with variable rates of blood flow because of which there is poor penetration of anticancer drugs.
(ix) Tumor may be located in areas with biological barrier such as brain or areas with poor blood supply.

8.3 Ideal Anticancer Drug Formulation

An ideal anticancer drug should have following characteristics:

(i) It should selectively target cancer tissues.
(ii) It should get accumulated at target tissues.
(iii) It should overcome biological barriers.
(iv) It should respond to the tumor environment by releasing the drugs.
(v) It should not show resistance.
(vi) It should have minimum toxicity.

8.4 Selective Targeting of Cancer Tissues

There are two ways by which cancer cells can be targeted. First one is passive targeting and the other one is active targeting (Hirsjarvi et al. 2011; Padhi and Behera 2020). Passive targeting can be done by either enhanced permeation and retention or electrostatic interaction. Active targeting involves targeting of tumor protein antigen or over expressed receptors by monoclonal antibodies or ligands. The anticancer drugs are coupled with antibodies or other ligands that recognize tumor associated antigens. So, active targeting (also known as smart targeting) increases the specificity (increases the exposure of drugs to malignant cells than normal cells) and reduces the side effects. There is another type of active targeting called lectin-carbohydrate interaction which targets whole organs and hence, is toxic to normal cells (Kutova et al. 2019; Yoo et al. 2019).

Enhanced Permeation and Retention Tumor blood vessels are leaky in nature. There is also poor lymphatic drainage. This results in enhanced permeation and retention effect (Khuroo et al. 2014; Behera and Padhi 2020). Small molecule drugs enter and exit tumors and normal tissues without being accumulated. Nano-sized drug delivery systems cause drug accumulation in tumors. Polymer protein conjugates of 80 kilo dalton molecular weight accumulate preferentially in tumor tissues. Intratumoral concentration of drug is increased by almost 70-fold due to enhanced permeation and retention effect (Duncan et al. 2013).

Electrostatic Interaction Tumor endothelial cells are negatively charged. So, the electrostatic interaction between the nanoparticles and tumor endothelial cell facilitates passive targeting. If the nanoparticles are positively charged, then they rapidly bind to tumor endothelial cells. But this may reduce the drug accumulation in the tumor. If the nanoparticles are negatively charged then they are taken up rapidly by the reticuloendothelial system in the liver and spleen. So, the nanoparticles should be either neutral or slightly negatively charged (Ramzy et al. 2017).

The limitations of passive targeting are:

(i) There may be release of the drug before tumor deposition which can cause inadequate efficacy.
(ii) The enhanced permeation and retention effect is incapable to ensure cellular internalization of nanoparticles.

8.5 Active Targeting or Smart Targeting

The basic principle underlying active targeting is that the delivery of anticancer drugs to cancer cells or cancer associated tissues such as tumor vasculature can be increased by associating the drugs with antibodies and small molecules such as folate and transferrin, which bind to antigens or receptors that are uniquely expressed

Table 8.1 Types of ligands specific to overexpressed receptors in solid tumors and their examples

Ligand type	Examples
Vitamins	Folic acid, biotin
Sugar residues	Hyaluronic acid
Amides	Anisamide
Peptide	Arginyl glycyl aspartic acid, endothelin, octreotide
Proteins	Transferrin, cytokine, lectin
Growth factors	Epidermal growth factor, vascular endothelial growth factor, fibroblast growth factor
Monoclonal antibodies	Anti-human epidermal growth factor receptor-2 monoclonal antibody (Trastuzumab)

or overexpressed on the cancer cells thereby reducing their toxicity on normal cells (Byrne et al. 2008). These antibodies or small molecules are also known as ligands. A list of ligands specific to over expressed receptors is given in Table 8.1. The drug carriers like liposomes, niosomes, micelles, polymerosomes and nanoemulsions can also be used for effective targeting. These drug carriers can also act as micro-reservoir systems (Behera et al. 2020a; Yao et al. 2016; Fonseca et al. 2014).

The drug is first loaded in drug delivery systems or drug carriers or smart vehicles after which they are conjugated with ligands specific to the over expressed receptors on the solid tumors. When administered, the ligands on the surface of smart vehicles attach to specific receptors in target tumor tissues. This results in internalization of the receptor complex to release the drug. In this way by smart targeting (Fig. 8.2) we can selectively target tumor tissues and decrease toxicity on healthy normal cells and organs thereby overcoming the major limitation of the traditional chemotherapy (Padhi et al. 2018; Xu et al. 2013).

8.6 Over-Expressed Receptors and Their Ligands in Solid Tumors

Over expressed receptors or specifically expressed receptors in solid tumors widely used for selective drug delivery through conjugated ligands are listed in Table 8.2. They are:

8.6.1 Epidermal Growth Factor Receptor

These receptors are also known as human epidermal growth factor receptor. They are over expressed in cancers of the lungs, breast, ovary and urinary bladder. The epidermal growth factor receptor family consists of four members. They are human epidermal growth factor receptor-1, human epidermal growth factor receptor-2,

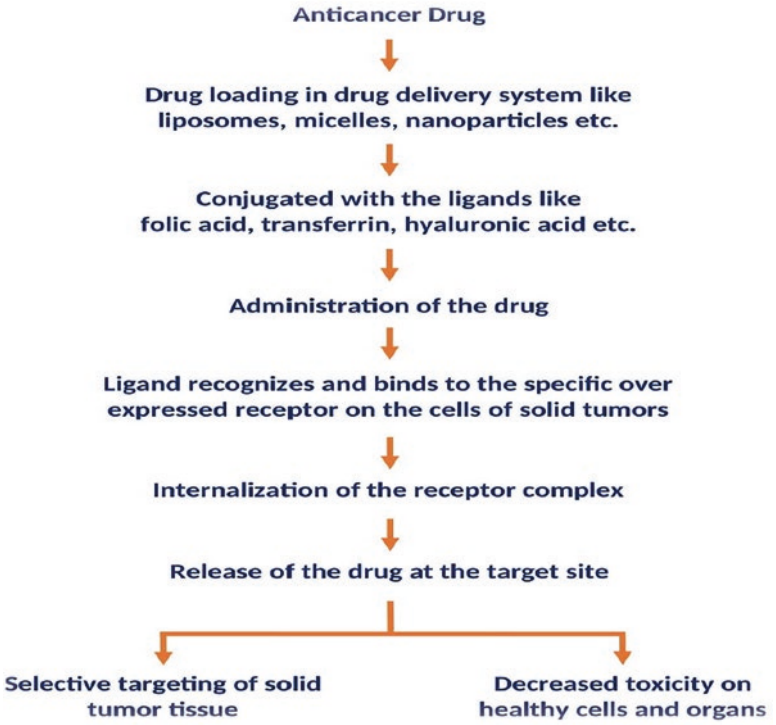

Fig. 8.2 Active targeting or smart targeting of anticancer drugs. The drug is first loaded in drug delivery systems after which they are conjugated with ligands specific to the over expressed receptors on the solid tumors. When administered, the ligands on the surface of drug delivery systems attach to specific receptors in target tumor tissues. This results in internalization of the receptor complex to release the drug

human epidermal growth factor receptor-3, human epidermal growth factor receptor-4. These receptors are tyrosine kinase linked receptors. Epidermal growth factor, transforming growth factor- α and histidine rich glycoproteins act as ligands on epidermal growth factor receptor. Monoclonal antibodies like anti- human epidermal growth factor receptor-2 monoclonal antibody can also act as ligand (Zhai et al. 2015).

8.6.2 Folate Receptors

Folate receptors are over expressed in cancers of uterus, ovary, brain, testis, lungs and pituitary cancers. There are 3 types of folate receptors: folate receptor-α, folate receptor-β, and folate receptor-γ. Folate receptor-α is mainly expressed at high levels in cancer cells. These receptors are cell surface glycosyl phosphatidyl inositol

Table 8.2 Over-expressed receptors in different solid tumors and specific ligands

Over expressed receptor	Type of solid tumor	Ligand
Epidermal growth factor receptor	Lungs, breast, ovary and urinary bladder	Epidermal growth factor, transforming growth factor-α, histidine rich glycoproteins
Fibroblast growth factor receptor	Breast, prostate and gastric cancer	Fibroblast growth factor
Folate receptor	Uterus, ovary, brain, testis, lungs and pituitary cancers	Folic acid
Cluster of differentiation-44 (CD44)	Epithelial, ovarian, colon, stomach	Hyaluronic acid
Integrin receptor	Brain, breast, pancreas, prostate, ovary, cervix	Asvitronectin, fibronectin, tenascin, osteopontin, fibrinogen, fibrillin and thrombospondin
Bombesin receptor	Lung, prostate, breast, pancreas, head/neck, colon, uterus, ovary and kidney	Bombesin
Sigma receptor	Breast, pancreas, neuroblastoma, lung and urinary bladder	Anisamide
Tansferrin receptor	Breast, ovary and brain	Transferrin
Vascular endothelial growth factor receptor	Breast, colon, lung, renal, gastric and oropharyngeal cancers	Vascular endothelial growth factor
Somatostatin receptor	Lung, prostate, breast, colorectal, gastric and liver cancer	Octreotide
Endothelin receptor	Melanoma	Endothelin family of polypeptides

anchored glycoprotein. There is receptor mediated endocytosis to internalize bound folates and folate conjugated compounds (Zwicke et al. 2012).

8.6.3 Fibroblast Growth Factor Receptors

Fibroblast growth factor receptors are over expressed in cancers of breast, prostate, bladder, and gastric cancers. There are 4 types of fibroblast growth factor receptors. Fibroblast growth factor receptor-1, fibroblast growth factor receptor-2 and fibroblast growth factor receptor-4 are over expressed in breast and prostate cancer. Fibroblast growth factor receptor-2 is over expressed in gastric cancer. Fibroblast growth factor receptor-1 and fibroblast growth factor receptor-3 are over expressed in papillary thyroid carcinoma. These are also tyrosine kinase linked receptors encoded by four fibroblast growth factor receptor genes. The mammalian fibroblast growth factor family consists of 18 ligands which act on the 4 types of fibroblast growth factor receptors (Akhtar et al. 2014).

8.6.4 Guanine Nucleotide Binding Protein Coupled Receptors

Many tumor cells over express or specifically express guanine nucleotide binding protein coupled receptors like bombesin receptor, somatostatin receptor and endothelin receptor.

The bombesin receptor family consists of receptors like the gastrin releasing peptide receptor, the neuromedin B receptor and the bombesin like receptor-3. The gastrin releasing peptide receptor is over expressed in cancer of lung, prostate, breast, pancreas, head/neck, colon, uterus, ovary and kidney. Somatostatin receptors are over expressed in lung, prostate, breast, colorectal, gastric and liver cancer. Endothelin receptor is of 2 types: endothelin receptor A and endothelin receptor B. The endothelin family of polypeptides (endothelin-1, endothelin-2 and endothelin-3) act as ligands on these receptors. Endothelin receptor B is over expressed in human melanoma (Sriram et al. 2019; Akhtar et al. 2014).

8.6.5 Integrin Receptor

Integrins are the transmembrane receptors which play an important role in the cell migration and invasion. Integrin $\alpha_v\beta_3$ receptors are upregulated in a wide range of cancers. Integrin α3 receptors are overexpressed in ovarian cancer, breast cancer and melanoma. Many ligands such as vitronectin, fibronectin, tenascin, osteopontin, fibrinogen, fibrillin and thrombospondin act as ligand on integrin receptor (Wu et al. 2019).

8.6.6 Sigma Receptor

Sigma receptor is a subtype of opioid receptors. Sigma-2 receptor is over expressed in a variety of human cancers like breast, pancreas, neuroblastoma, lung and urinary bladder. Anisamide acts as ligand on the sigma receptors (Collina et al. 2017; Ramzy et al. 2017).

8.6.7 Transferrin Receptor

There are two types of transferrin receptors: transferrin receptor-1 and transferrin receptor-2. Cancers of breast, ovary and brain overexpress transferrin receptor-1. Transferrin receptors are highly expressed in blood vessels (endothelial cells) forming blood brain barrier. Transferrin is a single chain glycoprotein acting as ligand on transferrin receptors (Daniels et al. 2012).

8.6.8 Vascular Endothelial Growth Factor Receptor

Vascular endothelial growth factor receptors are of 2 types: Vascular endothelial growth factor receptor-1 and vascular endothelial growth factor receptor-2. They are over expressed in breast, colon, lung, renal, gastric and oropharyngeal cancers. Vascular endothelial growth factor receptor-2 is up regulated in tumor endothelium (Liang et al. 2015).

8.6.9 Other Receptors

Other receptors which are over expressed in different type of cancers are follicle stimulating hormone receptor, biotin receptor, C-type lectin receptor and asialoglycoprotein receptor (Ramzy et al. 2017).

8.7 Factors Affecting the Selection of Targeting Ligand

In the active targeting or smart targeting approach, the selection of ligand is very crucial as the efficacy of the therapy depends on the ligand receptor binding on the tumor cells. The selection of the targeting ligand depends on the following factors (Allen 2002):

(a) *Receptor expression*: The receptors should be over expressed in cancer cells than normal cells. The receptor density should be high in cancer cells. A receptor density of 10^5 receptors per cell is required for efficacy of anti-human epidermal growth factor receptor-2 targeted liposomal doxorubicin.
(b) *Internalization of receptor*: For certain drug delivery system such as immunoliposomes, internalization of the drug receptor complex is necessary which occurs when the ligand binds to their receptors. For certain other drugs such as radiolabelled antibodies, internalization does not have any significant advantage. In case of antibody directed enzyme prodrug therapy, internalization is not required as the enzyme present at the cell surface must convert the inactive prodrug into active cytotoxic drug.
(c) *Type of ligand*: The ligand can be monoclonal antibodies or antibody fragments or non-antibody ligands. Monoclonal antibody or antibody fragments have the advantage of high specificity for the target tissue with wide range of binding affinities. However, they are expensive and time consuming to produce. There may be problems with their stability and storage. There may also be development of immunogenicity. Non antibody ligands are inexpensive, readily available and easy to handle. But their limitation is their lack of selectivity. They can bind to some non-target tissues. Some of the ligand like folate are found in diet and can compete for binding with targeted therapy.

(d) *Binding affinity and ligand density*: High binding affinity of the ligand for the receptor decreases penetration of solid tumors. For example, monoclonal antibodies have strong binding affinity but fail to penetrate or diffuse further into the tumor. Similarly, high ligand density may increase the binding to target cells. This can be achieved through liposomes or polymers. But this approach is expensive and difficult to achieve.
(e) *Solid tumor vasculature*: Several molecular targets are identified on the solid tumor vasculature which can be targeted to stop oxygen and nutrient supply to the tumor cells. Again, tumor blood vessels due to lack of tight endothelial cell junction are leaky and allow permeation or extravasation of the drugs from blood vessels to tumor interstitial space thereby increasing cytotoxicity.
(f) *Particle size*: Smaller particles have higher penetration into solid tumor tissue and slower clearance rates. Large particles such as immunoliposomes (100–150 nm size) can take more than 48 hours to reach peak levels in the tumor.
(g) *Drug ligand ratio*: Lower potency of the drug, higher the drug to ligand ratio is needed and vice versa. If the drug has low potency, then higher amount of ligand is required. This could saturate the binding sites thereby increasing the chance of immunological reactions (in case of antibodies) and become expensive.
(h) *Drug carrier*: Drug carriers such as liposomes can provide thousands of drug molecules per target. So, there will be no problem for drugs with low potency. However, drug binding, drug release and drug retention might affect the choice of a drug carrier.

8.8 Drug Delivery Systems, Drug Carriers and Smart Vehicles

There are 2 types of drug delivery systems i.e. targeted and non-targeted. Targeted drug delivery systems facilitate the active targeting of drugs to cancer cells. They have been developed to improve cancer therapy. These drug delivery systems or drug carriers are designed at nanoscale (10–1000 nm) (Chamundeeswari et al. 2019; Shreya et al. 2019) because

(i) Nanoparticles promote the solubility of drugs
(ii) Nanoparticles prolong circulation time
(iii) Nanoparticles achieve controlled release.
(iv) Nanoparticles reduce frequency of administration.
(v) Nanoparticles can be targeted to specific organs like brain, liver and lymph nodes

However, the efficacy of nanoparticles depends on factors like size of nanoparticles, stability of nanoparticles, ligand conjugation capacity, drug loading efficiency and retention time. The effective diameter should be 10–100 nm size. Particles with size less than 10 nm are readily excreted through urine. Particles with size more than

100 nm are susceptible to phagocytosis by the reticuloendothelial system (Sneider et al. 2017; Verma et al. 2017; Fonseca et al. 2014).

Drug delivery systems or drug carriers or smart vehicles (Fig. 8.3) for certain anticancer drugs effectively designed for active targeting through ligand and over-expressed receptor binding is given in Table 8.3. Many anticancer drugs like doxorubicin (Zhao et al. 2018), paclitaxel (Ma and Mumper 2013), docetaxel (Jose et al. 2019), vincristine (Wang et al. 2014), camptothecin drugs like topotecan (Behera and Padhi 2020; Wen et al. 2017; Padhi et al. 2015), methotrexate (Taheri et al. 2011), carboplatin (Sankar et al. 2012), 5-fluorouracil (Mansoori et al. 2020), gemcitabine (Van Waarde et al. 2015; Aggarwal et al. 2011; Saini and Bandyopadhyaya 2019), and bortezomib (Frasco et al. 2015) have been successfully designed for smart targeting (Juthi et al. 2020). The different types of drug carriers are classified as follows:

(i) **Lipid based nanoparticles**:

Liposomes (100–200 nm) and micelles (10–100 nm) are the lipid based nanoparticles (Padhi et al. 2020). They are the most commonly used nanoparticles. Liposomes are the vesicular structures composed of lipid bilayers whereas micelles are lipid monolayers. Liposomes enclose an aqueous core whereas micelles do not have internal aqueous compartment. Liposomes are made of polyethylene glycol.

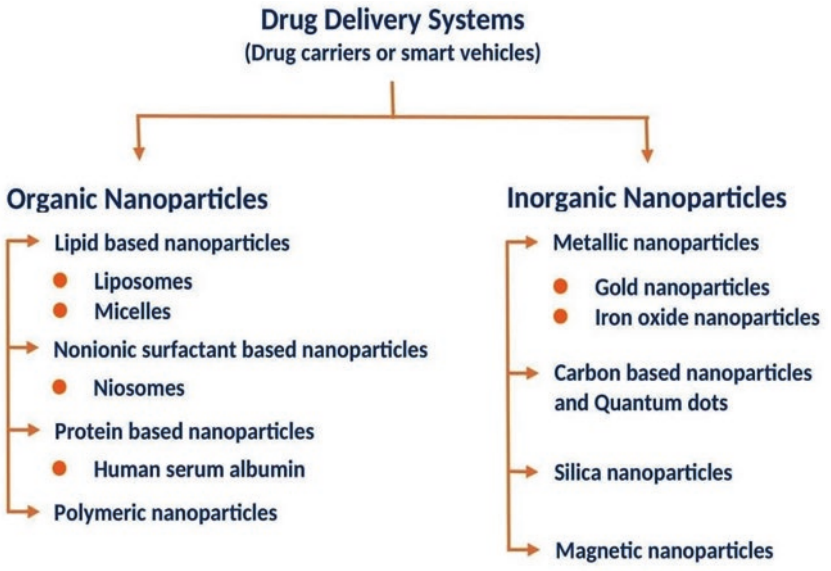

Fig. 8.3 Types of drug delivery systems. Drug delivery systems (or drug carriers or smart vehicles) can be either organic or inorganic nanoparticles. Organic nanoparticles may be lipid based (liposomes, micelles), nonionic surfactant based (niosomes), protein based (human serum albumin) or polymer based. The inorganic nanoparticles may be metal based, carbon based, silica based or magnetic

Table 8.3 Drug delivery systems or drug carriers or smart vehicles for some anticancer drugs successfully designed for active targeting through ligand-over expressed receptor binding

Drug	Smart vehicles	Over expressed receptor	ligand
Paclitaxel	Liposome	Bombesin receptor	Biotin
	Liposome	FGF receptor	Truncated bFGF
	Liposome	VEGF receptor	Anti-VEGF 165 monoclonal antibody
	Micelle	Integrin α3	Peptide
	Micelle	Folate receptor	Folic acid
	Micelle	CD44	Hyaluronic acid
	Chitosan nanoparticles	Folate receptor	Folic acid
	PBCA nanoparticles	CD44	Hyaluronic acid
	Magnetic nanoparticles	C-type lectin receptor	Lectin
	Gold nanoparticles	Bombesin receptor	Biotin
	Gold nanoparticles	Folate receptor	Folic acid
Docetaxel	Micelle	Transferrin receptor	Transferrin
	PLGA nanoparticles	Transferrin receptor	Transferrin
Doxorubicin	Liposome	FGF receptor 2	Truncated bFGF
	Liposome	EGF receptor	Heptapeptide
	Liposome	Integrin $\alpha_v\beta_3$	Arginyl glycyl aspartic acid
	Liposome	Folate receptor	Folic acid
	Liposome	Transferrin receptor	Transferrin
	Liposome	Somatostatin receptor	Octreotide
	Liposome	Bombesin receptor	Bombesin
	Liposome	VEGF receptor	Anti-VEGF monoclonal antibody
	Liposome	Sigma receptor	Anisamide
	Niosome	Transferrin receptor	Transferrin
	Micelle	Somatostatin receptor	Octreotide
	Micelle	Folate receptor	Folic acid
	Magnetic polymerosomes	Folate receptor	Folic acid
	PEGylated nanoparticles	CD44	Hyaluronic acid
Mitomycin C	PEGylated liposome	Folate receptor	Folic acid

(continued)

Table 8.3 (continued)

Drug	Smart vehicles	Over expressed receptor	ligand
Methotrexate	Human serum albumin nanoparticles	EGF receptor-2/ HER2	Trastuzumab (monoclonal antibody)
Bortezomib	PLGA nanoparticles	Transferrin receptor	Transferrin
Vincristine	Liposome	Folate receptor	Folic acid
Campothecin drugs like Topotecan	Mesoporous silica nanoparticles	CD44	Hyaluronic acid
	PEGylated nanoparticles	Folate receptor	Folic acid
Carboplatin	Liposome	Folate receptor	Folic acid
5-Fluoro Uracil	PEGylated nanoparticles	CD44	Hyaluronic acid
	PEGylated liposome	Folate receptor	Folic acid
Gemcitabine	PEG-Chitosan nanoparticles	Sigma receptor	Anisamide
	PLGA nanoparticle	EGF receptor	Monoclonal antibody
	Mesoporous silica nanoparticles	Transferrin receptor	Transferrin

FGF Fibroblast growth factor, *VEGF* Vascular endothelial growth factor, *CD* cluster of differentiation, *PBCA* Poly-butyl cyano acrylate, *PLGA* Poly (lactic-co-glycolic acid), *EGF* Epidermal growth factor, *PEG* Polyethylene glycol, *HER* Human epidermal growth factor receptor

Micelles (10–100 nm) are made of polyethylene glycol with another block of polylactic acid or polytrimethylene carbonate or cholic acid (Vanti 2021; Wang and Lu 2016; Miller 2013).

(ii) **Nonionic surfactant-based nanoparticles**:

Like liposomes, niosomes are also the bilayered vesicular structures composed of nonionic surfactants with or without incorporation of cholesterol. Compared to liposomes they are inexpensive and less toxic (Tavano et al. 2013).

(iii) **Protein based nanoparticles**:

Proteins like albumin, gelatin, gliadine, legumin, and ferritin are an attractive alternative to synthetic polymers to be used in nanoparticle formulation because of their safety. Their biocompatibility and biodegradability are their strengths. Human serum albumin nanoparticles are most commonly used among them (Hong et al. 2020).

(iv) **Polymeric nanoparticles**:

Poly (lactic-co-glycolic acid), poly (ethylene glycol), poly-butyl cyano acrylate, and chitosan are some of the polymers used to coat the surface of nanoparticles. Polymeric nanoparticles (10 nm) are single and linear. They may be homopolymers consisting of same monomer or heteropolymers consisting of different monomers.

Dendrimers (2–10 nm) are composed of many macromolecules. They are hyperbranched and look like a tree (Panigrahi et al. 2021; Bolhassani et al. 2014; Abbasi et al. 2014).

(v) **Metal based nanoparticles**:

Gold, iron, copper, zinc, silver, and platinum are the metals used in preparation of nanoparticles. Gold nanoparticles and iron oxide nanoparticles are the commonly used metal-based nanoparticles (Behera et al. 2020b; Sweet et al. 2012).

(vi) **Carbon based nanoparticles and quantum dots**:

Carbon based nanoparticles include carbon nanotubes, fullerenes, graphene oxide, carbon dots or graphene quantum dots. Quantum dots are the nanoparticles with size below 10 nm. They have considerable potential to be used as biomarkers for biomedical and pharmaceutical applications in nanomedicine (Maiti et al. 2019; Chow et al. 2011; Patnaik et al. 2021).

(vii) **Silica nanoparticles**:

These are made up of mesoporous silica. Mobile composition of matter number 41 silica has a porous structure. It is capable of encapsulating bioactive molecules (Yanes and Tamanoi 2012).

(viii) **Magnetic nanoparticles**:

Magnetic nanoparticles can be used to selectively target a particular tissue using an external magnetic field (Gul et al. 2019).

8.9 Conclusion

Due to lack of selectivity, anticancer drugs are highly toxic to normal tissues. Smart targeting the cancer tissues can minimize the toxicity. Among the various approaches, conjugating the anticancer drug with a ligand which has affinity for the over expressed receptors on the cells of solid tumors has gained prime importance in current anticancer research. This approach not only targets the cancer tissues selectively but also reduces the toxicity. Many anticancer drugs like doxorubicin, paclitaxel, docetaxel, vincristine, campothecin, methotrexate, cisplatin, carboplatin, 5-fluorouracil, gemcitabine, and bortezomib have been successfully designed for smart targeting. The over expressed receptors like folate, transferrin, sigma, bombesin, somatostatin and growth factors on the cells of solid tumors have been exploited. Nanoparticle smart vehicles like liposomes, niosomes, micelles, and polymerosomes have been effectively used as drug carriers to facilitate smart targeting. Identifying more uniquely expressed or over expressed receptors in the cancer cells would enable researchers to develop targeted drug loaded nanoparticles for selective targeting of cancer tissues.

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Chapter 9
Ligand Targeted Polymeric Nanoparticles for Cancer Chemotherapy

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Abbreviations

EDC 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
HER-2 human epidermal growth factor receptor-2

9.1 Introduction

Cancer remains to be the second deadliest disease in humans, characterized by unregulated cell growth and proliferation (Prabhu et al. 2015). In the last few decades, several advancements have been made in our basic concept of cancer and its biology, leading to improved diagnostic and treatment methods. Numerous therapeutic approaches are widely used for cancer treatment which involves a combination of surgery, radiation, hormone therapy, immune therapy, and chemotherapy (Masood 2016), among which chemotherapy is the prime approach for effective treatment. Traditional chemotherapy lacks specificity in the targeting of drugs to tumor cells, thereby affecting normal healthy cells by exhibiting several extreme side effects (Prabhu et al. 2015). Moreover, Rapid removal and non-specific distribution demand administration of a huge dose of the drug, which is costlier and also lead to unwanted toxicity. On the contrary, targeted cancer therapy is relatively more effective and imparts lesser side effects (Large et al. 2019).

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The application of nanotechnology in the field of drug delivery and diagnostics has grabbed significant attention due to its improved drug pharmacokinetics (Chamundeeswari et al. 2019). Nanotechnology endows chemotherapeutics with improved solubility, prolonged plasma half-life, and improved biodistribution profile (Kumari et al. 2015). Further, the small size of the nanomedicines facilitates them to penetrate various corners of tumor tissues by evading circulation. The frequently used nanoformulations for chemotherapeutics delivery include dendrimers liposomes, polymeric nanoparticles, inorganic and magnetic nanoparticles (Kumari et al. 2015).

Consequently, the ability of the nanoformulations to exploit the enhanced permeability and retention effect for targeting tumor tissues has made them a potential candidate for cancer treatment (Behera and Padhi 2020; Nehoff et al. 2014). Due to unregulated angiogenesis and/or over-expression of vascular permeability factors, the tumor microenvironment tends to show leaky vasculature with enhanced permeability. Moreover, the activation of proangiogenic and antiangiogenic molecules results in a porous endothelial layer in cancer vasculature with fenestration varying from 300 to 4700 nm in size. Additionally, due to the rapid proliferation of cells, the cancer microenvironment exhibits dysfunctional lymphangiogenesis and compressed lymphatic vessels, thereby leading to impaired lymphatic drainage. All this results in prolonged retention of the interstitial fluid containing the nanocarriers in tumor tissue when compared to normal tissue with functional vasculature and lymphatics. This phenomenon of gradual accumulation of nanomedicines in the tumor is termed as enhanced permeability and retention effect (Nehoff et al. 2014).

The delivery of drug encapsulated nanoparticles *via* systemic circulation takes place by two approaches i.e. passive targeting and active targeting. In passive targeting, nanoparticles utilize the advantage of leaky vasculature and enhanced permeability and retention effect, thereby resulting in enhanced accumulation of nanoformulations compared to the free drug in the tumor microhabitat. In the case of active targeting, nanoparticles avail additional advantage of site-specific targeted delivery due to the presence of specific homing moieties such as antibodies or ligands (Fig. 9.1) (Gagliardi et al. 2021; Kumari et al. 2015).

A wide array of materials is used for the preparation of drug encapsulated nanoparticles which includes polymers, ceramics, lipids, metal oxide etc. (Ulbrich et al. 2016; Sur et al. 2019). Polymeric nanomaterials possess several advantages over other materials, such as improved stability, biodegradability, lesser cytotoxicity, non-immunogenic, etc.. Additionally, the presence of multiple functional groups on these polymers facilitates their easy modification for ligation of drug molecules or targeting ligands (Werengowska-Ciećwierz et al. 2015). Hence, polymeric nanoparticles can be used as a potential candidate for the delivery of chemotherapeutics into the cancer microenvironment with enhanced efficacy and reduced cytotoxicity (Chan et al. 2010). In this chapter, we emphasize the application of various ligand conjugated polymeric nanoparticles for the delivery of cancer chemotherapeutics. Subsequently, this chapter aims to offer an overall outlook for developing

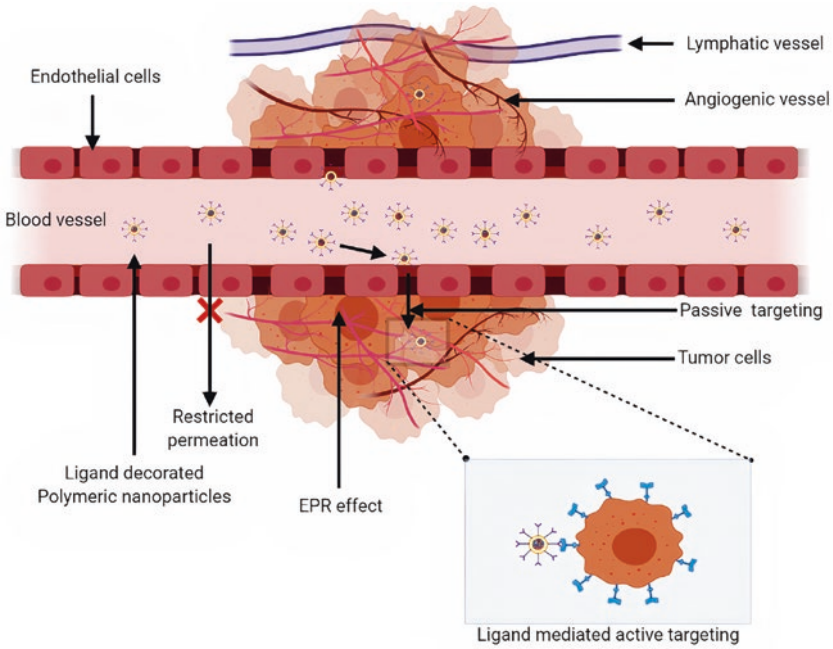

Fig. 9.1 Polymeric nanoparticles-mediated drug targeting approaches to tumor tissue. Passive targeting of nanoparticles occurs through leaky vasculature of tumor microenvironment and enhanced permeability and retention effect. Active targeting of tumor cells is facilitated by various ligand conjugated nanoformulation. Reprinted from "Biodegradable polymeric nanoparticles for drug delivery to solid tumors", Gagliardi et al. (2021)

effective target-specific polymeric drug delivery systems for future clinical applications.

9.2 Mechanism of Ligand Decorated Nanoparticles Uptake by the Cell

The active nanocarrier internalization by targeted cells occurs through a process of endocytosis which can be mediated through the mechanisms, namely micropinocytosis, clathrin-mediated endocytosis, caveolae-mediated endocytosis, and/or clathrin- and caveolae-independent endocytosis (Foroozandeh and Aziz 2018). Clathrin-dependent endocytosis (also known as receptor-mediated endocytosis) occurs in all mammalian cells, and it plays a key role in collecting essential nutrients for the cell, such as cholesterol through low-density lipoprotein receptors, iron through transferrin receptors. Once the ligand binds to the receptor, the ligand-receptor complex binds to cytoplasmic adaptor protein to form a clathrin lattice. The

GTPase activity of dynamin detaches the vesicle from the cell membrane, leading to the formation of clathrin-coated vesicles. Upon internalization, the acidic pH leads to loss of clathrin from coated vesicle as well as a disassociation of receptor-ligand complex. Nanoparticles like poly(ethylene glycol)-polylactide, chitosan, surface modified nanoparticles (modified with low-density lipoprotein, transferrin, etc.) use clathrin-dependent endocytosis process for the cellular entry (Fig. 9.2) (Bahrami et al. 2017).

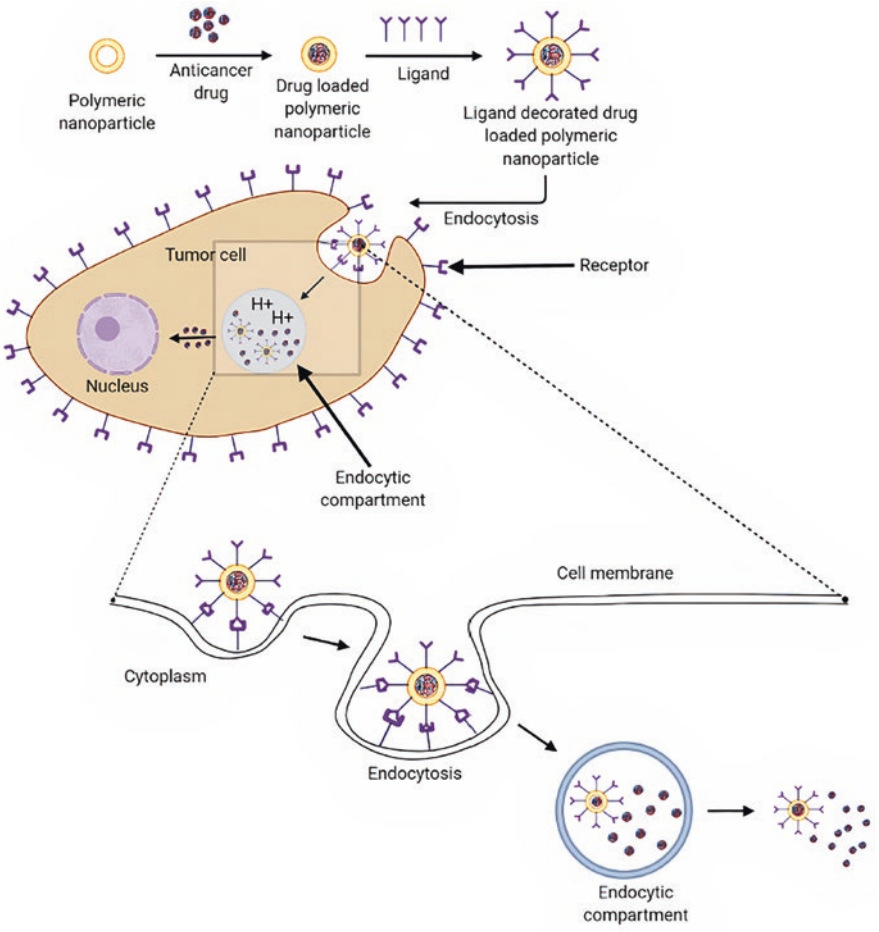

Fig. 9.2 Mechanism of ligand-conjugated nanocarrier internalization by targeted cells *via* endocytosis. The ligands decorated on the nanocarriers bind to its specific receptors overexpressed on cancer cells. This is followed by internalization of the nanocarriers attached to ligand-receptor complex into cytoplasm. Upon internalization, the acidic pH leads to dissociation of receptor-ligand complex. Reprinted from "Nanoparticles and targeted drug delivery in cancer therapy", Bahrami et al. (2017)

Caveolar endocytosis is characterized by caveolae, which are bulb-shaped plasma membrane invaginations. It is one of the prime mechanisms for the transcellular uptake of nanoparticles. Proteins that are known to utilize this mechanism include the folic acid receptor, platelet-derived growth factor receptor, epidermal growth factor receptor, etc. (Srinivasarao and Low 2017). Nanoparticles that utilize this mechanism for internalization generally escape degradation and show enhanced drug delivery to the nucleus or endoplasmic reticulum. It has been demonstrated that anionic nanoparticles generally undergo caveolae-dependent endocytosis (Perumal et al. 2008). Micropinocytosis is a growth factor-induced, actin-promoted endocytosis that encloses a large content of fluid-phase and is observed in almost all cells. Cationic nanoparticles like chitosan may use this pathway for internalization (Behzadi et al. 2017).

9.3 Ligand Directed Active Targeting for Cancer Treatment

Although the enhanced permeability and retention effect facilitates the entry of nanocarriers into the tumor microenvironment, it does not help in site-specific targeting. This drawback of passive targeting can be overcome by conjugating ligands of tumor-specific receptors with nanoparticles (Bahrami et al. 2017; Behera and Padhi 2020). This is termed active targeting, which has several advantages such as improved drug efficacy, lesser non-specific toxicity, enhanced therapeutic index etc. (Derakhshandeh and Azandaryani 2016). There is a wide range of targeting ligands that are used for surface modification of the nanocarriers, such as proteins, polysaccharides, vitamins, nucleic acid and glycoproteins (Tables 9.1, 9.2 and 9.3). Among various targeting moieties, transferrin, folate, epidermal growth factor are the most studied ligands for active targeting.

9.3.1 Small Molecule Directed Active Targeting Polymeric Nanoparticles

9.3.1.1 Folic Acid

Folic acid belongs to the family of vitamin B9. They are water-soluble, low-molecular-weight (441 Da) molecules (Chilom et al. 2018). It plays a crucial role in cancer cell proliferation as it is one of the key nutrients for the synthesis of pyrimidine and purines, which are required for DNA synthesis (Hagner and Joerger 2010). Apart from this, it also plays a role in DNA methylation, repair mechanisms, and cell division (Vivek et al. 2017). Live cells uptake folic acid *via* high-affinity folate receptors. Folate receptors are glycosyl phosphatidyl inositol-linked high-affinity membrane glycoproteins present on the cell surface (Song et al. 2013). They are highly expressed on the surface of various cancer cells. Though normal cells also

Table 9.1 Small molecule directed active targeting polymeric nanoparticles

Ligand name	Target receptor	Nanoparticle conjugate	Cargo	Targeted cancer cell lines	References
Folic acid	Folate receptor	Chitosan-coated poly(lactic-co-glycolic acid)	Carboplatin	HeLa cervical cancer cell line	Ji et al. (2015)
		Polycaprolactone-poly (ethylene glycol)-polycaprolactone	Doxorubicin and indocyanine green	Breast cancer	Hu et al. (2018)
		Bovine serum albumin	Chrysin (5, 7-dihydroxyflavone)	MCF-7 breast cancer cell line	Nosrati et al. (2018)
		Bovine serum albumin	Ginsenoside Rg5 (Rg5)	MCF-7 breast cancer cell line	Dong et al. (2019)
Biotin	Biotin specific receptors	Poly (ethylene glycol)-poly(lactic-co-glycolic acid)	15,16-Dihydrotanshinone I	HeLa cervical cancer cell line	Luo et al. (2018)
		Poly(lactic-co-glycolic acid)	Paclitaxel and tariquidar	Drug-resistant cancer cells	Patil et al. (2009)
		Biotin decorated Poly(lactic-co-glycolic acid)	7-ethyl-10-hydroxycamptothecin (SN-38)	4 T1 breast cancer cell line	Mehdizadeh et al. (2017)
		Poly (ethylene glycol)/ polycaprolactone blocked copolymer	Paclitaxel	Human uterine cervix adenocarcinoma (HeLa 229) cells	Kim et al. (2007)
Glycyrrhetinic acid	GA receptors	Bovine serum albumin	Curcumin	HepG2 liver cancer cell line	Li et al. (2015)
		Glycyrrhetinic acid-decorated alginate/ doxorubicin -modified alginate complex	Doxorubicin	Hepatoma carcinoma	Guo et al. (2013)
		Chitosan/poly (ethylene glycol)	Doxorubicin	Liver cancer	Tian et al. (2010)
		Hyaluronic acid conjugate	Paclitaxel	HepG2 and B16F10	Zhang et al. (2013)
		Alginate	Doxorubicin	Liver cancer	Zhang et al. (2012)

Table 9.2 Polysaccharide directed active targeting polymeric nanoparticles

Ligand name	Target receptor	Nanoparticle conjugate	Cargo	Targeted cancer cell lines	References
Hyaluronic acid	CD44, receptor for hyaluronan-mediated motility, and lymphatic vessel endothelial hyaluronan 1 receptors	Poly (lactic-co-glycolic acid)-hyaluronic acid/ camptothecin/curcumin nanoparticles	Camptothecin and curcumin	Colon-2 colon cancer cell lines	Xiao et al. (2015)
		Mesoporous silica nanoparticles	Paclitaxel	MCF-7 breast cancer cells	Li et al. (2017)
		Mesoporous silica nanoparticles	Doxorubicin	CD44-overexpressed cancer cells	Palanikumar et al. (2018)
		Methoxy poly(ethylene glycol)-poly(lactide) nanoparticles	Paclitaxel	CD44-overexpressed A549 epithelial cancer cells	Luo et al. (2020)
		Bovine serum albumin nanoparticles	Paclitaxel	SKOV3 and A2780 human ovarian cancer cell line	Edelman et al. (2017)
Galactosamine	Asialoglycoprotein receptor	Poly[N-(2-hydroxypropyl) methacrylamide]	Doxorubicin	Hepatocarcinoma	Julyan et al. (1999)
		Poly (ethylene glycol)-polycaprolactone copolymer	Doxorubicin	Liver cancer cells	Zhong et al. (2013)

Table 9.3 Protein molecule directed active targeting polymeric nanoparticles

ligand name	target receptor	nanoparticle conjugate	Cargo drug	targeted cancer cell lines	References
Transferrin	Transferrin receptor	Polyethylene glycol –dihydroartemisinin	Dihydroartemisinin	Lewis lung carcinoma (LLC) cells	Liu et al. (2016)
		Poloxamer 407 and 123	Doxorubicin	Doxorubicin-sensitive cells but also in the doxorubicin-resistant breast cancer cell lines	Soe et al. (2019)
		Poly(butylene adipate)/terephthalate	5-fluorouracil	HT29 colorectal cancer cell lines	Varshosaz et al. (2017)
		Poly(lactic-co-glycolic acid)	Docetaxel	MCF-7 breast cancer cell line	Jose et al. (2019)
		Chitosan-polyethylene glycol	Paclitaxel	HOP-62	Nag et al. (2016)
		Polyethylene glycol-polycaprolactone	Doxorubicin	Brain glioma	Pang et al. (2011)
Lactoferrin	Lactoferrin receptor	PEGylated-poly-lactide	Paclitaxel	Glioma cells (C6 and BCEC)	Miao et al. (2014)
		Poly(lactic-co-glycolic acid)	Tramadol	16HBE and SH-SY5Y cells	Lalani et al. (2012)
		Poly(lactic-co-glycolic acid)	Etoposide	Glioblastoma	Kuo and Chen (2015)
		PEGylated-poly(lactic-co-glycolic acid)	Shikonin	C6 glioma cells	Li et al. (2018)
		Chitosan	Curcuminoid	Brain glioma	Xu et al. (2017)
Epidermal growth factor	Epidermal growth factor receptor	2-methacryloyloxyethyl phosphorylcholine	Paclitaxel	A431 and H69 cell line	Shimada et al. (2009)
		Poly (amido amine) dendrimers conjugated with quantum dots	Paclitaxel	General cancer	Yuan et al. (2010)
		Poly (amido amine)/DNA	–	HepG2 cells	Li et al. (2016)

Lectin	Lectin receptor	C11Pc-polyethylene glycol-gold nanoparticles/jacalin/monoclonal antibodies	–	HT-29 and the SK-BR-3	Obaid et al. (2015)
		Biotinylated-lectin tagged fluorescent polymeric	–	MCF 7, Hela, and H929	Cho et al. (2014)
		Bauhinia purpurea agglutinin-modified PEGylated liposomes	Doxorubicin	DU145	Ikemoto et al. (2016)
Monoclonal antibodies	Epidermal growth factor receptor	Poly(lactic-co-glycolic acid)	Docetaxel	Lung cancer cells	Patel et al. (2018)
	Human epidermal growth factor receptor-2	Poly(2-methyl-2-carboxytrimethylene carbonate-**co**-d,l-lactide)-graftpoly(ethylene glycol)-furan	Doxorubicin	SKBR-3 breast cancer	Shi et al. (2009)
	Epidermal growth factor receptor	Poly(lactic-co-glycolic acid)	Temozolomide	U-87MG, SK-Mel 28, and SW480	Duwa et al. (2020)

express folate receptors, their expression is majorly limited to the apical surface of epithelial cells, which is inaccessible to circulating drugs. Also, their expression on cancer cells is 500 folds higher than normal cells. The receptors bind to folic acid and facilitate its internalization *via* receptor-mediated endocytosis (Jones et al. 2017). Consequently, due to such preferential expression of folate receptors, folic acid conjugated nanocarriers provide a unique approach for the targeted delivery of drugs to cancer cells.

Ji et al. formulated folic acid grafted chitosan-coated poly (lactic-co-glycolic acid) nanoparticles for the site-specific delivery of carboplatin to the cervical cancer cell lines (HeLa). The reported data showed that the IC_{50} value of the folic acid conjugated nanoparticles was 0.65 μg/ml while the IC_{50} value of chitosan-coated poly (lactic-co-glycolic acid) nanoparticles, poly (lactic-co-glycolic acid) nanoparticles, and the free drug was 1.08, 1.56, and 2.35 μg/ml, respectively. Thus this study implied that folate-targeted nanoparticles showed relatively higher cytotoxicity when compared to unconjugated nanoparticles and free drugs (Ji et al. 2015). In 2018, Hu et al. designed and developed redox-sensitive folic acid-doped polycaprolactone-poly (ethylene glycol)-polycaprolactone nanoparticles for combined delivery of doxorubicin and indocyanine green against breast cancer. When compared to free drugs, their constructed nanoparticles imparted enhanced tumor targeting abilities and improved accumulation in tumor tissues due to cumulative action of enhanced permeability and retention effect and folate-mediated targeting (Hu et al. 2018). Nosrati et al. synthesized folic acid decorated bovine serum albumin nanoparticles (97.5 ± 5.8 nm) for effective delivery of chrysin (5, 7-dihydroxyflavone) in breast cancer cell lines (MCF-7). They showed that folic acid-modified formulation was potentially more superior in suppressing cancer cell growth as compared to nontargeted nanoparticles (Nosrati et al. 2018).

9.3.1.2 Biotin

Biotin (vitamin H or vitamin B7), which belongs to the vitamin family, is known to play a pivotal role in cell growth and metabolism. Cancer cells require an increased amount of biotin for sustained growth and rapid proliferation (Li et al. 2013). To meet this demand, cancer cells overexpress biotin-specific receptors onto their surfaces as compared to healthy cells. Therefore, this phenomenon can be explored as a promising strategy for effective and specific tumor cell targeting. Accordingly, many research endeavors have proved the enhanced drug uptake by the cell treated with biotin decorated drug carriers as compared to non-conjugated same drug carriers. Thus, biotinylated nanoparticles have attracted particular interest as a novel drug delivery system for specific cancer cell targeting (Mehdizadeh et al. 2017). Notably, Luo et al. formulated biotin-conjugated polyethylene glycol-poly(lactic-co-glycolic acid) nanoparticles which served as an effective targeted drug delivery system for the delivery of 15,16-dihydrotanshinone I in the HeLa cell lines. Their formulated nanoparticles showed superior antiproliferative activity when compared to free 15,16-dihydrotanshinone I (Luo et al. 2018).

In another study, Patil et al. demonstrated the co-delivery of paclitaxel and tariquidar by biotin functionalized poly (lactic-co-glycolic acid) nanoparticles (222–240 nm) that resulted in increased cellular accumulation of the therapeutics and enhanced cytotoxicity in drug-resistant cancer cells (Patil et al. 2009). In 2017, Mehdizadeh and co-workers synthesized biotin decorated poly(lactic-co-glycolic acid) nanoparticles containing SN-38 for target-specific drug delivery in the 4 T1 breast cancer cell line. They showed that biotin decorated nanoparticles exhibit higher targeted cell toxicity (IC_{50} value of 0.32 μM) as compared to non-conjugated nanoparticles (IC_{50} value of 0.61 μM) (Mehdizadeh et al. 2017).

9.3.1.3 Glycyrrhetinic Acid

Glycyrrhetinic acid is the key bioactive metabolite extracted from licorice which is commonly used as a medicine for the treatment of hepatitis and hepatotoxicity (Zhong et al. 2014). In the early 90s, it was found that there were specific receptors for glycyrrhetinic acid on the cellular membrane of rat hepatocytes (Mishra et al. 2013). Because of their high binding affinity to hepatocytes, glycyrrhetinic acid has been used as a specific ligand for hepatoma-targeting drug delivery. Accordingly, Li et al. formulated glycyrrhetinic acid grafted bovine serum albumin nanoparticles for effective delivery of curcumin to the liver cancer cell line (HepG2), which exhibited a two-fold higher rate of apoptosis than non-glycyrrhetinic acid grafted nanoparticles (Li et al. 2015). Similarly, Guo and co-workers demonstrated inhibition of the hepatoma carcinoma cell growth by glycyrrhetinic acid-decorated alginate/doxorubicin-modified alginate complex nanoparticles. The rate of inhibition of tumor cell proliferation was 79.3% post glycyrrhetinic acid- alginate/doxorubicin-alginate nanoparticles treatment which was significantly greater than that of doxorubicin-alginate nanoparticles (62.7%) and free drug (48.5%). Further, no mice were sacrificed in the glycyrrhetinic acid-alginate/doxorubicin-alginate nanoparticles group, whereas the mortality rate was 40% in the doxorubicin hydrochloride group (Guo et al. 2013).

In another study, Tian et al. studied that glycyrrhetinic acid-doped chitosan/poly (ethylene glycol) nanoparticles have superior and effective targeting capabilities on hepatic carcinoma cells (QGY-7703 cells), thereby forming a potential candidate for liver cancer treatment. Their result indicated that chitosan/poly (ethylene glycol)-glycyrrhetinic acid nanoparticles accumulation in the cells was 2.6 times higher than that of chitosan/poly (ethylene glycol) nanoparticles (Tian et al. 2010). Zhang and co-workers designed glycyrrhetinic acid grafted-hyaluronic acid conjugate as a targeted drug delivery system to effectively deliver paclitaxel for the treatment of cancer. The result showed that paclitaxel encapsulated glycyrrhetinic acid grafted-hyaluronic acid nanoparticles exhibited more cytotoxicity to liver cancer cells (HepG2) than murine melanoma cells (B16F10) (Zhang et al. 2013). Zhang et al. synthesized doxorubicin-loaded glycyrrhetinic acid-modified alginate nanoparticles for targeted therapy against liver cancer. The result showed that the doxorubicin concentration was 2.8 and 4.7 fold higher than non-glycyrrhetinic acid-grafted nanoparticles (Zhang et al. 2012).

9.3.2 Polysaccharide Directed Active Targeting Polymeric Nanoparticles

9.3.2.1 Galactosamine

Galactosamine is a hexosamine derivative of galactose, which has a high affinity towards the asialoglycoprotein receptor. This receptor is specifically over-expressed on mammalian hepatocytes. Earlier, numerous saccharide (galactosamine and galactose) decorated nanoparticles have been synthesized for targeted cancer chemotherapy (Zhong et al. 2014). Thus, the asialoglycoprotein receptor stands as a potential target for hepatocyte-specific drug delivery. In 1999, Julyan and co-workers designed galactosamine functionalized doxorubicin-poly[N-(2-hydroxypropyl) methacrylamide] copolymer as a liver-targeted delivery of the therapeutics (Julyan et al. 1999). Zhong et al. formulated reduction-sensitive doxorubicin-loaded galactose-poly (ethylene glycol)-polycaprolactone copolymer (56.1–58.2 nm) for targeted delivery of doxorubicin into the nuclei of HeLa and HepG2 cancer cells, thereby resulting in enhanced anticancer efficacy as compared to the free drug (Zhong et al. 2013).

9.3.2.2 Hyaluronic Acid

Hyaluronic acid is a natural mucopolysaccharide, composed of repeating units of N-acetylglucosamine and D-glucuronic acid. It is found as a major part of the extracellular matrix in a wide variety of species. Functional groups of hyaluronic acid, such as hydroxyl, carboxylic, and N-acetyl groups, help in its chemical modifications (Kim et al. 2018). In biomedical science, there is a significant attraction towards the development of hyaluronic acid based nanoparticles, which is attributed to its superior physiochemical properties like high water-binding capacity, biodegradability, cell compatibility, non-immunogenicity, and non-toxicity (Rao et al. 2016). Numerous cancer cells over-expresses hyaluronic acid binding receptors such as CD44, receptor for hyaluronan-mediated motility, and lymphatic vessel endothelial hyaluronan receptors (Cai et al. 2017). CD44 is a glycoprotein receptor that is over-expressed in numerous cancers like ovarian, colon, and breast cancers (Dosio et al. 2016). Hyaluronic acid is imported by the cancer cells *via* CD44 receptor-mediated endocytosis, which is then followed by the enzymatic degradation of hyaluronic acid by the enzyme hyaluronidase (Zhang et al. 2016). Regarding the over-expression of CD44 receptors on cancer cells, hyaluronic acid has been frequently modified and widely used in various nano-formulations for targeted drug delivery to numerous cancer cells.

Accordingly, Xiao et al. prepared hyaluronic acid-modified poly (lactic-co-glycolic acid) nanoparticles (289 nm) for the co-delivery of camptothecin and curcumin in colon cancer cell lines (Colon – 2). They demonstrated that ligation of hyaluronic acid to poly (lactic-co-glycolic acid) nanoparticles increased the targeted

delivery and enhanced the cellular uptake of the formulations (Xiao et al. 2015). Another researcher reported that hyaluronic acid conjugated silica nanoparticles showed enhanced accumulation of paclitaxel drug in breast cancer cells (MCF-7) via CD44-mediated endocytosis. Additionally, the cellular uptake of hyaluronic acid-silica nanoparticles was 1.97 – fold higher than that of silica nanoparticles, thereby enhancing the cancer suppression potential of paclitaxel (Jinmao Li et al. 2017). Furthermore, Palanikumar et al. studied that the doxorubicin encapsulated hyaluronic acid modified mesoporous silica nanoparticles (130–165 nm) showed increased cellular uptake by CD44-overexpressed cancer cells (Palanikumar et al. 2018). In another study, Luo et al. demonstrated that hyaluronic acid-functionalized methoxy poly(ethylene glycol)-poly(lactide) nanoparticles (116 nm) exhibited enhanced internalization of paclitaxel by CD44-overexpressed epithelial cancer cells (A549). Moreover, the methoxy poly(ethylene glycol)-poly(lactide) nanoparticles showed enhanced tumor targeting ability and improved anti-tumor efficacy *in vivo* with a tumor inhibition rate of 75.9% when compared to other paclitaxel formulations (Luo et al. 2020).

9.3.3 Proteins Directed Active Targeting Polymeric Nanoparticles

9.3.3.1 Transferrin

Transferrin is a monomeric serum glycoprotein (80 kDa) which mediates iron transportation to various tissues such as the spleen, liver, and bone marrow *via* blood plasma (Ogun and Adeyinka 2018). It is mainly synthesized in the liver but also produced in the testis and brain in a lower amount (Anderson and Shah 2013). Transferrin receptor is the receptor protein for transferrin which imports iron by internalizing the iron-transferrin complex and thereby helps in maintaining iron homeostasis within the cells. This receptor is over-expressed on the surface of cancer cells which is attributed to its increased metabolism and high proliferation rate. This overexpression is utilized for selective delivery of anticancer drug encapsulated nanoformulations to these cells *via* transferrin receptor-mediated endocytosis (Liu et al. 2016). The binding of iron-bound transferrin to the transferrin receptor leads to the formation of clathrin-coated pits, which is then followed by internalization of the vesicle into the cytoplasm. Upon internalization, the acidic pH leads to loss of clathrin from coated vesicle as well as a disassociation of iron-transferrin complex, thereby facilitating iron release.

Accordingly, Liu et al. showed that transferrin-modified eight-arm-polyethylene glycol–dihydroartemisinin nanoparticles enhance cellular uptake and anticancer activity through receptor-mediated endocytosis in lewis lung carcinoma cells (Liu et al. 2016). Transferrin-conjugated poloxamer 407 and 123 nanoparticles (~90 nm) showed superior cellular uptake of doxorubicin and inhibit cell proliferation *in vitro*, not only in doxorubicin-sensitive cells but also in the doxorubicin-resistant

breast cancer cell lines (MDA-MB-231), as studied by Soe et al. (2019). Notably, transferrin-targeted poly(butylene adipate)/terephthalate nanoparticles were found to be safe for targeted delivery of 5-fluorouracil in colorectal cancer cell lines (HT29). Also, the targeted nanoparticles showed maximum significant cytotoxic effects on HT29 cells compared to the non-targeted nanoparticles (Varshosaz et al. 2017). Interestingly, Jose et al. formulated transferrin-conjugated docetaxel–poly (lactic-co-glycolic acid) nanoparticles for cancer chemotherapy. Its cytotoxicity studies confirmed that ligand-conjugated nanoparticles are more effective on MCF-7 cell lines (IC_{50} value of 4.392 μM/mL) than unconjugated nanoparticles (IC_{50} value of 6.24 μM/mL) (Jose et al. 2019). In 2016, Nag et al. designed transferrin functionalized chitosan-polyethylene glycol nanoparticles that exhibited increased cellular uptake of paclitaxel and higher intracellular toxicity (IC_{50} value of 0.3 μM) than unconjugated chitosan-polyethylene glycol nanoparticles (IC_{50} value of 2.0 μM) in lung cancer cell lines (HOP-62) (Nag et al. 2016).

9.3.3.2 Lactoferrin

Lactoferrin (80 kDa), also called lactotransferrin, is a multifunctional iron-binding globular glycoprotein of the transferrin family. It not only emerges as a promising antimicrobial, immunomodulatory, anticancer, and antiviral agent but also acts as an ideal and smart system for selective delivery of therapeutic drugs in the field of cancer therapy due to its well-recognized cytotoxic activity and effective anti-metastatic activity (Agwa and Sabra 2020). Lactoferrin overexpression is widely reported on the surface of the liver, respiratory epithelial, and cancer cells. Lactoferrin is decorated on the surface of nano-drug delivery systems to enhance their cellular uptake within the cancer cells through lactoferrin-mediated endocytosis. The cationic nature of lactoferrin facilitates its binding to anionic ligands (glycosaminoglycans) to enhance endocytic cell internalization *via* electrostatic interactions (Agwa and Sabra 2020). A promising biodegradable nano-drug delivery system comprising lactoferrin-decorated PEGylated-poly-lactide nanoparticles was designed as dual-targeted nano therapy for sustained brain delivery of paclitaxel in glioma cells (C6 and BCEC). The *in vitro* results showed enhanced cellular uptake of the formulated nanoparticles in glioma cells compared to unconjugated ones. Moreover, *in vivo* imaging studies showed higher brain accumulation and promising anti-glioma activity after intravenous administration of the cargo compared to uncoated ones (Miao et al. 2014).

In another approach, an intravenous administration of tramadol-incorporated poly (lactic-co-glycolic acid) nanoparticles modified with lactoferrin (158.8 nm) demonstrated analgesic effect, higher brain uptake, and prolonged sustained drug release profile *in vivo*. Conjugation of lactoferrin on poly (lactic-co-glycolic acid) nanoparticles surface showed a pronounced increase in the cellular internalization and accumulation in human bronchial epidermal cells (16HBE) and triple cloned neuroblastoma cells (SH-SY5Y) *in vitro*. Moreover, *in vivo* imaging of the brain recorded 3.85 fold higher cellular uptake after administration of modified

nanoparticles in comparison to unmodified ones (Lalani et al. 2012). Chen and co-workers designed and successfully constructed a novel etoposide-loaded poly (lactic-co-glycolic acid) nanoparticles by decorating its surface with folic acid and lactoferrin (83.3–181.9 nm) *via* 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) coupling reaction to inhibit the glioblastoma multiforme . *In vitro* data revealed higher cellular internalization of lactoferrinfolic acid-etoposide-poly (lactic-co-glycolic acid) nanoparticles driven strong cytotoxic effect compared to unmodified nanoparticles due to its high affinity to lactoferrin and folic acid receptors overexpressed on brain tissues and human tumors, respectively (Kuo and Chen 2015).

In another approach, an antiglioma drug named shikonin was loaded into lactoferrin-modified PEGylated-poly(lactic-co-glycolic acid) nanoparticles for targeting glioma cells *in vitro*. Results revealed strong antiglioma efficacy of lactoferin-poly (ethylene glycol)-poly (lactic-co-glycolic acid)/ shikonin nanoparticles against C6 glioma cells. Furthermore, *in vivo* study demonstrated prolonged biodistribution and higher brain accumulation (0.558 μg/g) of shikonin after intravenous injection of lactoferin-poly (ethylene glycol)-poly (lactic-co-glycolic acid)/ shikonin nanoparticles into sprague dawley rats compared to uncoated nanoparticles (0.101 μg/g) or free soluble shikonin (0.179 μg/g) (Li et al. 2018).

9.3.3.3 Epidermal Growth Factor

Growth factors play vital roles in stimulating several physiological functions such as cell proliferation, cell survival, wound healing, apoptosis, embryonic induction, and so forth. The epidermal growth factor receptor (HER, ErbB), which belongs to the receptor tyrosine kinases family, is a type-I transmembrane protein anchored in the cell cytoplasmic membrane. In the presence of the ligand, epidermal growth factor receptor undergoes dimerization which further activates tyrosine kinase-mediated signaling pathways, resulting in the regulation of several tumors mediated processes such as uncontrolled proliferation, metastasis, DNA synthesis, invasion, angiogenesis, and promoting motility of tumor cells. Epidermal growth factor receptor overexpression in solid tumors (breast cancer, melanoma, glioblastoma multiforme, lung cancer, pancreatic cancer, prostate cancer) is associated with a worse prognosis rate in cancer patients driven by aggressive behaviors of different metastatic cells worldwide (Duwa et al. 2020). Epidermal growth factor receptor signaling acts as a potential pharmacological target to treat cancer patients, and much global effort has been spent in the development and approval of potent epidermal growth factor receptor inhibitors.

In 2009, Shimada et al. designed paclitaxel entrapped epidermal growth factor-conjugated 2-methacryloyloxyethyl phosphorylcholine nanoparticles (50–75 nm) as a novel drug delivery system against epidermal growth factor receptor overexpressed cancer cells (A431 and H69 cell line) (Shimada et al. 2009). For instance, functionalization of paclitaxel-entrapped polymeric lipid-based nanoparticles with epidermal growth factor resulted in significant tumor growth inhibition in the mice

model. Administration of epidermal growth factor – coupled poly (amido amide) dendrimers conjugated with quantum dots led to site-specific delivery of nucleic acid and tumor imaging (Yuan et al. 2010). Similarly, the therapeutic efficacy of epidermal growth factor functionalized poly (amido amide)/DNA nanoparticles were proved *in vitro* by epidermal growth factor receptor – expressing HepG2 cells. The results indicated that this nanoformulation efficiently delivers tumor-targeting genes to the liver cancer cell line (HepG2) (Li et al. 2016).

9.3.3.4 Lectin

Carbohydrate-binding proteins (lectins) as recognition molecules mediate several biological processes, such as protein targeting, cell-molecule, and cell-cell adhesion, intracellular signaling events. Lectins are ubiquitous and are present in a wide range of organisms, including plants, animals, insects, and humans (Gupta 2020). Moreover, the expression of lectin can be modulated under different physiological conditions, including cancers leading to high expression of tumor-associated carbohydrate antigens for the design of promising anti-cancer vaccines. Tumor-associated carbohydrate antigens are crucial factors associated with tumor angiogenesis and metastasis (Freire-de-Lima et al. 2016). Moreover, the interaction of cell surface carbohydrates with binding sites of lectins leads to glycan accumulation within cells *via* the lectin-mediated endocytotic process. Several intracellular lectins, namely phosphomannosyl receptors (P-type), calnexin and calreticulin (calnexin family), ERGIC-53 (L-type), mannosidases (M type) are involved in various biological trafficking, targeting, secretion, sorting, and maturing glycoproteins, whereas extracellular lectins including galectins (siglecs), mannose-binding lectins (C selectins) mediate cell signaling, recognition of foreign pathogens, adhesion and so forth. The versatile glycan-based site-specific drug delivery strategy, also called "glycotargeting" has been the subject of extensive cancer-associated research for two decades for the development of customized glyconanocarriers (Brannon-Peppas and Blanchette 2012).

Cho et al. successfully designed biotinylated-lectin tagged fluorescent polymeric nanoparticles (35 nm) using biocompatible polymers for detecting the expression of sialic acid on the surface of a cell. Sialic acid represents the common clinical biomarker for cancer detection and diagnosis. *In vitro* assessment revealed successful detection of sialic acid overexpressed on cells (HeLa, MCF 7 and L929 fibroblasts) with high specificity; hence this fluorescent polymer-based nano cargo can be established as a potential bioimaging probe for cancer cells detection (Cho et al. 2014). In another study, *in vitro* active administration of hydrophobic photosensitizer zinc phthalocyanine (C11Pc) and hydrophilic PEGylated gold nanoparticles *via* jacalin revealed enhanced cellular uptake and internalization of cargo by the colorectal adenocarcinoma cells (HT-29) and the breast cancer cells (SK-BR-3) *via* endocytosis mechanism (Obaid et al. 2015). Ikemoto and team developed *Bauhinia purpurea* agglutinin-modified PEGylated liposomes loaded with doxorubicin for the treatment of prostate cancer cells (DU145). The modified nanocarrier significantly

showed enhanced anticancer activity against DU145 cells in comparison to unmodified PEGylated liposomes at the same dose. This *in vitro* experiment suggested the nanoformulation be a potent nano-drug delivery system for the treatment of prostate cancers in humans (Ikemoto et al. 2016).

9.3.3.5 Monoclonal Antibody

An antibody, also known as immunoglobulins, is highly specific for protein antigen. In recent years, the use of monoclonal antibodies as a drug delivery system in cancer therapy has resulted in the targeted delivery of potent chemotherapeutics within the metastatic cells that overexpresses the target tumor antigen on their surface, thereby affecting less to the normal healthy cells. The conjugation of intact monoclonal antibodies and potent anti-cancer drugs can be directly formulated *via* covalent bonds forming biopharmaceutical antibody-drug conjugates or even surface functionalization of the nanoparticles in a controlled fashion, which further might act as "magic bullets" to shoot the target cells.

Patel et al. studied that anti-epidermal growth factor receptor antibody conjugated docetaxel-loaded poly (lactic-co-glycolic acid) nanoparticles (128.4 nm) showed enhanced cytotoxicity and site-specificity when used as a drug delivery system to target epidermal growth factor receptor, which is highly expressed on non-small lung cancer cells (Patel et al. 2018). In another study, conjugation of cetuximab (anti-epidermal growth factor receptor) to temozolomide loaded poly (lactic-co-glycolic acid) (cetuximab-temozolomide-poly (lactic-co-glycolic acid) nanoparticles (266.40 nm) enhanced chemotherapeutic effects in three epidermal growth factor receptor overexpressing cancer cell lines (U-87MG, SK-Mel 28, and SW480). The targeted nanomedicine showed a higher cytotoxic effect and cellular uptake accompanied with increased biomarker (γ-H2AX) level compared to temozolomide-poly(lactic-co-glycolic acid)-nanoparticles or free soluble temozolomide (Duwa et al. 2020). Shi et al. prepared anti-human epidermal growth factor receptor 2 (HER-2) antibody grafted doxorubicin-loaded poly (2-methyl-2-carboxytrimethylene carbonate-co-d,l-lactide)-graftpoly(ethylene glycol)-furan nanoparticles for the effective delivery of doxorubicin in SKBR-3 breast cancer cell line (Shi et al. 2009).

9.4 Conclusion

In the era of nanotherapeutics, several immunotherapeutic approaches, including tumor antigen vaccine, monoclonal antibodies, administration of immunomodulators, and immune checkpoint inhibitors or their polytherapy, have led to limited anti-tumor strategy due to some critical issues challenging controlled and high-specific targeted delivery. The majority of monoclonal antibodies and vaccine candidates didn't achieve success as preventive measures because of their high toxic

doses. Therapeutic administration of immunomodulators such as cytokines and monoclonal antibodies contributes to the development of systemic toxic effects. Moreover, adaptive anti-tumor T cells therapy is associated with off-target (normal healthy cells) autoimmunity. To encounter the above challenges and limitations associated with the above-mentioned pitfalls, extensive research has been emphasized in the nanomedicine and nanobiotechnology fields for improving the profile of tumor vaccination strategies by utilizing specific ligands against receptor molecules overexpressed in aggressive and metastatic cancers. The promising and unique properties of versatile polymeric nanocarriers include their biocompatibility, biodegradable nature, high stability, prolonged and sustained drug release capacity, high drug (hydrophilic and hydrophobic) loading capacity, and viable routes of administration.

Additionally, the development of effective polymer-based drug delivery nanocarriers can also co-deliver multiple pharmaceutical agents in the body for their synergistic effect. Certain adverse side effects and complications associated with conventional chemotherapy can be reduced through the administration of ligand-modified drug encapsulated nanoparticles, whereas the unmodified polymeric nanoparticles could partially reach this goal. Engineering the surface of polymeric nanocarrier by targeted ligands can efficiently control the biodistribution and circulation kinetics of cargo *in vivo*. Consequently, several biocompatible polymers were conjugated with ligands as "magic bullets" against tumor-associated biomarkers for facilitating specific tumor targeting *via* efficient cellular uptake and internalization. The anticancer strategy of several ligands (transferrin, epidermal growth factor receptors, lactoferrin, folic acid, carbohydrates (lectin ligands)) against tumor-specific markers has been developing extensively towards targeted drug delivery. To date, very limited human clinical trials have been conducted, and there exists no comprehensive study related to the comparative study of several targeting small molecules involved under the same physiological conditions. Therefore, it is the need of the hour to design and evaluate the comparative oncology study accompanied by early initial human clinical trials to reach a rational decision.

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Chapter 10
Polymeric Nanoparticles as Theranostics for Targeting Solid Tumors

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Abbreviations

DNA	Deoxyribonucleic acid
HepG2	Human liver cancer cell line
MCF-7	breast cancer cell line
MDA-MB-231	Human breast cancer cell line

10.1 Introduction

The term "theranostics" was devised by John Funkhouser that combines the therapeutic and imaging modalities in a system, thus emerging as a more effective and targeted treatment for various pathological diseases, especially cancer. Initially, the concept of theranostics was used in 1964 for the treatment of thyroid cancer (Ali et al. 2020). Over the past few years, the stated domain has evolved as a new field in the drug delivery sphere with an interest in the simultaneous diagnosis, treatment, and progress in the monitoring of the disease. Theranostic materials can be used to monitor the pharmacokinetic and pharmacodynamic parameters of the drugs or other molecules injected into the body.

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10.2 Theranostic Polymeric Nanomedicine for the Treatment of Solid Tumor Cancer

The application of nanotechnology (Gautam et al. 2021) in the medical field is referred to as nanomedicines. It offers a plethora of scientific and medical methodologies or techniques that are effective, improved, and rewarding in nature. One of the methodologies involves the use of nanoparticles in theranostics. The aim of engineering nanomedicines as theranostics is to treat a disease condition and also to follow up patient-specific clinical outcomes as prognosis (Shrivastava et al. 2020). Nanoparticles can be easily manipulated and functionalized. Also, they could be made highly target tissue/organ-specific. All of these properties render them of high medicinal value in the field of medicine.

As compared to conventional therapy, nanoparticles can easily cross biological barriers without premature destruction or degradation by the physiological process. Due to the high surface and volume ratio, nanoparticles can carry and transport various molecules without the usage of superfluous materials. Symptomatically, cancer is considered to be a heterogeneous group of diseases that is caused and characterized by uncontrolled and rapid cell growth. It is caused by the genetic and epigenetic changes which occurred in the affected patients. Currently, the therapies used for cancer include chemotherapy, surgery, immunotherapy, and radiotherapy (Fig. 10.1). Chemotherapy has its limitations due to the sub-therapeutic amount of active pharmaceutical ingredients generally reaching the targeted sites of the tumor. In addition, some other drawbacks such as the development of resistance towards treatment during the therapy and precipitation of toxic side effects of drugs on the normal cells and organs of the body limit its widespread usage.

To overcome these limitations of conventional chemotherapeutic treatment, the role of nanomedicine for targeted drug delivery could be appreciated. Drug delivery systems employing polymeric nanoparticles allow molecular targeting and make sure that a higher amount of active pharmaceutical ingredients reaches targeted sites. Therefore, in recent years, approaches based on nanotechnology for the drug delivery area have been extensively investigated by researchers of the varied domain (Khuroo et al. 2014). Mainly studies have been done to determine how nanomedicines trigger the release of drugs at specific sites actively and passively. These studies also ensure that drugs bypass the off-target tissues, ultimately leading to a high therapeutic index.

Nanoparticles are the ideal candidates for the diagnosis and treatment of cancer because they can rapidly cross biological barriers and accumulate at specific cancer tissues (Padhi and Behera 2020). After accumulation, they emit specific signals in response to respective biomarkers and subsequently release the drugs. The best examples of nanomedicines showing substantial clinical applicability are polymer-drug conjugates, polymeric micelles, polymeric nanoparticles, metallic nanoparticles, mesoparticles, and liposomes (Thakur et al. 2019; Gautam et al. 2020). These nanocarriers have longer half-lives as compared to conventional drugs, which are easily eliminated from the bloodstream. The inclusion of imaging modalities with

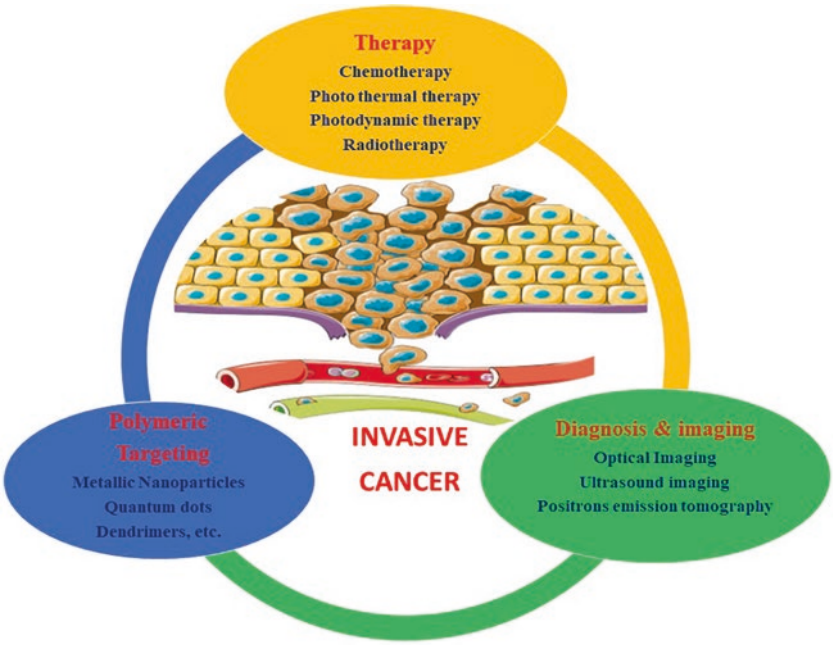

Fig. 10.1 Diagnosis and treatment strategies in cancer treatment. The metastasis process explored in this figure along with the strategies for the treatment. In green box different type of preliminary diagnostic test have been shown to identify the cancer in the primary stage or find out there progression pattern. In yellow box different therapy involved while the treatment is taking care along with the therapeutics. In blue box showed that how the different targeting polymeric carrier can be used to target the cancer site either by ligand conjugation, detection using quantum dots or other nano-carriers

nanomedicines works as a diagnostic part of theranostics (Gautam et al. 2019). These strategies have an immense scope and would enable efficient and effective management, particularly of carcinomas.

10.2.1 Polymeric Gold Nanoparticles

Toxicity is an important parameter that needs to be considered while fabricating nanoparticulate systems (Patra et al. 2018). Gold nanoparticles are chemically stable, biocompatible, and exhibit relatively low toxicity (Cui et al. 2017; Yang et al. 2015; Zhang et al. 2012, 2014, 2015). However, to maintain the electrostatic stability of gold nanoparticles in the complex biological environment, polymeric gold nanoparticles are prepared. Polymeric gold nanoparticles are more stable than free gold in biological and physiological conditions. Polymeric coatings/shells increase

the stability in biological and physiological conditions which favours their cellular uptake. In cancer therapy, gold nanoparticles increase radio-sensitization and also act as a carrier for drug delivery while in the area of diagnosis, they serve as contrast agents in computed tomography, magnetic resonance tomography, photoacoustic imaging, and fluorescence imaging (Thakur et al. 2017).

Radiotherapy is the key terminator of cancer cells. However, the high vulnerability of healthy tissues compared to radiation-resistant tumors is the main limitation in the usage of radiotherapy. The solution to this pertinent problem is the use of radio sensitizing agents. Thus the off-target dose deposition does not cause severe toxicity as the presence of radio sensitizing molecules or materials in the targeted tumors selectively increases the dose accumulation at the target that receives the radiation (Wang et al. 2018). Metallic (high atomic number materials) nanoparticles, structurally composed of a core, a shell (base), and a surface (specific targeting units), have been developed for sensitizing radiotherapy (Fukumori and Ichikawa 2006). The core consists of high atomic number materials (mostly of gold (Z = 79)) to increase X-ray absorption. X-ray absorption by high atomic number materials produces the release of photo- or auger- electrons which induce the emission of locally damaging secondary electrons (delta rays) (McMahon et al. 2008; Retif et al. 2015). The nanoparticle size, structure, and choice of stabilizing ligand were found to influence auto-absorption of secondary electrons, tumor-targeting efficiency, bio-distribution, and bio-elimination (McMahon et al. 2011). These nanoparticles could enhance tumor tissue damage in two ways: (i) directly by accelerating deoxyribonucleic acid (DNA) damage and (ii) indirectly by producing reactive free radicals (Rosa et al. 2017; Sicard-Roselli et al. 2014; Wang et al. 2018). It was reported elsewhere that polystyrene beads (100–200 nm) with citrate-stabilized gold nanoparticles (4.5 nm) were able to successfully radio sensitize breast cancer (radiation-resistant) and prostate cancer cells (Belhout et al. 2016; Bennie et al. 2020). The authors reported significant radiation dose enhancement, enhanced cellular uptake with minimal toxicity.

Polymeric gold nanoparticles have reportedly been effective against the hindering biological barriers and difficult bio elimination for feasible drug delivery as compared to inorganic nanoparticles. Modified gold nanoparticles with polymeric materials offered increased ease of surface modification, thereby leading to enhancement of circulation time, targeting efficiency, drug loading capacity, stability, and biocompatibility. Chemotherapeutic drugs like cisplatin, magnolol, doxorubicin, methotrexate, curcumin, oxaliplatin, docetaxel, and paclitaxel were successfully incorporated by applying ionic, covalent, and physical means.

Feng et al. reported 80 nm dithiol-poly (ethylene glycol) nanospheres appended with drug molecule and epidermal growth factor peptide (Feng et al. 2017). The nanospheres were internalized by the brain tumor cells mediated through epidermal growth factor receptors. A pH-dependent drug release was triggered and the nanospheres displayed an easy elimination from the body. Banihashem et al. reported the formulation of 24 nm gold nanoparticles surface modified by thioglycolic acid ligand with chitosan grafted poly(N-vinyl caprolactam) coating (loaded with cisplatin) (Banihashem et al. 2020). Elhabak et al. developed 136 nm-sized poly

(lactic-co-glycolic acid) nanoparticles surface-functionalized with trastuzumab (encapsulating magnolol and gold nanoparticles) (Elhabak et al. 2020). These fabricated approaches successfully targeted breast cancer cells (MCF-7) and exhibited temperature/pH-controlled cisplatin release and magnolol cytotoxicity with photo thermal effect.

Gold nanoparticles can potentially revolutionize an early cancer diagnosis as well. Polymeric conjugation imparts hassle-free delivery of many imaging molecules with an enhancement in therapeutic properties. Commonly, imaging moieties are combined with the photothermal killing property of the gold nanoparticles to give a theranostic effect. Zhu et al. prepared gold nanoclusters (2 nm in size) grafted with fluorescent conjugated polymer (100 nm in size) (Zhu et al. 2020). It has been observed that there is a vast difference between the nanoparticle uptake mechanism by a healthy and cancerous cell. The main difference arises from the cell-specific and non-specific interaction of polymeric nanoparticles. On the basis this observation, Huang et al. developed polymeric nanoparticles for accurate cancer diagnosis by the dissipative particle dynamics simulation (Huang et al. 2019). The nanoparticles contained folic acid conjugated superparamagnetic iron oxide nanoparticles for dual targeting. Ge et al. created gold nanorod coated with light-responsive ruthenium -complex and polyethylene glycol (Ge et al. 2020). The vesicles incorporated near-infrared cyanine dye for fluorescence and photoacoustic imaging of targeted tumor cells (Table 10.1).

10.2.2 Polymeric Micelles

Polymeric micelles are composed of nano-sized (between 10 and 100 nm) core/shell assembly of amphiphilic block-co-polymers (Deshmukh et al. 2017). The spherical, supramolecular colloidal particles are thermodynamically self-assembled together in aqueous polymer chains that exceed the critical micelle concentration (Torchilin et al. 2003). The micelle has a hydrophobic core that acts as a carrier for hydrophobic drug molecules loaded by chemical, physical, or electrostatic methods and a hydrophilic shell that provides compatibility in the biological environment evading host defense. Enhanced permeability and retention effect (due to extensive angiogenesis) in cancer cells lead to preferential passive accumulation of small-sized, prolonged circulating polymeric micelles (Fang et al. 2011; Maeda 2001; Maeda et al. 2000; Behera and Padhi 2020). The condensed hydrophilic polymeric mesh on the micelle surface offers an easy surface functionalization for targeted tumor direction and stimuli-sensitive drug release especially when specific copolymers are used.

Amphiphilic micelles copolymers have a hydrophilic block chemically attached to a hydrophobic block to form a di-block polymer, a triblock polymer, or a graft copolymer (Adams et al. 2003; Aliabadi and Lavasanifar 2006; Harris 2013). Table 10.2 enlists some commonly used hydrophilic shell-forming and hydrophobic core-forming polymers used in various combinations to form micellar carriers

Table 10.1 Polymeric gold nanoparticles in various combinatorial preparations for cancer theranostics

Polymer	Use	Other components	Size	Target	Cellular intake	References
Polystyrene beads	Targeting, radiation sensitizing	Citrate-stabilized gold nanoparticles	100–200 nm	Breast cancer cell line (MDA-MB-231)	Phagocytosis or clathrin- mediated	Belhout et al. (2016) and Bennie et al. (2020)
Dithiol-polyethylene glycol sphere	Targeting, drug delivery	Epidermal growth factor peptide and anticancer drug	~80 nm nanospheres	Brain tumors	Target epidermal growth factor receptors	Feng et al. (2017)
Polyethylenimine and poly(ethylene glycol)	Targeting, drug delivery	Doxorubicin folic acid attached superparamagnetic iron oxide nanoparticles	–	Breast cancer (MCF-7)	Folic acid and external magnetic field mediated	Huang et al. (2019)
Chitosan-poly (N-vinylcaprolactam)	Targeting, drug delivery	Thioglycolic acid ligand and cisplatin anticancer drug	24–19 nm gold/ thioglycolic acid nanoparticles	Breast cancer cells (MCF-7)	Receptor-mediated	Banihashem et al. (2020)
Trastuzumab monoclonal antibody anchored polylactic-glycolic acid	Drug delivery, photothermal effect	Magnolol (phenolic polyhydroxy compound) and gold nanoparticles	~136 nm	Breast cancer cells (MCF-7)	Monoclonal antibody – Human epithelial growth factor receptor-2 interaction	Elhabak et al. (2020)
Poly (methoxy-ethylhexyloxy)-phenylenevinylene) (MEH-PPV)	Imaging, photothermal killing	Gold nanoparticles modified with polyethyleneimine, fluorescent nanoparticles	MEH-PPV 100 nm; gold-nanoparticles, 2 nm	Lung epithelial cell line (A549)	Endocytosis	Zhu et al. (2020)
Gold nanorods conjugated with light-responsive drug rutheniumu-complex and poly (ethylene glycol)	Fluorescence and photoacoustic imaging	Near infrared-II cyanine dye	–	–	Endocytosis	Ge et al. (2020)

Table 10.2 Commonly used polymers in micelle formation

Hydrophilic polymer block	Micellar core polymer blocks	
Poly (ethylene-oxide) or Poly (ethylene-glycol)	Polyethers (prone to hydrolysis, physical encapsulation)	Poly (propylene-oxide)
Chitosan	Polyesters (prone to hydrolysis, physical encapsulation)	Poly (L-lactide)
Poly (N-vinyl-pyrrolidone)		Polycaprolactone
Poly (nisopropylacrylamide)		Poly (lactide-co-glycolic acid)
	Poly-amino acids (provides functionaliseable groups for bonding with drugs)	Poly (L-histidine)
		Poly (L-aspartic acid)
		Poly (β-aminoesters)
	Lipids (aliphatic chains, do not disintegrate easily)	Dioleoyl (phosphatidylethanolamine)
		Distearoyl (phosphatidylethanolamine)

(Biswas et al. 2016; Braunová et al. 2017; Kwon 2003; Rapoport et al. 2004). Popularly known amphiphilic block copolymer for example pluronics consists of the triblock, i.e. poly (propylene-oxide) attached to poly (ethylene glycol) (Danson et al. 2004). Block copolymer combinations like pluronic, poly(ethylene oxide)-poly (propylene oxide), poly(ethylene oxide)-poly(ester), and poly(ethylene oxide)-poly(amino acid) exhibit appreciable ability to solubilize and deliver hydrophobic compounds, such as paclitaxel, propofol, amphotericin B and photosensitizers (Kwon 2003; Lavasanifar et al. 2002; Elhasi et al. 2007).

Micellar systems are being developed for stimuli-sensitive action (like pH, reduction, thermal, light, magnetic field, and ultra-sound sensitive) by using the different combinations of stimuli sensitive copolymers or by conjugating site targeting ligand molecules like micelle-antibody conjugates, referred to as immune micelles (Torchilin 2004; Torchilin et al. 2003). Overproduction of p-glycoprotein (efflux pump) in tumors and cancer cells causes multidrug resistance. Polymeric micelles, especially the micelles containing poly (propylene oxide), show potential inhibition of p-glycoprotein in drug-resistant tumors (Braunová et al. 2017, 2019). Thus they extend a potential way to increase drug absorption and overcome drug resistance in cancer cells (Kim et al. 2008).

For imaging applications, polymeric micellar nanoparticles are used as carriers for (i) signal emitting compounds (like near-infrared dyes), (ii) contrast agents (like gadolinium complexes for magnetic resonance tomography), and (iii) imaging probes for diagnosis (like near-infrared fluorophore indocyanine) (Cagel et al. 2017). The micelle provides enhanced biodistribution, tissue-specific uptake, biocompatibility by reducing toxicity and circulation time in the blood (Kim et al. 2004; Sant et al. 2005; Valle et al. 2004). Theranostic applications of polymeric micelles have gained immense popularity due to their ease of functionalization and self-assembling characteristics. Mi et al. presented calcium phosphate micelles hybridized with poly (ethylene glycol)-polyanion block copolymers with

gadolinium (III) chelates the magnetic resonance tomography contrast agent for non-invasive magnetic resonance imaging cancer diagnosis and neutron capture therapy as gadolinium emits γ-rays with high energy following a neutron capture reaction with electrons of low energy. The micelles showed significant therapeutic efficacy as the high internalization of the micelle delivered gadolinium gadolinium (III) chelates the magnetic resonance tomography contrast agent gadolinium-diethylene triamine penta acetic acid in tumor tissues and subsequently damaged the cancer cells by γ-ray or electron emission. Significantly efficient delivery of gadolinium (III) chelates the magnetic resonance tomography contrast agent gadolinium-diethylene triamine penta acetic acid with improved plasma clearance, on-site accumulation, magnetic resonance tomography contrast, and anti-tumor pharmacodynamics effect have highlighted their potential (Mi et al. 2015; Shiraishi et al. 2009).

Increased cellular uptake of doxorubicin was noted when delivered using polymeric micellar particles. Cuong et al. prepared triblock copolymer poly (ethylene glycol)-polycaprolactone-polyethylene glycol micelle to deliver anticancer drug doxorubicin in the breast cancer cell line (MCF-7). Treatment with the fabricated micellar carriers did not develop adverse effects even after multiple doses (Cuong et al. 2011). Polymeric micelles conjugated with an anti-cancer monoclonal antibody and a chelator for Copper-labelling were reported (Guo et al. 2014). These multi-functional carriers offered pH stimuli sensitive drug release, the potential for non-invasive imaging, and passive as well as active cellular uptake leading to cellular drug accumulation.

There is a constant need for new contrast agents for better image sensitivity and resolution. Hoang et al. validated computed tomography imaging with enhanced sensitivity and resolution by using ^{111}Indium-labeled (^{111}In) encapsulated micelle. ^{111}In- diethylene triamine pentaacetic acid–b–polycaprolactone micelle exhibited adequate cellular uptake by breast cancer cell (MDA-MB-231)-induced tumor in mice with better imaging properties (Hoang et al. 2009). In such a scenario, it is apt to state that tissue distribution can be simultaneously quantified by non-invasive nuclear imaging. Oleic acid-coated superparamagnetic iron oxide nanoparticles micelles (5–10 nm) were formulated using polymer poly(ethylene glycol)-poly[N-(2-hydroxypropyl)] (Talelli et al. 2009). The micelles were highly stable for 7 days in the presence of fetal bovine serum. Easy preparation protocol and nano- size (~200 nm) made them suitable and effective for image-guided drug delivery.

The ligand cyclic arginine-glycine-aspartate targets $\alpha_v\beta_3$ proteins on tumor endothelial cells to initiate receptor-mediated endocytosis and superparamagnetic iron oxide nanoparticles loaded poly (ethylene glycol)-poly (D, L-lactide) and poly-(ethylene glycol)–poly (D, L-lactide) copolymer micelle were reported to impart stable high contrast for magnetic resonance imaging (Nasongkla et al. 2006). The system was stable for a prolonged duration within the body and efficiently internalized by the cells. Celastrol, the *nuclear factor kappa B* inhibitor and sulfasalazine, glutathione inhibitor employing micellar system enhanced the antitumor activity. Celastrol and oleic acid-coated superparamagnetic iron oxide nanoparticles were

entrapped in the sulfasalazine micellar core (Elhasany et al. 2020). These micelles accomplished both magnetic tumor targeting and magnetic resonance diagnosis.

Micelle hybrid hydrogel has been developed for chemodynamic therapy of skin tumors by using photothermal hyperthermia. Bioenzyme glucose oxidase loaded pluronic F127 based micelles and molybdenum disulphide-manganese ferrite nanocomposites were incorporated into a hydrogel of chitosan-dihydrocaffeic acid (Zheng et al. 2021). The constant release of bioenzyme glucose oxidase by the hybrid hydrogel consumed the intratumoral glucose and produced hydrogen peroxide, which increased acidity. Enhanced acidity in the tumor accelerated the release of iron ions from molybdenum disulphide-manganese ferrite nanocomposites. Iron catalyzes the disintegration of hydrogen peroxide into highly toxic free radicals to induce cell death. The hydrogel synergistically enhanced skin tumor eradication (Table 10.3).

10.2.3 Polymeric Superparamagnetic Nanoparticles

Superparamagnetism is a property observed in nanoparticles when they are in a magnetized state, but magnetization randomly flips, where the average magnetization in the absence of an external magnetic field appears to be zero. In this state, they are highly susceptible to magnetization by external magnetic fields. In biomedical science, superparamagnetic materials form a significant class of material with drug delivery applications, magnetic resonance imaging, and hyperthermia cancer therapy. The most advantageous property they offer is the on-site controlled delivery due to an external magnetic field. Among the various pure metal ions and metal oxides, the most widely used magnetic nanoparticles are ferric oxide or ferroferric oxide due to their comparatively less toxicity (Andhariya et al. 2011). Surface properties of magnetic nanoparticles can limit or enhance their biological environment effectiveness and these properties include surface charge and hydrophobicity. Hydrophobic magnetic nanoparticles are more susceptible to opsonization and clearance. Surface charge leads to non-specific internalization into oppositely charged cells via absorptive endocytosis. Binding or functionalizing magnetic nanoparticles with synthetic and natural polymers reduces non-specific interactions and toxicity while increasing blood circulation time. The polymeric superparamagnetic iron oxide nanoparticles are coupled with polymeric vesicles and micelles which effectively increase their activity range. Superparamagnetic iron oxide nanoparticles have adequate drug payload capacity with protracted blood circulation, particle size distribution, biocompatibility, stability, and programmable drug release. Non-invasive cancerous tissue imaging is highly beneficial for an accurate and early diagnosis and treatment planning for cancer patients. Magnetic nanoparticles are physio-chemically efficient magnetic resonance contrast agents which increase the resolution and contrast between healthy and cancerous cells. The cancerous tissue is

Table 10.3 Polymeric micelles in various combinatorial preparations for cancer theranostics

Polymer	Use	Additions	Size	Cell line	Uptake	References
Calcium phosphate micelles hybridized with polyethylene glycol	Imaging, thermal neutron irradiation therapy	Gadolinium chelates-diethylene triaminepentaacetic acid	100 nm	Colon adenocarcinoma (C26) cells	Endocytosis	Mi et al. (2015)
Poly (ethylene glycol)-polycaprolactone-poly (ethylene glycol)	Drug delivery	Doxorubicin	100 nm	Breast cancer cells	Endocytosis	Cuong et al. (2011)
Poly(2-hydroxyethyl methacrylate) with poly(L-lactide)-block-poly(ethylene glycol) side chains	Drug delivery, *positron emission tomography* imaging	Doxorubicin, anti-CD105 monoclonal antibody, copper-labelling chelator	100 nm	Positive human umbilical vein endothelial cells (CD105)	CD105-mediated endocytosis	Guo et al. (2014)
Poly(ethylene glycol) ε-caprolactone	Computed tomography imaging	^{111}Indium-labeled (^{111}In)		Tumor-bearing mice	Endocytosis	Hoang et al. (2009)
Poly(ethylene glycol)-b-poly[N-(2-hydroxypropyl)]	Magnetic resonance imaging, drug delivery	Magnetic resonance imaging contrast agent superparamagnetic iron oxide nanoparticles	200 nm	–	Endocytosis	Talelli et al. (2009)
Mal-poly (ethylene glycol)-poly (L-lactide) and meo-poly (ethylene glycol)-poly(L-lactide)	Drug delivery, magnetic resonance imaging	Doxorubicin, cyclic tripeptide ligand, magnetic resonance contrast agent superparamagnetic iron oxide nanoparticles	45 nm	Tumor SLK endothelial cells	$\alpha_v\beta_3$- mediated endocytosis	Nasongkla et al. (2006)
Mal-poly (ethylene glycol)-poly(L-lactide)and meo-poly (ethylene glycol)-poly (L-lactide)	MR imaging, therapeutic delivery	Lung cancer-targeting peptide, superparamagnetic iron oxide nanoparticles, doxorubicin	50 nm	Lung cancer cells (H2009)	Endocytosis	Guthi et al. (2010)

N3-poly (ethylene glycol)-NH_2 and poly (ethylene glycol)-NH_2	Drug delivery	Doxorubicin, probe X (antiangiogenesis agent)	–	–	Endocytosis	Sun et al. (2020a, b)
Polysaccharide chondroitin sulfate	Magnetic tumor targeting therapy	Celastrol, sulfasalazine	154.4 nm	Breast cancer cells (MCF-7)	Uptake by a magnetic field	Elhasany et al. (2020)
Poly (amino acid) methoxy poly (ethylene glycol)-poly aspartic acid-polylysine	Magnetic resonance imaging, drug release	Chemotherapeutic paclitaxel, superparamagnetic iron oxide nanoparticles	134.5 nm	Liver hepatocellular carcinoma (Bel-7402) cancer cells	Endocytosis	Xiao et al. (2020)
Amphiphilic chitosan derivative micelles	Imaging, photothermal therapy	Bimetallic metal nanoclusters containing platinum and silver (Pt_1Ag_{28})	60 nm	–	Positively charged Pt_1Ag_{28}- amphiphilic chitosan derivative micelles taken in by negatively charged cancer cells	Yang et al. (2020)
Chitosan-dihydrocaffeic acid and Pluronic micelles	Photothermal hyperthermia	Glucose oxidase, molybdenum disulphide-manganese ferrite nanocomposites-	–	Skin cancer	Endocytosis	Zheng et al. (2021)

distinguished from surrounding tissue based on the internalization of magnetic nanoparticles (passive targeting) or ligand-mediated interaction (active targeting). Huang et al. fabricated folic acid-modified superparamagnetic iron oxide nanoparticles with an encapsulated anti-cancer drug, doxorubicin. The external magnetic field can control the doxorubicin-folic acid- superparamagnetic iron oxide nanoparticles aggregation in the breast tumor cell line (MCF-7) and inhibit the cancer cells by delivering the anticancer drug *in vivo*. The accumulation of superparamagnetic iron oxide nanoparticles at the tumor site when monitored in real-time by magnetic resonance imaging revealed no relevant side effects in the liver, lung, kidney, and heart histology in mice (Huang et al. 2017). Similarly, superparamagnetic iron oxide nanoparticles and doxorubicin were co-encapsulated in multi-functional micelles equipped with the lung cancer-targeting peptide leading to a significant increase in $\alpha_v\beta_3$-dependent cell targeting in lung cancer cells (Guthi et al. 2010). Ling et al. used prostate stem cell antigen antibodies as active tumor-targeting agents in poly (ethylene glycol) nanoparticles loaded with docetaxel and Superparamagnetic iron oxide nanoparticles. The use of antibodies led to highly specific targeting and magnetic resonance imaging (Ling et al. 2011). Yang et al. achieved better targeting efficacy by developing poly (ethylene glycol) – superparamagnetic iron oxide nanocarriers with pH-sensitive bonds with the anti-cancer drug molecules. Cancer cells targeting ligands, cyclo (arginine-glycine-aspartate- aspartate-phenylalanine-cysteine) peptides and positron emission tomography copper chelators, were conjugated at the ends of polyethylene glycol arms (Yang et al. 2011). Yallapu et al. developed multi-layer water-dispersible superparamagnetic iron oxide nanoparticles. Iron oxide core nanoparticles were developed by the precipitation of iron salts in the presence of ammonia. The particles were then complexed with β-cyclodextrin and pluronic polymer coatings. Effective encapsulation of the anticancer drug in the formed polymeric carrier led to prolonged drug release along with magnetic resonance imaging and hyperthermia properties.

Macrophages did not engulf the nanoparticles, hence were hemocompatible and exhibited increased apoptosis in cancer cells (Yallapu et al. 2011). To overcome the rapid filtration in the liver and spleen and bypass the opsonization by phagocytes, surface modifications were done. However, polymers like polyethylene oxide had produced immunogenic responses, leading to oxidative stress thereby influencing cellular processes (Ulbricht et al. 2014). Therefore, alternative polymers with improved features such as poly(2-oxazolines) were being developed for the next generation of therapeutics (De La Rosa 2014; Luxenhofer et al. 2012). Synthetic conjugates of natural polysaccharide glycogen which are known to be inherently biocompatible have been used as suitable carrier for superparamagnetic iron oxide nanoparticles. Glycogen carriers were passively accumulated due to the enhance permeability and retention effect and showed great potential in curbing cancer (Gálisová et al. 2020) (Table 10.4).

Table 10.4 Polymeric superparamagnetic iron oxide nanoparticles (SPIONs) in various combinatorial preparations for cancer theranostics

Polymer	Drug	Targeting Agent	Size	Cell line	Uptake	References
Poly(ethylene-glycol) and polyethylene imine	Doxorubicin, folic acid	–	67 nm	Breast tumor	Folic acid receptor	Huang et al. (2017)
Maleimide-poly(ethylene-glycol)-polylactic acid and methyl poly (ethylene glycol)-polylactic acid	Doxorubicin	Lung cancer-targeting peptide	60 nm	Lung cancer cells	Avβ6-dependent	Guthi et al. (2010)
Poly (ethylene-oxide)	Doxorubicin	Tri-mellitic chloride-folate	100 nm	Liver cancer	Folate receptor	Maeng et al. (2010)
Poly (lactic-glycolic) acid	Docetaxel	Prostate stem cell antigen antibodies	147 nm	Prostate cancer	Endocytosis	Ling et al. (2011)
β-cyclodextrin and pluronic polymer	Curcumin	–	175 nm	Ovaria, breast & prostate cancer	Endocytosis	Yallapu et al. (2011)
Poly (amidoamine) with poly (ethylene glycol)	Doxorubicin	–	>100 nm	Breast tumor	Endocytosis	Chen et al. (2014)
Polyethylene glycol/polyethylene imine/polysorbate	Doxorubicin	–	58 nm	Glioma growth	Endocytosis by an external magnetic field	Xu et al. (2016)
Poly(D, L-lactic-co-glycolide) core and poly (ethylene glycol) lipid shell	–	Folate, paramagnetic di ethylene triamine pentaacetic acid-gadolinium	439 nm	Hela cells	Folate-binding effect on the cell membrane	Liao et al. (2011)
Pluronic® L-121 with fluorescent label (Alexa Fluor® 647)	Camptothecin	Peptide (bombesin)	< 200 nm	Prostate cancer cells (PC-3)	Endocytosis	Bleul et al. (2013)
Poly (ethylene glycol)	Doxorubicin	Methoxy/folate groups	23 × 200 nm	Hela human cervical tumor cell	Folate receptor-mediated endocytosis	Yang et al. (2010)
Oleylamin		Protoporphyrin, trastuzumab	7 nm	Breast cancer cells	Trastuzumab –human epidermal growth factor-2 receptor mediated	Khaniabadi et al. (2020)
Glycogen		Gadolinium chelate, near infrared fluorescent dye.		Tumor in rat	Passive accumulation	Gálisová et al. (2020)

10.2.4 Quantum Dots-Loaded Polymeric Nanoparticles

Quantum dots are inorganic nanoscale (2–10 nm) semiconductor crystals that can transport electrons. They have exclusive optical and electronic attributes that differ from bulk particles (Valizadeh et al. 2012). They show strong photoluminescence in ultraviolet light, as an electron in quantum dots can be excited to conductance band from valance band, which then drops back to valance band, and release energy in the form of visible light. The colour of the light depends on the distance between the valance and conduction band. Quantum dots are gaining attention as a diagnostic and imaging agent, as the emitted light is more stable and brighter against photo bleaching than standard fluorescent indicators. However, quantum dots have shown signs of toxicity by the generation of reactive oxygen species (Walling et al. 2009). To enhance their biocompatibility, quantum dots are further hybridized with other agents such as polymers, proteins, polysaccharides, or lipids. The generation of hybridized quantum dot moieties offers improved cellular targeting and uptake with controlled release and prolonged circulation in the blood (Zayed et al. 2019).

Nifontova et al. reported fluorescent live-cell targeting agent quantum dot microcapsules, surface-functionalized with human epidermal growth factor receptor 2 targeting ligand, trastuzumab in breast cancer cells. The microcapsules were prepared by layer deposition of polymers and quantum dot solution on a calcium carbonate nanoparticle template. Polyallylamine hydrochloride and poly (styrene-sulfonate) are oppositely charged polymers. Negatively charged quantum dots aqueous solutions were used for coating sequentially, and the surface of the resultant sphere was activated with carbodiimide to enhance optical and dispersion properties (Nifontova et al. 2019). Mesoporous and hollow carbon spheres were reported to be synthesized using pyrrole and aniline precursors, wherein nitrogen-doped quantum dots were trapped. *In vitro* results proved that human oral cancer cells show good fluorescence quantum yield with a significant photothermal ablation effect on the infrared laser (Das et al. 2019).

Gold shell with quantum dot core is an effective combination with enhanced photostability of quantum dots, high surface plasmon absorption, and light scattering property. In another study, poly (N-hydroxyethyl)-dl-aspartame polymeric micelles were surface tagged with lipoic acid and folic acid and hydrophilic chains of polyethylene glycol. The polymeric micelle carrier was further loaded with antitumor drug (doxorubicin) and core-shell gold quantum dots for drug delivery and photothermal action along with real-time imaging. The whole system was found to be very effective against the breast cancer cell lines (MCF-7) (Li Volsi et al. 2018).

Beta-cyclodextrin nanosponges (Nagma et al. 2020) were hybridized with carbon quantum dots and loaded with doxorubicin. The 300 nm nanosponges showed high biocompatibility, bright blue fluorescence yield, and pH-sensitive drug delivery properties. *In-vitro* studies on hepatic cancer cells (HepG2) showed appreciable drug accumulation and tumor regression (Pei et al. 2018). Indium phosphide/zinc sulfide quantum dots core was conjugated with co-polymers poly lactide-poly (ethylene glycol) to form a hybrid micelle and was additionally integrated with

anti-epidermal growth factor receptor targeting moiety and the drug, aminoflavone. The quantum dot core expressed stable near-infrared fluorescence for optical imaging, encapsulated amino-flavone drug was effective against the tumor with no observed adverse effects (Wang et al. 2017).

Nanohybrids for gene therapy and imaging were formulated by encapsulation of quantum dot in (ethanolamine-functionalized poly (glycidyl methacrylate)) functionalized dextran. The use of hydrophilic ethanolamine-functionalized poly (glycidyl methacrylate))-dextran carrier resulted in biocompatibility and pH-responsive self-destruction characteristics. The system showed effective delivery of the anticancer p53 gene in breast cancer as tracked live by quantum dot fluorescent guided imaging. The self-destruction of vesicles led to effective gene transfection and showed effective tumor suppression (Liu et al. 2019).

In photothermal therapy, the near-infrared laser intensity declines before reaching deeply located tumors. To overcome this limitation, photothermal therapy is combined with radiation therapy to cause local DNA damage to the tumor. Polyaniline and molybdenum disulfide quantum dot nanohybrids have been developed to enhance imaging, photothermal therapy, and radiation therapy. The enhanced oxygenation under mild photothermal therapy enhanced the effect of radiation therapy (Wang et al. 2016). Prussian blue has high efficiency in absorbing near-infrared and has gained approval for radioactive treatments. Magnetic prussian blue nanoparticles were functionalized with hyaluronic acid and bovine serum albumin-coated quantum dots to significantly enhance fluorescence and infrared imaging. Enhanced real-time imaging proved accumulation in target cancer cells which led to successful and effective ablated photothermal therapy. The presence of hyaluronic acid increased the internalization efficiency by CD44 overexpressed cervical cancer cells (HeLa) in the presence of external magnetic field (Yang et al. 2017) (Table 10.5).

10.2.5 Polymeric Nanoparticles Containing Dendrimers

Dendrimers are symmetrically repetitive, extensively branched chemical structures known for their structural perfection. They have a core surrounded by symmetric branched structures in all directions, giving it a three-dimensional spherical morphology. These molecular properties and functionality depend on the functional groups and molecular moieties attached to the core branches. Their accessibility to functionalization surface, monodispersity, and compound encapsulation capacity renders them potential theranostic agents (Cabral and Kataoka 2010). The structure of the dendrimer is divided into three main parts: (i) core, (ii) inner shell, and (iii) outer shell. They can be designed to have different properties according to the attachments used (Table 10.6). The synthesis of dendrimers is an elaborate multistep process and requires high precision to protect the active sites (Jain and Jain 2016).

Difficulty in loading of anticancer drug, cisplatin has been reported in large polymeric vesicles due to steric hindrance. Nguyen et al. synthesized carboxylated polyamidoamine dendrimer and sonicated them in the presence of cisplatin, which

Table 10.5 Polymeric quantum dots in various formulations for cancer theranostics

Polymer	Use	Additions	Size	Cell line	Uptake	References
Polyallylamine hydrochloride and poly(styrene sulfonate)	Fluorescence imaging	Quantum dots, trastuzumab (monoclonal antibody)		Breast cancer cells	Endocytosis	Nifontova et al. (2019)
Pyrrole and aniline precursors	Fluorescence imaging, photothermal effect	Nitrogen-doped quantum dots	~65–70 nm	Human oral cancer cells	Endocytosis	Das et al. (2019)
Polymeric micelles of α,β-poly(N-hydroxy ethyl)-aspartamide with lipoic acid; functionalized with polyethylene glycol, and folic acid	Fluorescence imaging, photothermal effect, drug delivery	Doxorubicin, gold core-shell quantum dots	~100 nm	Human breast cancer cells (MCF-7)	Endocytosis	Li Volsi et al. (2018)
B-cyclodextrin	Fluorescence imaging, drug delivery	Carbon quantum dots, doxorubicin	~300 nm	Immortal cell line (HepG2 cells)	Endocytosis	Pei et al. (2018)
Polylactideb-poly(ethylene glycol)	Fluorescence imaging, drug delivery	Indium phosphide/ zinc sulfide quantum dots, anti-epidermal growth factor receptor) nanobodies, aminoflavone	~20 nm	Epithelial growth factor receptor overexpressing breast cancer cells	Endocytosis	Wang et al. (2017)
Poly(glycidyl methacrylate)-functionalized dextran	Fluorescence imaging, gene therapy	Quantum dot, anti-oncogene p53	~320 nm	Breast cancer	Endocytosis	Liu et al. (2019)
Polyaniline	Fluorescence imaging, photothermal/ radiation therapy	Molybdenum disulfide quantum dots	~20 nm	Murine breast cancer cells (4T1 cells)	Endocytosis	Wang et al. (2016)
Hyaluronic acid and bovine serum albumin coated	Fluorescence imaging, photothermal treatment	Copper indium disulfide-zinc sulfide) quantum dots, magnetic prussian blue ferric oxide) nanoparticles		Hela cells	Endocytosis	Yang et al. (2017)

Table 10.6 Commonly-used moieties are used to make the core and exterior of the dendrimer structure

Sl.No	Core	Exterior functional groups
1	Polypropylene	Amine
2	Polyamidoamine	Carboxyl
3	Diaminobutyl	Alcoholic
4	Ethylenediamine	Amine

increased the drug retention capacity of the dendrimer carrier. *In vitro* studies showed increased biocompatibility and effectiveness in the suppression of lung cancer (Nguyen et al. 2015). To decrease the toxicity of the magnetic resonance imaging contrast agents, Mekonnen et al. prepared polyamidoamine dendrimers containing superparamagnetic gadolinium-ferrite nanoparticles. *In vitro* studies confirmed the absence of toxicity, controlled drug release with effective cancer tumor suppression by magnetic resonance imaging-guided delivery in the presence of an external alternating magnetic field (Mekonnen et al. 2019).

A combination of superparamagnetic ferrosoferric oxide nanoparticles with polyamidoamine dendrimers exhibited significant photothermal properties along with chemotherapeutic outcomes. Superior contrast properties in magnetic resonance live imaging were also noted (Jędrzak et al. 2019). Poly (amido amine) is currently one of the most widely used materials to prepare dendrimers. Fan et al. designed a phosphorus dendrimer-based copper complex without any chemotherapeutic drug, which caused the suppression of pancreatic cancer cell line by inducing apoptosis. Ultrasound was used to enhance the cellular uptake of nanoparticles and to induce a chemotherapeutic effect (Fan et al. 2020).

Xiong et al. devised a theranostic messenger RNA carrier system for simultaneous cancer detection and anticancer messenger RNA delivery. Messenger ribonucleic acid is emerging as potential anticancer agent. Lipid based nano dendrimers demonstrated pH-responsive noninvasive magnetic resonance imaging (Xiong et al. 2020). Some promising combinations of polyamidoamine dendrimers with differential applications are reported such as amine conjugated quantum dot for imaging (Ghosh et al. 2019), copper nanoparticles for radiotherapy (Fan et al. 2019), gold nanoparticles for photothermal effect and imaging (Kesharwani et al. 2019) and aptamer attached a nano luminescence probe for drug delivery (Zhang et al. 2020) (Table 10.7).

10.2.6 Polymeric Nanoparticles Along with Carbon Nanotubes

Carbon nanotubes are cylindrical sheets of graphene which have excellent physical, chemical, and mechanical properties. These have attracted exceptional interest in the field of drug delivery and cancer therapy. Based on the number of graphene

Table 10.7 Polymeric dendrimers in various combinatorial preparations for cancer theranostics

Polymer	Use	Additions	Size	Cell line	Uptake	References
Carboxylated poly(amidoamine) dendrimer	Drug delivery	Cisplatin	3–8 nm	Lung cancer cell	Endocytosis	Nguyen et al. (2015)
Poly(amidoamine) dendrimer	Drug delivery, magnetic resonance imaging	Gadolinium/iron oxide nanoparticles, doxorubicin	92 nm	HeLa cells	Endocytosis	Mekonnen et al. (2019)
Poly (amidoamine) dendrimers	Magnetic resonance imaging, chemo- and photothermal therapy	Polydopamine coated ferrosoferric oxide nanoparticles, doxorubicin	4–7 nm	Liver cancer cells	Endocytosis	Jędrzak et al. (2019)
Phosphorus dendrimer-copper complex	Magnetic resonance imaging, therapy	–	1–8 nm	Pancreatic cancer cell line	Endocytosis	Fan et al. (2020)
Dendrimer -based lipid nanoparticle	Deliver messenger RNA, detect cancer	Messenger RNA	10 nm	Liver cancer cells	Endocytosis	Xiong et al. (2020)

sheets used in a single nanotube, carbon nanotubes are regarded as single-wall carbon nanotubes or multi-wall carbon nanotubes. With rapid development and application in recent years, it is also exploited in the field of nanomedicines. Although being spherical in structure, these carbon nanotubes have different mechanisms to interact with biological cells. Due to ease of functionalization, carbon nanotubes have the ability to bind drugs or biological cells in multiple ways. Carbon nanotubes also have a tremendous ability to interact with aromatic molecules and hydrophobic drugs via supramolecular interactions, which mainly rely on π-π interactions and weak van der Waals forces.

Though carbon nanotubes have been extensively studied and explored for their applications, yet they have so many drawbacks like insolubility issues, toxicity and safety concerns, difficulty to purify, and involvement of complex mechanisms in the interaction between carbon nanotubes and biological systems. To overcome some of these stated issues, the polymer-based approach appears to be more promising, wherein carbon nanotubes are conjugated with the polymeric system via the covalent or non-covalent bond. One of the major drawbacks associated with carbon nanotubes is their short half-life in blood circulation and uptake by the reticuloendothelial system.

The PEGylation of carbon nanotubes plays an important role because PEGylated carbon nanotubes-based nanomaterials have a long circulation time, where poly (ethylene glycol) as such helps to lower the immunogenicity of carbon nanotubes thus preventing their non-specific uptake by the reticuloendothelial system. There are some reports which reveal that PEGylated carbon nanotubes could accumulate within spleen and liver macrophages for 16 weeks with good compatibility (Schipper et al. 2008). In 2006, Liu et al. (2007) synthesized a polymeric conjugated carbon nanotubes based system to incorporate various functional species such as therapeutic agents (drugs), targeting moiety, and diagnosis agents (dyes). They reported that chemotherapeutic molecules such as doxorubicin and daunorubicin along with diagnostic agents bind with the pre-functionalized single walled carbon nanotubes via non-covalent interaction (π-π stacking). They used this theranostic system for pH controlled targeted drug delivery and as an imaging platform for cancer detection. To deliver the drug to the cancer cells, poly (amidoamine) is known to be another polymeric material which has been used as a carrier. Poly (amidoamine) based dendrimers have been widely exploited for cancer therapy. Shi et al. reported the use of poly (amidoamine) dendrimers to bind multi-wall carbon nanotubes for targeting as well as imaging the cancer cells. They used folic acid as a targeting moiety and fluorescein isothiocyanate for imaging purposes (Shi et al. 2009).

Polyethyleneimine is a cationic polymer frequently used for the administration of small interfering RNA. Shen et al. conjugated polyethyleneimine to multi-walled carbon nanotubes, and then these polymeric- multi-walled carbon nanotubes conjugates were subjected to *in vitro* cytotoxicity evaluation against human thyroid cancer cell line (FRO cells). Results demonstrated that the surface characteristic of the conjugated nanomaterials played an important role in their biocompatibility which were easily altered by chemical modification (Mingwu et al. 2009).

Some of the researchers also employed block-copolymer to enhance the solubility and compatibility of carbon nanotubes (Adeli et al. 2011). One of the best examples of this application is poly citric acid–polyethylene glycol–poly citric acid-based tri-block copolymer which has been used for functionalization of multi-walled carbon nanotubes and was further conjugated with an anticancer drug, cisplatin. The resulting nanomaterial hybrid showed higher toxicity than free drug when subjected to endocytosis and cisplatin release within murine colon adenocarcinoma tumor cell lines (C26). The same group of researchers also synthesized a magnetic carbon nanotubes-based polymeric theranostic system, where they deposited iron oxide nanoparticles onto the surface of multi-wall carbon nanotubes. Tri-block copolymer of poly citric acid and polyethylene glycol was used in a previously reported manner. Again they used cisplatin as an anticancer prodrug and non-covalently conjugated it with the magnetic nanoparticle nanohybrid system. Along with water-solubility and biocompatibility, these magnetic nanoparticles carbon nanotube-based polymeric nanohybrid systems also showed tumor-targeting ability under the influence of an external magnetic field. The result found that these systems have effective cytotoxicity efficacy in the connective fibroblast adhesive cell line (L929) group (Mehdipoor et al. 2011).

10.2.7 Miscellaneous Carriers

10.2.7.1 Polymeric Liposomes

Liposomes are spherical vesicles of cholesterol and phospholipids having unilamellar or multivesicular lipid bilayer structures. They are composed of similar components as biological cells. Owing to their bilayer structure, these vesicles have the ability to contain both hydrophobic and hydrophilic drugs and other molecules. They are strongly biocompatible and non-toxic to the cells. They showed enhanced permeability and retention effect and accumulated passively at cancer sites owing to their nano-size range. But there are some limitations associated with liposomes as they are quite unstable, show very slow drug release kinetics with drug leakage from the vesicles. To overcome these problems, the surface of these liposomes is coated with polymeric materials like poly (ethylene glycol), hyaluronic acid, or other polymers. These liposomes are called polymeric-liposomes.

In the year 2014, Chiang et al. synthesized stable, pH-sensitive, and extracellular matrix targeted polymeric liposomes. They used methoxy-poly (ethylene glycol)–b–poly (N-2-hydroxypropyl methacrylamide-co-histidine)-cholesterol as copolymer and biotin 2-polyethylene glycol as a crosslinker. These polymeric liposomes had a better antitumor activity with low systemic toxicity in the liver and renal tubules (Chiang and Lo 2014). To enhance the stability of liposomes, they were coated with natural polymers such as chitosan and hyaluronic acid. In the case of CD44 over-expressing cancer cells, hyaluronic acid is widely used. Accumulation

of poly (ethylene glycol)- hyaluronic acid liposomeS in solid tumors was noted to be similar to poly (ethylene glycol) liposomes (Qhattal et al. 2014).

10.2.7.2 Polymersomes

Polymersomes are defined as self-assembled synthetic amphiphilic vesicles of block co-polymer, enclosing both hydrophilic and hydrophobic functional moieties. As compared to liposomes which are mainly composed of phospholipids, polymersomes possess significant biological properties with superior stability and extended circulation time. Moreover, the surface properties of the block-copolymer can be manipulated by functionalization with various active moieties. Structural properties of the polymersomes are defined by the ratio of hydrophilic to hydrophobic block volume fraction. These may be micelles shaped like spherical, prolate, or oblate, or vesicles shaped polymersomes (Levine et al. 2008). These polymersomes can work as theranostic nanomaterials. As an example, amphiphilic block co-polymers such as poly (ethylene oxide)-block-poly(butadiene), when loaded with porphyrin-based fluorescence imaging with near-infrared, could produce a signal with sufficient intensity to penetrate through a tumor. This encapsulation process produces a polymeric complex that can be utilized for *in vitro* diagnosis and *in vivo* cellular tracking (Bermudez et al. 2002).

10.3 Polymeric Nanoparticles in Clinical Trials

Many of the polymeric nanoparticles already got approval from the Food and Drug Administration, and also a large number of polymeric nanoparticles are in phase-II or phase-III clinical trials. The first-ever polymer-based nanoformulation, which was approved by the food and drug administration for cancer therapy is Doxil® containing doxorubicin hydrochloride. This is a liposomal nanoformulation (80 nm) that contains doxorubicin as a chemotherapeutic drug encapsulated with poly (ethylene glycol) polymer to enhance water solubility and biocompatibility of the formulation (Gewirtz 1999).

Another example of PEGylated formulation is NKTR-102, which contains irinotecan, an anticancer drug that is in phase-III of clinical trials. The results of the trials showed that the PEGylation of these nanomaterials enhanced circulation time. More exposure to cancerous cells ultimately increased the therapeutic efficacy (Awada et al. 2013). Besides PEGylation, other hydrophilic polymers were used to enhance the circulation time of drugs. A polymeric nanoparticle formulation of polyglutamic acid conjugated with paclitaxel is currently under phase III clinical trials. Results showed a significant increment in the life expectancy of patients having non-small cell lung cancer when treated with this formulation (Paz-Ares et al. 2008). Researchers have reported synthesizing hydrophobic polymer-based nanoparticles which provided controlled and sustained release of the encapsulated drugs.

Poly (lactic-co-glycolic acid) is one of the best examples of the polymer used under this category. A nanoformulation based on poly (lactic-co-glycolic acid) is Eligard®, which contains the drug, leuprolide and is used for the treatment of prostate cancer (Berges 2005). Some micellar-based polymeric nanoformulations are under clinical trials. A polymeric micelle of poly (ethylene oxide) 5000-b-polyaspartic acid 4000 contains doxorubicin in its core as an anticancer drug and is currently under phase II clinical trials in Japan. The study suggested that the formulation was found to be very effective in animal models with fewer side effects (Matsumura et al. 2004). Another polymeric micelle-based formulation is poly (ethylene oxide) 2000 – poly (D, L-lactide) micelles loaded with paclitaxel. Results revealed that these micellar formulations had a three times higher maximum tolerated dose as compared to the non-micellar formulation of taxols (Kim et al. 2001). Camptothecin, an anticancer drug used to inhibit DNA topoisomerase-I, was encapsulated in cyclodextrin-polyethylene co-polymers. The formed nanoparticles were in a size range of 20–50 nm. These polymeric nanoparticles were named CRLX101 and licensed by Cerulean Pharma Inc. They have been evaluated following phase I and phase II clinical studies and are used in ovarian, rectal, and peritoneal cancer patients (Svenson et al. 2011) (Table 10.8).

10.4 Conclusion

Polymeric nanoparticles could be a powerful tool as theranostics for cancer treatment and diagnosis. These are the safe and biologically relevant molecules that can act as drug carriers in cancer therapy. Polymeric nanoparticles have proven to be successful candidates to mitigate cancer also its diagnosis and management. These nanoparticles are not only used for cancer therapy but also have the scope in the field of gene and radiation therapy. They have a promising future to deliver drugs, proteins, genes, vaccines, and antibiotics to the targeted area of cells. Even though these nanoparticles showed potential results in cancer treatment, still more research studies are needful as these nanoparticles are not specific to detect cancer at the early stages. Apart from this, they also suffer selectivity and specificity issues in some cases, like brain cancer. Nevertheless, these nanoparticles can be used as a platform strategy for a variety of biomedical applications. It also needs to be noted that some of the polymeric nanoparticles have a complex structure that can be difficult to handle during the synthesis and preparation process. Also, it is essential to evaluate the pharmacokinetics and pharmaco-dynamics in a detailed manner. Lastly, all the preclinical and clinical trials need to be done appropriately in a large number of cancer patients. At the end of the day, the usage of polymeric nanoparticles may save the lives of millions and billions of peoples in the near future. A list of recently engineered polymeric nanoparticle as theranostics is enlisted in Table 10.9.

Table 10.8 Polymeric nanoparticles in clinical trials

Nanocarriers	Name	Polymer	Therapeutic drugs	Application target	Status
Polymeric nanoparticles	BIND-014	Polyethylene glycol-poly (lactide-co-glycolic acid)	Docetaxel	Lung cancer	Phase II
	Nanotax		Paclitaxel	Neoplasm	Phase I
	ABI-008	Albumin	Docetaxel	Breast and prostate cancers	Phase II
	CALAA-01	Cyclodextrin	Small interfering RNA	Solid tumors	Phase I
	ProLindac	2-Hydroxypropyl methacrylate	Dichloro (1,2-diaminocyclohexane) platinum (II)	Ovarian cancer	Phase I
	Docetaxel-P nanoparticles	–	Docetaxel	Solid tumors	Phase I
	ABI-009	Albumin	Rapamycin	Solid tumors	Phase II
	Mitoxantrone-loaded poly butyl cyanacrylate nanoparticles	–	Mitoxantrone	Hepatocellular carcinoma	Phase II
	BA-003	–	Doxorubicin	Hepatocellular carcinoma	Phase III
Polymeric micelles	Paclical	–	Paclitaxel	Ovarian cancer	Phase III
	SP1049C anthracycline chemotherapeutic agent	Pluronic L61 & F 127	Doxorubicin	Lung cancer	Phase II
	NC-4016	–	Oxaliplatin	Solid tumors	Phase I
	NC-6300	Polyethylene glycol-b-Poly (allylamine hydrochloride)	Epirubicin	Solid tumors	Phase I
	NK012 polymeric micelle formulation	Polyethylene glycol–poly (glycolic acid)	SN-38 (antineoplastic drug)	Lung and colorectal cancer	Phase II
	NC-6004	Polyethylene glycol-poly(glycolic acid)	Cisplatin	Gastrointestinal and genitourinary cancer	Phase III
	NK105	Poly (ethylene glycol) – per acetic acid	Paclitaxel	Breast and gastric cancer	Phase III
	NK911	Poly (ethylene glycol) – per acetic acid	Doxorubicin	Solid tumors	Phase I
	Lipotecan	–	Camptothecin	Liver and renal cancer	Phase II

Table 10.9 Recent engineered approaches using polymeric nanoparticles in cancer therapeutics

Polymers	Modifications	Model/ cell line	Remarks	References
Poly(lactic-co-glycolic) acid	Poly(lactic-co-glycolic acid) hybrid carbon nanodots	Breast cancer (MDA-MB-231)	Photothermal and fluorescent properties with good drug loading capacity.	Mauro et al. (2020)
	Poly (lactic-coglycolic) acid-poly (N-isopropyl acrylamide) porous nanoparticles with drug toxol	Human liver cancer cell lines (HepG2 cell line)	Great surface functionalization and payload capacity.	Amgoth et al. (2020)
	Poly (lactic-co-glycolic acid) nanoparticles with bovine serum albumin functionalized to delivery near infrared dye indocyanine green and drug doxorubicin	EMT-6 tumors in nude mice	Guided chemo photothermal cancer theranostics	Shen et al. (2019)
	Poly (lactic-coglycolic) acid nanoparticles embedded with molybdenum octahedral cluster	Ovarian cancer cell line	Promising photosensitization in photodynamic therapy.	Brandhonneur et al. (2018)
	Poly (lactic-co-glycolic acid) nanoparticles tagged with vascular endothelial growth factor targeted small interfering RNA encapsulating quantum dots, superparamagnetic ferrosoferric oxide nanoparticles, and doxorubicin	HeLa cells (immortal cancerous cells)	Shows great potential for tumor-specific targeting, drug/gene (small interfering RNA) delivery and, magnetic resonance and fluorescence cancer imaging	Shen et al. (2017)
Poly-caprolactone	Poly-caprolactone/gelation scaffold fortified with Chlorin e6 and graphene oxide with protonated graphitic carbon nitride	Breast cancer cells	Chlorin e6 is sono/photosensitizer and carbon nitride boost electrostatic interactions enhance inactivation of cancer cells.	Sun et al. (2020a, b)
	Poly-caprolactone-poly(amidoamine) linear dendrimers with near infrared theranostic agent (C3)	S2 melanoma cells	Cancer cells with C_3 when exposed to near-infrared light cause effective cell death	Chandrasiri et al. (2020)
	Polyethylene imine-poly-caprolactone and di ethylene triamine pentaacetic acid gadolinium (III)-polyethylene glycol-polycaprolactone hybrid micelle carrying doxorubicin and microRNA-34a	Breast cancer cell lines (MDA-MB-231)	Gadolinium-enhanced the magnetic resonance imaging and micelle accumulation boosts the anticancer effect of drug doxorubicin and therapeutic gene miR-34a	Xie et al. (2019a, b)
	Poly-caprolactone nanoparticles with gold nanoparticles and iron hydroxide nanoparticles and anticancer drugs	Mice tumor *in-vivo*	Effective chemo-photothermal anticancer effect by doxorubicin and docetaxel drugs, and near-infrared, computed tomography, and magnetic resonance photothermal effect of gold and iron nanoparticles	Zhang et al. (2018)

Chitosan	Chitosan nanoparticles conjugated with octadecanoic acid and gadopentetic acid, loaded with chlorin e6	4 T1 cells tumor model	Powerful magnetic resonance guided cancer inhibition by phototherapy compared to Gd-DTPA model	Zhao et al. (2020)
	Chitosan-acrylic acid/multiwalled carbon nanotubes with adsorption of scandium (III)	–	The quality control tests verify high purity and validity for cancer theranostics	Gizawy et al. (2020)
	Ferrosoferric oxide nanoparticles coated with folic acid-chitosan to deliver curcumin and 5-fluorouracil	Folate receptor-positive, human breast cancer (MDA-MB-231) cell lines	Showed good magnetic resonance imaging contrast, uptake, and anticancer release	Nejadshafiee et al. (2019)
	Gold octahedron core and porous shell in chitosan nanoparticles carrying drug	Breast cancer cell (MCF-7 cells)	Near-infrared stimuli-based drug-delivery, Photothermal therapy, surface-enhanced raman scattering bioimaging	Sarkar et al. (2019)
Inorgani cnanoparticles	Silica nanoparticles coated with bovine serum albumin-based gadolinium/gold nanoparticles and a folate targeting agent carrying doxorubicin	KB human oral epidermoid carcinoma	Effective targeting and magnetic resonance/ fluorescence imaging capacity	Yang et al. (2019)
	Triplet tellurophene-based semiconducting polymer nanoparticles	4 T1 tumor cells	Great infrared absorption and low cytotoxicity	Wen et al. (2019)
	Titanium nanosheets	Human hepatocellular carcinoma cells (SMMC-7721)	Titanium nanoparticles are biocompatible and have a strong absorption ability for imaging and photothermal therapy	Xie et al. (2019a, b)

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Chapter 11
Oral Delivery of Polymeric Nanoparticles for Solid Tumors

Priyanka Dash, Sayantan Ghosh, and Bismita Nayak

11.1 Introduction

The burden of non-communicable and chronic diseases has contributed to the increase of global death or disability, with higher frequencies of cancer-related deaths (9.8 million) as estimated by the World Health Organisation in 2018. Non-small cell lung cancer represents the standard form of lung cancer accompanied with the highest incidences of mortality followed by breast, prostate, cervical, thyroid, and colon cancer, respectively (Gridelli et al. 2015; Ahuja et al. 2020).

Conventional cancer therapy includes radiation, surgical resection, chemotherapy, or polytherapy of these effective treatment options. The traditional therapies primarily interfere with the DNA replication and transcription of fast-growing cancer cells, causing their death (D'Souza et al. 2016). The inevitable destruction of healthy cells during chemotherapy and use of high anticancer drug doses results in aggravating cellular toxicity driven development of drug resistance risk which further results in mortality among cancer patients (D'Souza et al. 2016).

Over the years, enormous strides have been made in the field of biomedical research that has resulted in remarkable progress towards the understanding of the underlying pathophysiology and mechanisms associated with a broader range of life-threatening diseases. Fueled with this knowledge, the most promising novel and innovative therapy-based regimen are developed that could have a significant positive impact on chronic disease management and healthcare enhancement. These therapies majorly belong to the pharmaceutical class i.e., biologics, including growth factors, peptides, nucleic acids, and so forth. Moreover, an amalgamation of

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active pharmaceutical ingredients with material science has paved the way for the engineering and clinical development of nanomedicines, as a start-up of a new promising discipline in the twenty-first century.

This development encompasses the utilization of particles at the nanoscale level, which possibly diverge from the larger-scale particles of the same materials used for a nano-scale particular assembly. The era of flexible nanoscale-driven biotechnological advances could equip real-time developments in idioms of effective and cost-efficient healthcare services, forming an axial element in remedies fabrication and making medications accessible and affordable (Khalid and El-Sawy 2017). All nanocarriers such as nanoemulsions, polymeric nanoparticles, lipid based nanoparticles, bimetallic nanoparticles, quantum dots, etc. designed for implementation in the biomedical field are biocompatible and also display a highly targeted delivery of drug molecules in diseased cells without affecting the healthy ones (Chamundeeswari et al. 2019).

Moreover, they are recognized by their prolonged circulating half-life and extended shelf life. Diversified chronic disease burden would be beneficial from the outstanding application of nanomedicine, requiring prolonged treatment. Despite these promising findings, the administration of these emerging nano-therapeutics is currently restricted to continuous parenteral methods. Injection-based administration severely faces poor patient compliance and reduced therapeutic efficacy associated with adverse reactions and greater complaints of side-effects. Other administration routes, such as buccal, pulmonary, oral, nasal, rectal, ocular, and so forth, are currently under investigation to improve their therapeutic efficacy. Among these approaches, Oral drug delivery remains the most convenient and comfortable route of drug administration due to its non-invasiveness, ease of ingestion, low cost, the design flexibility of the dose formulations, and most importantly, high and improved patient compliance (Homayun et al. 2019). In terms of clinical trial studies, oral drug administration has proved its efficient safety profile due to favorable pharmacokinetics in critically-ill patients (Taheri et al. 2019). With major advances made in areas of molecular biology and biotechnology, the realm of therapeutic compounds repertoire has extended beyond small molecule compounds to include biological macromolecules, large supramolecular structures proteins, peptides, nanomedicine, and even intact whole cells or tissue, facilitating their large scale production.

However, the oral drug delivery of these biological therapeutics possesses substantial challenges in achieving convenient and efficient clinical outcomes due to the multitude of formidable biological barriers, inherent instability, and low permeability posed by the gastrointestinal tract that a small-therapeutic molecule must navigate through. Hence, the improved oral bioavailability of such therapeutic compounds requires the design of an ideal oral drug delivery system prior to successful human clinical trial studies with an in-depth understanding of intestinal physiology. At present, extensive and robust research on biopolymers together with nanotechnology as an oral drug delivery framework have ideally attracted considerable attention because of their exceptional non-immunogenic property and thereby

significantly improving the pharmacokinetics of orally administered pharmaceutical agents (Patra et al. 2018). To date, several biopolymeric nanoparticles based chemotherapeutics have been designed, but the majority of them are diffused into the body *via* intravenous infusion. Hence, the field of oncology therapy still is in dire need of developing oral delivery platforms. Potential oral delivery platforms reduce the drug toxicity by adjusting the therapeutic doses (*via* dose banding) to maintain strong anti-cancer activity without elevating toxicity to healthy cells. Several novel biopolymeric-based nanocarriers, such as nanomicelles, nanoliposomes, and so forth represent the new face in drug delivery and cancer therapy which ensures the protection of chemotherapeutic drugs from gastric degradation with improved bioavailability (Chivere et al. 2020). Furthermore, the development of surface-modified oral delivery platforms impart site-specific and targeted drug delivery (Fig. 11.1).

This review presents the physiology of the gastrointestinal tract and summarize the multitude of intestinal barriers to drug absorption and provide a concise incursion into the use of biopolymeric nanoparticles strategies to overcome such barriers. Current advances in the use of biopolymers as oral delivery platforms against solid tumors are also discussed in association with future opportunities.

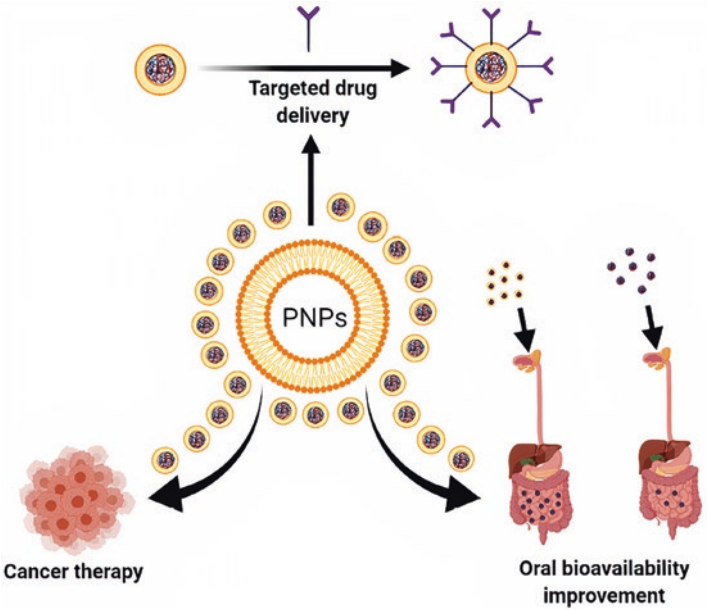

Fig. 11.1 Basic polymeric nanoparticles (PNP) backbone, including its versatile characteristics and its applications. Polymeric nanoparticles improves oral bioavailability and targeted drug delivery for cancer therapy

11.2 Gastrointestinal Tract Physiology

The use of particulate oral-based delivery systems can be used to treat an assortment of gastrointestinal tract disorders locally or to facilitate active biologic therapeutics to the systemic circulation. After oral administration, the therapeutics must pass through the gastrointestinal tract and then can be readily absorbed into the bloodstream. The gastrointestinal tract functions as a compartmentalized mechanochemical processing center for ingested food sources, and its lining forms a formidable barrier against any potential invading pathogens and the by-products of digestion. The upper small intestine or small bowel is the primary site that regulates the absorption of nutrients extracted from food through specific transport pathways. The presence of macroscopic folds and microscopic finger-like projections (called villi) in the internal surface significantly leads to a high absorption surface area which forms a primary target site for drug absorption (Tortora and Petti 2002).

The intestine wall (also known as the brush border) is covered with an epithelial layer organized into long folds arranged circularly or helical, known as circular folds (plicacirculares). The epithelium cells of the villi as a polarized cell monolayer acts as a tightly regulated barrier to mediate the selective transport of intestinal luminal materials to the lamina propria (Chen et al. 2011). The villi of lamina propria extend into a rich blood capillary network that aids in the absorption of molecules, and the presence of a blunt-ended lymphatic vessel called a lacteal in the center of the villi facilitates the absorption of larger particles such as fats and fat-soluble vitamins. The hair-like projections on the apical surface of enterocytes called microvilli increase the absorptive surface area (Pridgen et al. 2015). Another specialized microfoldcells (also known as M cells) are found in association with the gut-associated lymphoid tissue of the intestinal peyer's patches and mucosal-associated lymphoid tissue. M cells function to transport luminal microbial antigens to sub-epithelium *via* transcytosis for efficient systemic immune responses (Dillon and Lo 2019).

11.3 Physiological Barriers as Hurdles for the Oral Delivery of Chemotherapeutics

The major hurdles and limitations faced by oral drug delivery systems are a result of complex obstacles (pH environments, enzymatic degradation and poor permeability) presented by the gastrointestinal tract anatomy, biochemistry, and physiology. The skin represents the primary and the largest epithelial interface (approximately $2m^2$) between the human body and its external environment. So designed primarily to facilitate the assimilation of complex nutrients, electrolytes and fluids, the healthy gastrointestinal tract must also simultaneously function as the crucial first line of defense includes biochemical, cellular permeability, and mucus

diffusional to an intruding pathogen and its exogenous toxins, as it has a larger surface area of 300–400 m^2 (Walker and Owen 1990).

Therefore, the administration of biologics *via* the oral route poses significant clinical challenges and disadvantages. The swallowed drug enters the gastrointestinal tract regions and is released at the intestinal site and further proceeds by diffusing the mucus layer (Fig. 11.2) (Ahadian et al. 2020). In contrast to the colon and stomach, the mucus layer in the regions of the small intestine is thin and discontinuous. In addition, the presence of absorption barriers in the intestinal epithelium also limits the intestinal transportation of such drugs. Thus, to design efficient and effective oral delivery platforms, the understanding of the different characteristics of these barriers needs to be considered (Table 11.1). The drug absorption site is determined by the drug type, driven by local environmental conditions such as pH, enzymes, mucosal barriers, drug residence time, and surface area of the gastrointestinal tract.

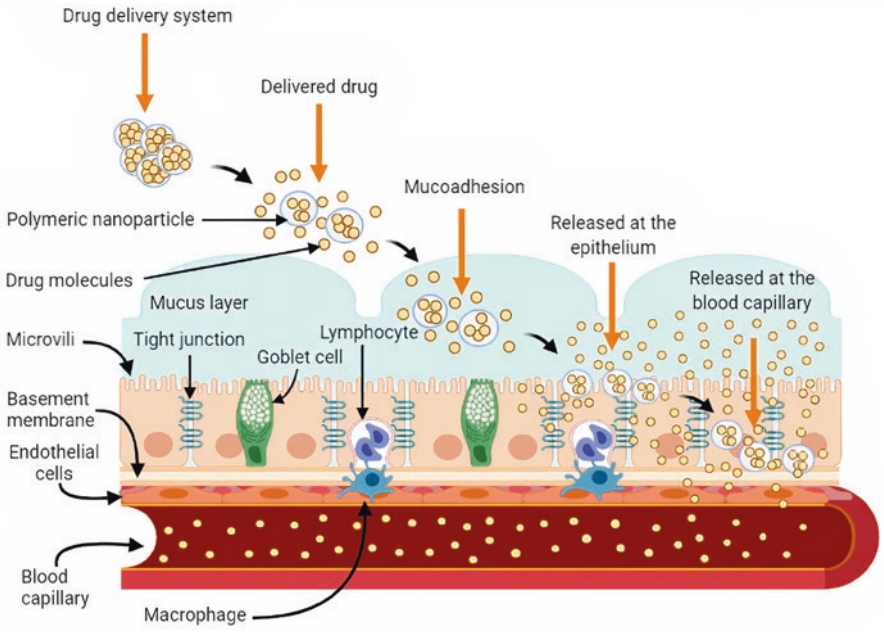

Fig. 11.2 Structure of the intestinal epithelium and mechanism involved in the uptake of orally administrated drug into the gastrointestinal tract regions. The drug released at the intestinal site gets diffused into the mucus layer followed by its absorbtion into the large surface area of the intestinal epithelium. Reproduced from "Micro and nanoscale technologies in oral drug delivery", Ahadian et al. (2020)

Table 11.1 Physical characteristics of the gastrointestinal tract in normal adults

Organs of body	pH	Length (cm)	Mucus thickness (μm)	Mucus turnover rate (hours)
Stomach	1.0–5.5	20–25	245 ± 200	24–48
Duodenum	5.0–7.0	20–30	16.0 ± 0.5	24–48
Jejunum	6.5–7.0	150–260	16.0 ± 0.5	–
Ileum	7.0–7.5	200–350	16.0 ± 0.5	–
Colon	7.0–8.0	90–150	135 ± 25	24–48

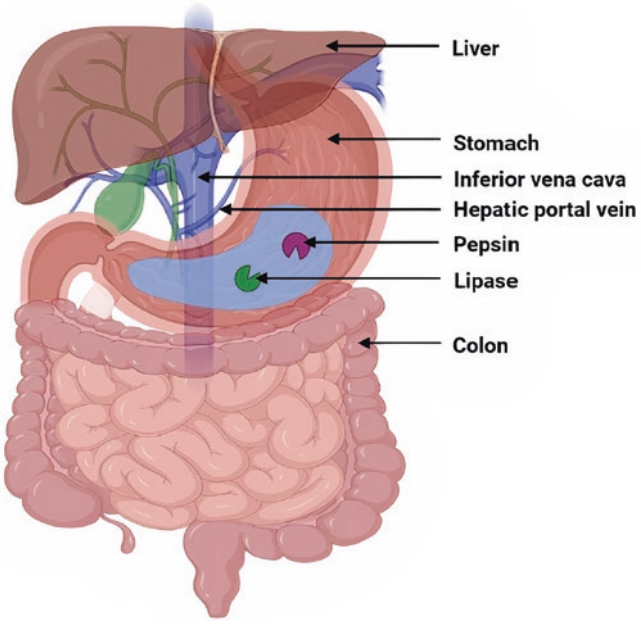

Fig. 11.3 Biochemical barriers in oral drug delivery system. The interaction of drug and numerous hydrolytic enzymes, as well as the extreme acidic conditions, can trigger bioactive compounds to hydrolyze, oxidise, or deamidate, resulting in a significant loss of efficacy. Represented from "Micro and nanoscale technologies in oral drug delivery", Ahadian et al. (2020)

11.3.1 Biochemical Barrier

The combined degradation action of the intestinal enzymes and harsh pH environment act together as the critical biochemical barriers for the enhanced oral bioavailability of the therapeutics (Fig. 11.3) (Ahadian et al. 2020). To be effective, any oral biologics must transit through the gastrointestinal tract, allowing increased adherence and infiltration through the unstirred mucus gel layer, diffuse across the intestinal epithelium through tight-junctional areas, finally enter into the hepatic portal vein, and reach the target systemic circulation. However, many anti-cancer drugs are very susceptible to the extreme pH variation in the gastrointestinal tract. The

parietal cells and cardiac gland in the stomach releases hydrochloric acid and mucus respectively, which maintain its acid homeostasis. The gastrointestinal tract pH varies from being highly acidic in the stomach (pH 1.0–3.0) to about pH 8.0 in the intestine (Koziolek et al. 2015). Exposure of the chemotherapeutics to these extreme pH environments causes hydrolysis, oxidation, or deamidation of the bioactive molecules, resulting in a drastic loss of their effectivity. In addition to stomach acid, different gastric enzymes like pepsin and lipase also act as an obstacle to the bioavailability of the biopharmaceuticals resulting in enzymatic degradation. Exposure to these drug degrading enzymes and the extremely acidic environment can cause hydrolysis, oxidation, or deamidation of the bioactive molecules, resulting in a drastic loss of their effectivity (Raja et al. 2019).

Apart from this, the small intestine also secretes digestive enzymes like carboxypeptidases, trypsins, chymotrypsins, and elastases in higher concentrations which can denature the biomolecules prior to absorption (Brown et al. 2020). If these hurdles are overcome, the drug molecules must penetrate the mucosal barrier for absorption in the small intestine.

11.3.2 Mucosal Barrier

Absorption barriers encountered by the administration of oral chemotherapeutics are very challenging. The mucus barrier, one of the enzymatic and diffusional barriers, represents the second physiological obstacle faced by such therapeutics. Physiologically, the mucus is continuously secreted, which further sheds from the epithelial surface and is recycled every 4–5 h (Birchenough et al. 2015). Besides the biochemical barriers (pH and enzymes), mucus with a complex hydrogel-like structure creates a dynamic semi-permeable barrier that can trap and immobilize the therapeutics before reaching the epithelial surface but remain permeable to small molecules, water, and nutrients (Fig. 11.4) (Seo et al. 2021).

Gastrointestinal tract mucus has a viscoelastic property which is essential for the protection and lubrication of the gastrointestinal tract. There are two mucus layers, the outer loosely adherent layer and the inner firmly adherent layer, which hampers the direct contact of therapeutics with epithelial cells (Brown et al. 2020). Mucus is continuously secreted by goblet cells to protect the exposed epithelial surface from pathogens and other potentially harmful compounds. The major constituent of mucus is mucin glycoproteins, and it also contains proteins, lipids, carbohydrates, salts, nucleic acids, antibodies, bacteria, and other active proteins. Gastrointestinal mucus creates a safeguard and maintains a nutrient rich environment for colonization by commensal or ‘good’ bacteria while acting as an obstacle to pathogenic bacteria and foreign particulates and rapidly clearing them (Schroeder 2019).

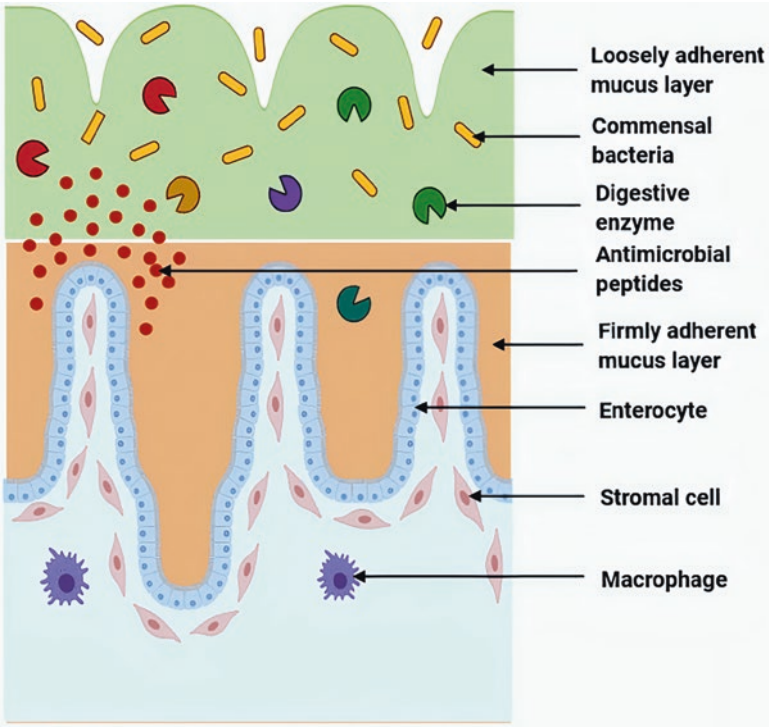

Fig. 11.4 Mucosal barriers in oral drug delivery system. The mucus, which has a dense hydrogel-like substance, forms a dynamic semi-permeable barrier that traps and immobilises drugs before they reach the epithelial surface while allowing small molecules, water, and nutrients to pass through. Reproduced from "The role of mucosal barriers in human gut health", Seo et al. (2021)

11.3.3 Cellular Permeability Barrier

The intestinal epithelium (outermost layer) is composed of microfold cells (M-cells), enterocytes, and goblet cells (Fig. 11.5) (Peterson and Artis 2014; Han et al. 2019). Enterocytes facilitate the active transport of water and nutrients from the gastrointestinal tract to the bloodstream. M-cells are specialized epithelial cells that cover peyer's patches and are responsible for sampling antigen and are presented as easy drug targets as they are less shielded by mucus (Brown et al. 2020). The mechanism of permeation of the clinical therapeutics through the epithelium into the bloodstream may occur through the transcellular passive diffusion (through epithelial cells) or paracellular (through the tight-junctional areas) routes, lymphatic absorption *via* M-cells, receptor, and transcytosis-mediated endocytosis pathway (Brown

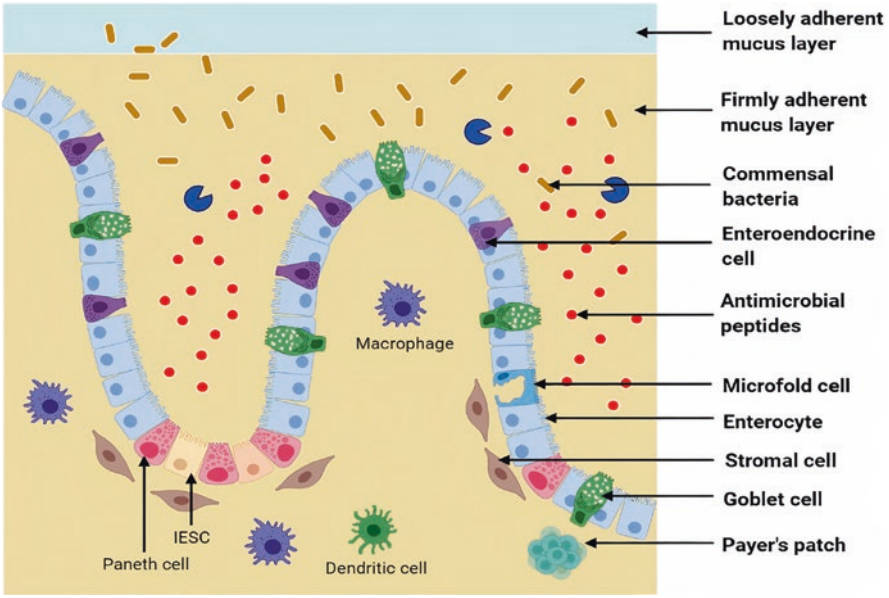

Fig. 11.5 Cellular permeability barrier in oral drug delivery system. The diffusion of drug through the paracellular pathway is inhibited due to the regulation of tight-junctional regions between adjacent intestinal epithelial cells such as microfold cells, goblet cells, and enterocytes. Reproduced from "Intestinal epithelial cells: Regulators of barrier function and immune homeostasis", Peterson and Artis (2014)

et al. 2020). Permeation *via* the paracellular route is restricted due to the regulation of tight-junctional areas between adjacent intestinal epithelial cells. Only hydrophilic drugs of small molecular weight (<200 Da) can easily access their transportation through the paracellular pathway (Laksitorini et al. 2014).

Consequently, despite the availability of different permeation routes, several therapeutics possess an inadequate bioavailability *via* the oral route. Nanoparticles can be directly taken up by M-cells *via* the transcellular routes. Supramolecular nanoparticles, due to their large size, cannot permeate through the cells by passive diffusion mechanism. In contrast to this, drug-loaded nanoparticles rely largely upon the active diffusion pathway as they are energy-dependent. The active transcellular diffusion (phagocytosis, micropinocytosis, caveolin, and clathrin-mediated) of drug encapsulated nanoparticles follows the endocytosis process. Both phagocytosis and caveolin/clathrin-mediated endocytosis follow receptor-mediated pathways, while micropinocytosis (actin-dependent process) internalizes large extracellular liquid and dissolved solutes (des Rieux et al. 2006).

11.4 Biopolymeric Nano-Drug Delivery Systems for Oral Administration of Chemotherapeutics

Biopolymeric nanoparticles (hydrophobic or hydrophilic) have attracted considerable attention as versatile oral delivery platforms for the controlled and targeted delivery of chemotherapeutics due to their well-characterized safety profile. Hydrophilic polymers, such as pullulan, chitosan, and their derivatives, have gained therapeutic popularity as exceptional candidates due to their excellent mucoadhesive properties, biocompatibility and, permeabilization capacity. Formulations of various polymeric materials modulate the physicochemical properties of nanoparticles (e.g., surface charge and mucoadhesive) that enable them to control the release of encapsulated drug using principles of stability, low toxicity, and solubility in an aqueous physiological environment. Table 11.2 represents a summary of the biopolymers designed as nanoenabled oral delivery platforms for cancer therapy.

11.4.1 Protein-Based Biopolymers

11.4.1.1 Gelatin

The natural biopolymer is extracted from collagen *via* acid/alkaline hydrolysis, which has been clinically explored for the design of several drug delivery systems as it can be chemically crosslinked with other compounds using N-hydroxysuccinimide, glutaraldehyde, carbodiimides, and genipin. The modification of the gelatin yields stealth carriers, which further helps in evading the reticuloendothelial uptake and protects the drug from degrading enzymes and harsh pH environments, which is very crucial for oral drug delivery systems to enhance their circulation time and accumulation in the leaky vasculature of the tumor microenvironment. Moreover, gelatin can be tailor modified for mediating the incorporation of targeting ligands for site-specific oral drug delivery to ensure that the metastatic cells are only targeted and damaged.

In 2016, Singh et al. synthesized redox-responsive gelatin nanoparticles modified with epidermal growth factor receptor for targeted delivery of gemcitabine for orthotopic pancreatic cancer treatment. The PEGylated surface of epidermal growth factor receptor - targeted gelatin nanoparticles increased the circulation time in association with the target specificity of gemcitabine. The obtained *in vivo (*orthotopic pancreatic adenocarcinoma tumor-bearing severe combined immunodeficiency beige mice and *in vitro* results showed an improved cytotoxic profile of loaded gemcitabine against the orthotopic pancreatic cancer cells, and the epidermal growth factor receptor - targeted nanoparticles could actively administer the chemotherapeutic drug *via* oral route with more efficacy as compared to an intravenous solution of gemcitabine in succinimidyl 3 – [2 – pyridyldithio] – propionate. Gemcitabine- succinimidyl 3 – [2 – pyridyldithio] – propionate showed an IC_{50}

Table 11.2 Oral delivery platforms, their possible polymeric complexes, and encapsulated chemotherapeutics

Polymers	Polymeric complex	Encapsulated drug	Route	Tumor type	References
Chitosan	Lecithin-chitosan	Tamoxifen	Oral	General tumor	Barbieri et al. (2015)
	Chitosan-grafted poly(methacrylic acid)/ graphene oxide	Doxorubicin	Oral	General tumor	Abbasian et al. (2018)
	Chitosan nanoparticles	Carboplatin	Oral	Breast cancer	Khan et al. (2017)
	Chitosan/ carboxymethyl chitosan nanoparticles	Doxorubicin	Oral	Liver, spleen, and lung tumor	Feng et al. (2013)
Poly(lactic-co-glycolic acid)	Magnetic poly(lactic-co-glycolic acid) nanoparticles	Paclitaxel	Oral	Breast cancer	Cui et al. (2017)
	Poly(lactic-co-glycolic acid) nanoparticles	Cetuximab	Oral	Lymphoma tumor	Kaushik and Sharma (2018)
	Polyethylene glycol-poly(lactic-co-glycolic acid) nanoparticles	Piperine	Oral	Breast cancer	Pachauri et al. (2015)
	Poly(lactic-co-glycolic acid)-polyethylene glycol nanoparticles	Gemcitabine and betulinic acid	Oral	Pancreatic cancer	Saneja et al. (2019)
Collagen	Collagen nanoparticles	Doxorubicin	Oral	Liver cancer	Zhong Luo et al. (2011)
Hyaluronic acid	Hyaluronic acid nanoparticles	Cisplatin	Oral	Ovarian cancer	Liu et al. (2015)
	Hyaluronic acid nanoparticles	Doxorubicin and paclitaxel	Oral	Breast cancer	Pramanik et al. (2019)
Gelatin	Redox responsive Gelatin nanoparticles	Gemcitabine	Oral	Pancreatic cancer	Singh et al. (2016)
	PEGylated gelatin nanoparticles	Doxorubicin and betanin	Oral	Breast cancer	Amjadi et al. (2019)
	Gelatin-methoxxy poly (ethylene glycol) - porous nanosilica particles	Doxorubicin	Oral	General tumor	Vo et al. (2019)
Pullulan	PH responsive pullulan nanoparticles	Doxorubicin and sorafenib	Oral	Breast cancer	Sui et al. (2017)
Alginate	Disulfide cross-linked sodium alginate nanoparticles	Paclitaxel	Oral	Colon cancer	Ayub et al. (2019)
	pH-responsive alginate nanoparticles	Doxorubicin	Oral	Liver cancer	Guo et al. (2013)

(continued)

Table 11.2 (continued)

Polymers	Polymeric complex	Encapsulated drug	Route	Tumor type	References
Polyglutamic acid	Polyglutamic acid-g methoxy polyethylene glycol nanoparticles	Cisplatin	Oral	Bone cancer	Li et al. (2015)
	Chitosan-polyglutamic acid grafted polyethylene glycol nanoparticles	Doxorubicin	Oral	Lung cancer	Deng et al. (2015)
	Polyethylene glycol -b-(polyglutamic acid)-b-poly(phenylalanine) nanoparticles	Paclitaxel and cisplatin	Oral	Ovarian cancer	Desale et al. (2013)

value of 8.39 ± 1.79μM, as compared to the target-specific nanoparticles (IC_{50} value of 17.08 ± 2.32μM) (Singh et al. 2016).

In another study, Amjadi et al. formulated pH-responsive doxorubicin and betanin encapsulated PEGylatedgelatin nanoparticles (162 nm) for the successful delivery in the breast cancer cell line (MCF-7). The resulted nanocarriers showed loading capacity of about 20.5% and 16.25% for doxorubicin and betanin, respectively. Moreover, the research showed synergistic efficacy of the smart nanocarrier with decreased viability of MCF-7 cells with a sustainable release of the loaded drugs in comparison to free soluble ones (Amjadi et al. 2019). In another study, Vo and coworkers designed doxorubicin-loaded gelatin-methoxy poly (ethylene glycol)-porous nanosilica particles (69.60 ± 3.27 nm) that have a high potential for effective delivery of doxorubicin into the cancer cells. The nanoformulation showed pH-dependent and controlled release behavior of loaded doxorubicin up to 96 h in acidic media, without a burst release with respect to unlabelled nanocarrier (Vo et al. 2019).

11.4.1.2 Collagen

Collagen is a natural biopolymer that is especially present in the connective tissue of mammals. It is widely used as an excellent oral delivery platform because of its extraordinary properties such as biocompatibility, biodegradability, high absorbability, no-antigenicity, and has shown a highly synergistic effect when administered in the form of polytherapy with other bioactive compounds (Sahithi et al. 2013). The presence of active functional groups in the biodegradable collagen can be therapeutically modulated to produce highly specific oral drug delivery characteristics. In addition, the collagen scaffold has also proved to resemble the tumor microenvironment in the *in vivo* model. Luo et al. have formulated redox responsive collagen capped mesoporous silica nanoparticles for the controlled release of doxorubicin into the target-specific cancer cells. In this study, they investigated the

targeted delivery and cellular uptake properties of the formulated nanoparticles in HepG2 and endothelial cells. Due to its biocompatibility 2.2 fold increase in targeted delivery, and excellent cellular uptake property, it can potentially be used as a carrier for the orally administrated anticancer agent for cancer treatment as compared to the intravenous solution of the free drugs (Zhong Luo et al. 2011).

11.4.2 Poly-Amino and Poly-Ester Based Biopolymers

11.4.2.1 Poly(Lactic-Co-Glycolic Acid)

Over the years, researchers have been exploring a wide variety of natural and synthetic polymers for the preparation of nanoparticles. Poly(lactic-co-glycolic acid) has been extensively investigated in the medicinal industry for the synthesis of nanomedicine because of its biocompatibility and biodegradability properties. It is usually less toxic inside the body as the polymeric chain of poly(lactic-co-glycolic acid) gets hydrolyzed into lactic acid and glycolic acids, which are then metabolized and excreted as carbon dioxide and water (Mir et al. 2017). It has been approved by the *United States food and drug administration* (US FDA) as a suitable carrier for various hydrophobic anticancer drugs resulting in improved bioavailability and controlled release of drugs (Rathore et al. 2020).

In a recent study, Cui et al. have formulated paclitaxel-encapsulated magnetic poly(lactic-co-glycolic acid) (50:50) nanoparticles (150 ± 20 nm) coated with transferrin. As compared to the free paclitaxel and unmodified poly(lactic-co-glycolic acid) nanoparticles, this formulated nanoparticle exhibited increased cellular uptake and improved cytotoxicity against breast (MCF-7) and glioma cells (U87MG) (Cui et al. 2017). In another study, Kaushik et al. used poly(lactic-co-glycolic acid) (85:15) to formulate novel biodegradable cetuximab loaded poly(lactic-co-glycolic acid) nanoparticles (115–270 nm). The *in vivo* studies showed controlled release and enhanced drug targeting efficacy of radiolabelled cetuximab-poly(lactic-co-glycolic acid) nanoparticles into dalton's lymphoma solid tumor cells after oral administration with respect to the free soluble drug (Kaushik and Sharma 2018).

Pachauri et al. studied the anti-cancer property of piperine-encapsulated poly (ethylene glycol)- poly(lactic-co-glycolic acid) nanoparticles in MCF-7 breast cancer cell lines. In conclusion, aptamer-decorated piperine-poly (ethylene glycol)-poly(lactic-co-glycolic acid) nanoparticles exhibited strong antiproliferative activity against MCF-7 cells and hence can improve the clinical therapeutic potential of several other combinatorial anti-cancer medications as an adjuvant therapy against multiple drug-resistant breast solid tumors (Pachauri et al. 2015). In 2019, Saneja et al. performed an experiment where they synthesized gemcitabine and betulinic acid co-encapsulated poly(lactic-co-glycolic acid)- poly (ethylene glycol) nanoparticles (<200 nm) to improve the therapeutic efficacy of these drugs against cancer cells. The gemcitabine-betulinic acid-poly(lactic-co-glycolic acid)-poly (ethylene glycol) nanoparticles exhibited greatly enhanced cytotoxicity in pancreatic cancer

cell lines (Panc1) as compared to native drugs. The enhancement in cytotoxicity activity was associated with cellular reactive oxygen species production, resulting in increased cell apoptosis. *In vivo* mice, trials showed increased anticancer efficacy of combinatorial nanoparticles relative to native drugs. The obtained results in the present study showed that the solid tumor treated with gemcitabine/betulinic acid/poly(lactic-co-glycolic acid)-poly (ethylene glycol) nanoparticles formulation had a mean volume of 195.5mm^3, while the tumors treated with the injectable solution of gemcitabine/betulinic acid/poly(lactic-co-glycolic acid)-poly (ethylene glycol) had a mean volume of 213.5 mm^3 (Saneja et al. 2019).

11.4.2.2 Polyglutamic Acid

The polyamino acid forms active conjugates with chemotherapeutics which further enhances the therapeutic efficacy of the drug with reduced toxicity towards normal cells. Hydrophilic polyglutamic acid emerges as an excellent non-toxic polymeric candidate for the delivery of water insoluble anticancer drugs *via* the oral route (Zhiting Luo et al. 2016). Natural polyglutamic acid secreted by *Bacillus* spp. is composed of repeating units of extensively viscous D-glutamic acid/L-glutamic acid units or both (Khalil et al. 2016; Ogunleye et al. 2015). In 2013, Desale and coworkers performed an experiment where they formulated copolymers containing poly (ethylene glycol), glutamic acid, and phenylalanine for the co-delivery of cisplatin and paclitaxel against ovarian cancer cells. The therapeutic combination of binary drugs in cross-linked micelles represented synergistic toxicity against A2780 cells (human ovarian cancer) and exhibited an enhanced antitumor efficacy in comparison to monotherapy of individual drug-loaded micelles or free soluble cisplatin *in vivo* xenograft models of variable cancers (Desale et al. 2013). In another study, Li et al. studied the potential anticancer property of cisplatin entrapped polyglutamic acid-g-methoxy poly (ethylene glycol) nanoparticles against osteosarcoma cells (MNNG/Hos). In an *in vivo* trial for MNNG/Hos osteosarcoma tumor-bearing mice, cisplatin-nanoparticles and cisplatin-nanoparticles loaded cyclic tumor penetrating peptide (iRGD) showed marginally improved anticancer activity and a considerably lower body weight loss relative to free cisplatin, suggesting the effectiveness of cisplatin-nanoparticles over free cisplatin in showcasing anticancer activity with minimal known systemic side effects for the treatment of osteosarcoma (Li et al. 2015).

In 2015, Deng et al. designed pH-sensitive complex nanoparticles of chitosan-polyglutamic acid-grafted - poly (ethylene glycol)-doxorubicin nanoparticles that exhibited enhanced cellular uptake and increased cytotoxicity against tumor cells. The formulated long-circulating nanovehicles exhibited excellent biotherapeutic efficacy accompanied with lower systemic toxicity in comparison to the intravenous solution of free soluble doxorubicin (10μg/mL). Moreover, long-circulating polyglutamic-grafted-polyethylene glycol-hyd-doxorubicin nanoparticles showed increased accumulation in tumor region with controlled and sustainable release of doxorubicin within tumor cells due to intracellular acid-trigged hydrolysis of

hydrazone bonds, hence resulting in an efficient cancer based therapy with minimal known side effects, when compared with free soluble doxorubicin. Hence, the designed nanoformulation effectively overcame the obstacles faced during oral administration and systematically improved the instillation efficiency of doxorubicin *via* the oral route, which thereby offered a promising biomedical platform for the success of oral chemotherapy (Deng et al. 2015).

11.4.3 Polysaccharide-Based Biopolymers

11.4.3.1 Chitosan

Chitosan is derived from N-deacetylated chitin and is composed of β-(1,4)-2-acetamido-D-glucose and β-(1,4)-2-amino-D-glucose with good biocompatible, biodegradable properties (Rathore et al. 2020). Due to its efflux pump inhibitor and mucoadhesive properties, anticancer drugs can easily enter inside the cells, and they also enhance the therapeutic efficiency of drugs (Kumar et al. 2016). Some chitosan modifications are also fabricated for sustained release of drugs which can influence the pharmacokinetic property of the nanoparticle. Chitosan is mainly used to encapsulate hydrophobic and acid-labile anti-cancer drugs. In 2015, Barbieri et al. formulated tamoxifen-loaded lecithin-chitosan nanoparticles, which showed increased permeability to the intestinal epithelium because of its mucoadhesive property. *Ex vivo* experiments revealed that the active encapsulation of tamoxifen in lecithin/chitosan nanoparticles significantly improved the transportation of non-metabolized drugs across the intestinal tissue of rats (Barbieri et al. 2015).

In 2018, Abbasian et al. synthesized doxorubicin-loaded chitosan-grafted-poly(methacrylic acid)/graphene oxide nanoparticles for sustained release of doxorubicin in MCF-7 breast cancer cell line. The experimental findings showed that the developed doxorubicin encapsulated chitosan-grafted-poly(methacrylic acid)/graphene oxide nanocomposite led to extended-release profiles, greater therapeutic efficacy, promising biodistribution, and minimization of the drug-associated side effects during cancer chemotherapy (Abbasian et al. 2018). In a recent study, Khan et al. developed carboplatin-loaded chitosan nanoparticles (277.25 ± 11.37 nm) as an effective formulation for breast cancer (MCF-7) treatment. They concluded that the carboplatin encapsulated chitosan nanoparticles also exhibited superior cellular drug uptake, controlled intracellular drug retention, and improved tumor-targeted anti-proliferative activity against cancer cells (Khan et al. 2017).

In another study, Feng et al. prepared doxorubicin-encapsulated chitosan/carboxymethyl chitosan nanoparticles which exhibited enhanced intestinal absorption of doxorubicin. The findings showed that the doxorubicin-chitosan/carboxymethyl chitosan nanoparticles oral administration was efficient in distributing doxorubicin into the blood, with 42% absolute bioavailability, thereby presenting a highly effective and safe oral delivery system of doxorubicin. *In vivo* experiments revealed that the tissue distribution of doxorubicin-chitosan/carboxymethyl chitosan nanoparticles

in rats had a reduced level of doxorubicin accumulated in the kidneys, liver (5.87μg/g tissue), spleen (3.65μg/g tissue) and lungs (2.58μg/g tissue) and heart (Feng et al. 2013).

11.4.3.2 Pullulan

The hydrophilic pullulan is a biocompatible and linear homopolysaccharide of glucose that is secreted primarily by thermally bi-morphic fungi (*Aureobasidium pullulans*). The unbranched polymer is formed by repeating units of maltotriose linked by α-1,6 glycosidic bonds (Scomparin et al. 2011). Because of its outstanding physicochemical and biological properties, like biodegradability, low toxicity, and solubility in an aqueous physiological environment and few polar solvents, this biopolymer has become a promising component for chemical modification and biomedical applications. It serves as a versatile nanocarrier for the targeted delivery of hydrophobic chemotherapeutics in the spleen, liver, brain, and lungs (Singh et al. 2017). In 2017, Hui Sui et al. designed stable pH-sensitive sorafenib encapsulated pullulan - doxorubicin conjugate nanoparticles as a synergistic delivery system against breast carcinoma in a murine model with a 65.34% of drug loading capacity. In contrast to free drugs, the cytocompatibility of sorafenib encapsulated pullulan - doxorubicin conjugate nanoparticles was better against 4 T1 cells under *in vitro* conditions. *In vivo* animal model experiments showed there was an enhanced accumulation of the synergistic combinatorial pullulan-doxorubicin/sorafenib nanoparticles in cancer cells as compared to the free drug (Sui et al. 2017).

11.4.3.3 Hyaluronic Acid

Hyaluronic acid, non-sulfated glycosaminoglycans, is comprised of *alternating* D-*glucuronic acid* and D-N-*acetylglucosamine* units connected by β (1–3) and β (1–4) glycosidic bonds. The desired functional groups (hydroxyl, N-acetyl, carboxyl) expressed on the hyaluronic acid surface establish it as an excellent oral delivery platform for sustained and controlled drug release activity. Biocompatible hyaluronic acid exhibits high viscoelasticity with desired properties, such as nonimmunogenicity and targeted drug delivery, as they are highly overexpressed in the tumor microenvironment (Widjaja et al. 2014). Hyaluronic acid receptor, CD44, is overexpressed in cancerous cells, thus making it an excellent targeting nanocarrier for cancer therapy (Mattheolabakis et al. 2015). In a recent study, Liu et al. has formulated cisplatin encapsulated titanium dioxide nanoparticles coated with hyaluronic acid for target-specific delivery into the tumor cells. The results indicated that cisplatin-loaded hyaluronic acid- titanium dioxide nanoparticles cause increased endocytosis of the conjugates in A2780 ovarian cancer cells and exhibited enhanced antitumor activity *in vitro*. *In vivo* real-time studies showed that nanoformulation has better tumor-targeting potential to mitigate the harmful side effects of cisplatin

in clinical use. Cisplatin-loaded hyaluronic acid- titanium dioxide nanoparticles demonstrated an 8.31-fold higher distribution of encapsulated drug after 4 h of incubation as compared to that of intravenous solution of free cisplatin (Liu et al. 2015).

In another study, Pramanik et al. made a unique delivery system by conjugating hyaluronic acid to the graphene oxide for target-specific co-delivery of doxorubicin/paclitaxel against cancer cells. They showed that the nanoformulation could specifically kill CD44-expressing breast cells (MDA-MB-231) but were unable to eradicate CD44 negative BT-474 cells. They also conjugated iron oxide nanoparticles onto the graphene oxide-hyaluronic acid-drug formulation for enabling magnetic hyperthermia property, which results in the significantly better killing of cancer cells when compared to the individual system (Pramanik et al. 2019).

11.4.3.4 Alginates

Alginate is a linear polyanionic polysaccharide composed of repeating units of α-L-guluronic acid and β-D-mannuronic acid connected by 1,4-glycoside linkage. The unique physicochemical characteristics (biocompatibility, biodegradability, cytocompatibility, and mucoadhesive property) of sodium and potassium-based alginates have led to their controlled and targeted drug delivery, hence emerging as favorable oral delivery platforms for solid tumor treatment (He et al. 2020). The mucoadhesive property of alginate enhances the anti-cancer drug absorption in the surface of the intestinal epithelium, therefore, increasing the oral bioavailability for the therapeutic molecules (Sosnik 2014). Ayub et al. developed paclitaxel-loaded cysteamine-based disulfide cross-linked sodium alginate nanoparticles for targeted delivery of the anticancer drug to the human colon cancer (HT-29) and human colon normal (CR L1790) cells. The work showed successful cellular internalization of the formulated nanomedicine into the cancer cells (Ayub et al. 2019). Guo and coworkers published a report on the pH-sensitive targeted intracellular delivery of doxorubicin by glycyrrhetinic acid-modified alginate/doxorubicin-loaded alginate complex nanoparticles with outstanding anti-cancer activity against hepatoma carcinoma cells. Moreover, experiments performed in ectopic tumors exhibited 78.91% anticancer efficacy of the smart nanoformulation (Guo et al. 2013).

11.5 Conclusion

Over the past decade, scientists have had great leverage to design nanodelivery systems enabling safe oral administration of several biologics into the tumors. Attention towards the tremendous research of biopolymeric nanoparticles as oral drug delivery systems is increasing at a global scale due to their favorable properties, such as non-immunogenicity, non-toxicity, biocompatibility, and so forth. Utilizing biopolymeric oral delivery platforms in the treatment of solid tumors improves drug solubility, dose-reduction, site-specific sustained delivery, and most importantly, the

safety of the therapeutics from the extreme gastric environment. In comparison to the intravenous route, continuous oral dosing facilitates prolonged exposure of the drug, which is a crucial factor for chemotherapeutics. Additional research necessitates comparing the stability of oral and parenteral-based chemotherapeutics *in vitro* and *in vivo* using established diseased models over a longer duration of evaluation followed by gathering enough data focussing on their toxicity profile. Soon, oral delivery platforms will acquire the potential to modulate the delivery of chemotherapeutics drugs, as they offer key solutions to drug solubility problems, the oral bioavailability of poorly soluble drugs, and site-specificity challenges.

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Chapter 12
Polymeric Nanoparticles to Target Glioblastoma Tumors

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12.1 Introduction

Cancer is one of the diseases with the maximum mortality tolls in the world, amid major medical developments in the field of oncology (Padhi et al. 2015). There are many types of cancers (Fig. 12.1), out of which glioblastoma multiforme is one of the most severe and most frequent brain tumors of all human cancers in adults, responsible for about 50% of all primary gliomas (Fernandez et al. 2012). According to the World Health Organization, the prevalence is roughly 5–10 instances in a population of one lakh (Omuro and DeAngelis 2013). Bailey and Cushing initially identified glioblastoma multiforme in the year 1926, and they identified it as irregular glioblasts (glial cell growth) in the brain (MacKenzie 1927). A more frequently identified central nervous system tumor in adults is glioblastoma multiforme, which is specified as grade IV astrocytoma as described in the guidelines of the World Health Organization. Usually, the tumor shows dispersed boundaries and a large penetration into the surrounding healthy brain tissue of individual tumor cells that exacerbates surgical removal (Maher et al. 2001). In addition, conventional chemotherapy results in by low drug partitioning through the blood brain barrier leading to poor selectivity and lower therapeutic efficacy. Treatment methods presently offered

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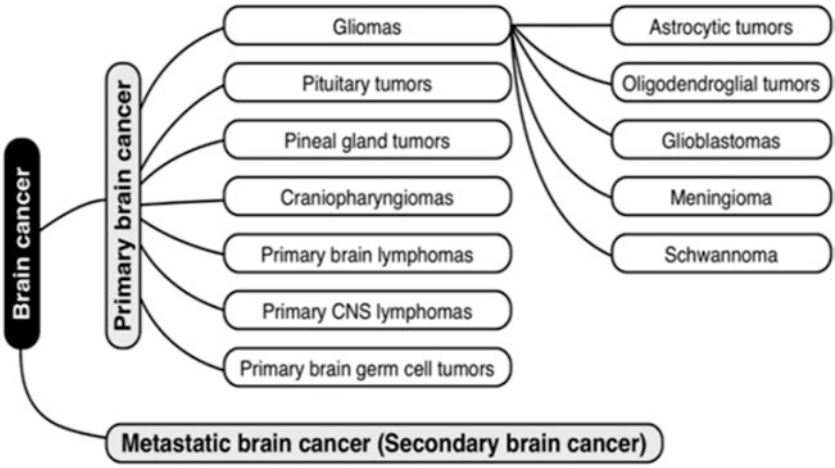

Fig. 12.1 Common types of brain tumors. Adapted from the Journal of Cancer Metastasis and Treatment, OEA Publishing Inc. from EL Amrawy et al. (2016), an open access article distributed under the creative commons attribution license that permits unrestricted use, distribution, and reproduction in any medium

have not proven very successful in improving the conditions of patients. It offers a median approximate life expectancy of just 12–16 months, presumably the presence of residual tumor cause the glioma to relapse.

Restricted and far less successful choices for the management of such extremely aggressive glioblastoma multiforme pushed the research scientists to develop new strategies furthering to improvement of existing technologies. Multiple options for overcoming the disadvantages of conventional delivery of anticancer agents to the brain through the use of nanocarriers have been implicated in the recent years. In curing the stated disease, nanoscience has played a vital role and specifically polymeric nanoparticles have shown the ability to penetrate the blood brain barrier and persist in glioblastoma multiforme tissues for a prolonged period of time. Nanoparticles are especially suitable carriers for deoxyribonucleic acid (DNA), ribonucleic acid (RNA), chemotherapeutic agents and proteins (Pitorre et al. 2020). Current nanotechnology based approaches aim to increase the active targeting of drug to the targeted tissues for the purpose of delivery of chemotherapeutic drugs and offer good imaging studies (Padhi et al. 2018; Behera and Padhi 2020).

The aim of the present chapter is to elaborate the progress in the field of nanotechnology that has helped enormously in the targeted delivery of chemotherapeutic drugs in glioblastoma multiforme tissues. The potential of polymeric nanoparticles in the management of malignant gliomas also will be addressed, along with the significance of their coating and functionalization for their ability to cross the blood brain barrier.

12.2 Glioblastoma

Glioblastoma multiforme remains as one of mankind's biggest life threatening ailments and each year impacts lakhs of patients worldwide. Early in its pathogenesis, this malignancy infiltrates the brain and renders total neurosurgical resection which is nearly inevitable (Abrudan et al. 2014). Gliomas (54%) and primary brain tumors (16%) are categorized under glioblastoma multiforme. Malignant gliomas accounts for approximately 11,000 deaths globally (Behin et al. 2003). The standard treatment protocol pertains to surgical removal accompanied by temozolomide administered orally simultaneously with chemotherapeutic. The life expectancy of patients with glioblastoma multiforme following its preliminary identification is only 15 months (Roger Stupp et al. 2005). Hence, there arises a certain need to develop novel strategies for treating patients with glioblastoma multiforme. The lack of existing therapeutic strategies for malignant gliomas has so far been due to the presence of a subpopulation of malignant glioma cancer stem cells that have the strength to tolerate chemotherapy and ionizing radiation built on some of their distinctive attributes such as high anti-apoptotic protein expression, high ATP-binding cassette pump expression, and outstanding DNA properties (Stupp et al. 2005, 2010).

A bunch of nanoparticulate drug delivery systems, including polymeric nanoparticles, nanoemulsion, liposomes, iron oxide nanoparticles, and polymeric micelles have been widely studied as carriers for an array of drugs in the treatment of various disease conditions in the recent past (Patnaik et al. 2021; Behera et al. 2020a, b; Hassan et al. 2021) Passive and active targeting are the central approaches which are employed for targeting nanocarriers to specific sites (Padhi and Behera 2020). Passive targeting allows the accumulation of nanoparticles in tumor tissues owing to the typical attributes of the tumor microenvironment which is termed as enhanced permeability and retention effect (Verma et al. 2017). Enhanced permeability and retention effect allows the retention of nanomaterials in tumor tissues via passive targeting. At present, convection-enhanced delivery is applied to increase the uptake of nanomaterials into brain tumor tissues. Nanomaterials along with small-interfering RNA are used to suppress the gene function that makes glioblastoma multiforme highly aggressive. More importantly, these nanomaterials can be used to deliver chemotherapeutic agents specifically to the tumor tissues without causing systemic toxicity (Michael et al. 2018). A combination of conventional and nanotechnology-based therapies has provided promising outcomes in this regard (Abrudan et al. 2014).

12.3 Advances in the Development of Novel Therapeutics for Glioblastoma

Nanotechnology has revolutionized the preceding years in the drug delivery domain (Padhi et al. 2020). The past few years have witnessed major developments in the studies related to targeted therapies for amelioration of tumors. Owing to the specific chemical and physical characteristics that lead to precise distribution and

accumulation of encapsulated drugs in precise organs and tissues, polymeric nanoparticles have proven as outstanding transport carriers for biologically active molecules or drugs (Abrudan et al. 2014). Polymeric nanoparticles employing biodegradable polymers like poly (ethylene glycol), and poly (butyl-cyanoacrylate) encapsulating an array of chemotherapeutic agents have garnered varied application and have resulted in improved survival rates. Polymeric nanoparticles have also demonstrated enhanced therapeutic efficacy with a reduction in adverse effects to the surrounding healthy tissues (Abrudan et al. 2014; Maier-Hauff et al. 2011; Khuroo et al. 2014).

Malignant gliomas are one among the deadly types of brain cancer. Particularly the administration of hydrophilic drugs in neat form leads to diminished targeted delivery at the tumor site due to inadequate blood brain barrier penetration. Furthermore, drugs of low molecular weight do not undergo sufficient accumulation in cancerous tissues and are characterized with a lower $t_{1/2}$ in the systemic circulation. The nanoparticles may be engineered with suitable ligands for crossing the blood brain barrier leading to targeted delivery in the brain, thereby enhancing their therapeutic efficacy as compared to drugs in its native form. Surface decoration of polymeric nanoparticles with suitable ligand is known to improve the therapeutic effectiveness with reduced off-target side effects (Mahmoud et al. 2020).

There has been a significant improvement in nanomedicine and cancer care over the past few decades. However, due to its complicated pathophysiology, cancer continues to be a daunting health issue. As shown by the american cancer society, the number of cancer occurrences is projected to increase to 27.5 million by the year 2040. The major brain tumors, which can seldom be healed, are among the most problematic malignant cancers, with a 5 years average lifespan. Gliomas are by far the most prevalent type of primary malignant brain tumor in adults (Lapointe et al. 2018). The primary factors that decide whether glioma cells belong to low grade (WHO I and II) or high grade (WHO III and IV) category are their capacity to penetrate and metastasize into surrounding tissues of brain. Gliomas have the ability to penetrate the underlying tissue, and hence it becomes difficult to define their margins. This results in the failure of conventional treatment approach to provide a curative effect. The prevalent chemical and physical barriers challenging the biological milieu possess a major challenge for the effective delivery of the drugs at the target site (Cornago et al. 2014; De Boer and Gaillard 2007). The barrier that prohibits suitable delivery of drugs across the brain is the blood brain tumor barrier and the blood brain barrier (Fig. 12.2). The multipotent stem cells that culminate into glioma cells are capable of self-renewal and often relapse (Binello and Germano 2011). With the advancement of targeted strategies for drug delivery to the brain, attempts have been explored to address physical barriers, however, all of these methods are found to be intrusive and hazardous with severe side effects. Utilizing polymeric nanoparticles for drug delivery and targeting is one among the innovative treatment options. Positive results for drug-loaded nanoparticles targeting gliomas are reported in many research studies, which are discussed later in this chapter.

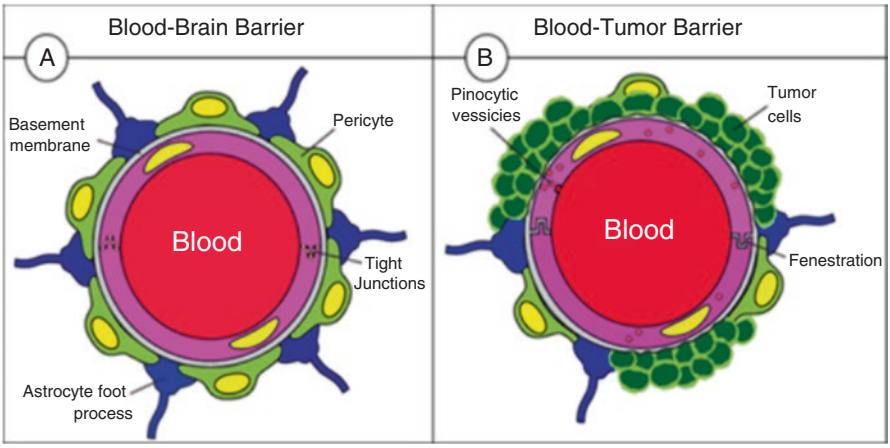

Fig. 12.2 Differences between blood brain barrier and blood brain tumor barrier. Blood brain barrier, a diffusion barrier, protects the brain and maintain brain's homeostasis by controlling influx of blood components into the brain. Brain capillary endothelial cells and other cell types such as pericytes, astrocytes, and neuronal cells that play an important role in its function form the blood brain barrier. Tight junctions of brain capillary endothelial cells prevent paracellular transport of small and large water-soluble compounds (**a**). Under some pathological conditions like tumors, the structure and functions of blood brain barrier are altered. In such case, the barrier is called as blood-brain tumor barrier. In high-grade gliomas, the blood brain tumor barrier becomes disrupted and leaky in nature (**b**). Adapted from the Journal of Cancer Metastasis and Treatment, OEA Publishing Inc. from EL Amrawy et al. (2016), an open access article distributed under the creative commons attribution license that permits unrestricted use, distribution, and reproduction in any medium

Further attempts should indeed be made to enhance these nanomedicines to improve their ability to target gliomas (Mahmoud et al. 2020).

12.4 Drug Delivery to the Brain

Paracellular permeability is not provided by the normal physiology of the blood brain barrier. In central nervous system diseases such as glioblastoma multiforme, however, it may take place if the blood brain barrier is damaged, which may allow drug distribution to the brain. Immune cells like leukocytes are transferred through chemotaxis and diapedesis processes to the brain parenchyma in conditions like neuroinflammation, or glioblastoma multiforme. This process could be exploited in the production of nanoparticles or drugs that could be phagocytosed by leukocytes and then transmitted into the brain. This has been shown to improve the effectiveness of free drugs and nanoparticles supplied by such a natural process, which is also regarded as the trojan horse mechanism. This process makes it possible to penetrate the brain with larger particles, but their larger size will also contribute to

enhanced toxicity. To circumvent the blood brain barrier, there seems to be a range of choices some of which are mentioned below.

Intracerebroventricular delivery is done directly into the brain *via* an aggressive skull invasion technique and drug injection. To administer the medication *via* an outlet catheter, a pump or an implantable reservoir is employed. At high levels, they facilitate the flow for a steady drug supply. The procedure of intracerebroventricular is highly aggressive and may contribute to augmented intracranial pressure and infections.

Intraparenchymal/Intracerebral management includes injecting medications straight into the brain tissue either through stereotactic injection or through implant formulation, which can be inserted throughout resection surgical procedure (Gliadel®), or through stereotactic surgical treatment. The problem of this delivery technique is that the medication dissemination happens gradually from the injection/implantation site (penetrates only 2 mm inside) that helps the drug to escape.

Convection enhanced delivery is a surgical technique that is marginally less intrusive whereby catheters are positioned within the brain parenchyma interstitial space. Using a pump the solution of drug is administered inside the brain underneath a positive pressure gradient, resulting in a greater amount being visually presented for intracerebral/intraparenchymal treatment. Although this technique is also invasive and could increase the threat for patients like illness, tissue damage, and air bubbles. Besides, the solution of drug might escape into vulnerable parts of the brain, like subarachnoid space, because of the high pressure being utilized (Bennewitz and Saltzman 2009).

Intrathecal administration is regarded to be among the lowest medical interventions in which medicines are inserted through a lumbar puncture into another subarachnoid space of the spinal cord, touching the central nervous system parenchyma into the cerebral spinal fluid. Though, according to this process, potential adverse effects known as adverse immune responses and infections may follow. Furthermore, while the intracerebroventricular and intrathecal techniques may resolve the obstacles of cerebrospinal fluid and blood brain barrier, the glial cells and ependymal cell layer reside between the cerebrospinal fluid and the brain parenchyma, restricting the effectiveness of diffusion of the drug by these strategies to enter the brain parenchyma (Mahmoud et al. 2020).

Intratympanic administration uses the middle ear pathway to administer medications that are transmitted by pinocytosis, ultimately accessing the brain whereby they circumvent the blood-labyrinthine barrier. For therapeutics upto 1 μm scale, this route may be acceptable. Poly (D, L-lactide-co-glycolide) nanoparticles have been utilized with encouraging effectiveness to deliver drugs through this pathway.

Intranasal distribution is a method of drug delivery that is non-invasive and used for circumventing blood brain barrier into nasal cavity *via* spraying medications, whereby they disperse drugs extracellularly or by convection. Another direction is through the intra-neuronal transport of olfactory sensory neurons or trigeminal nerves, called intra-neuronal transport (Lochhead and Thorne 2012). In addition to becoming effective for customers, the intranasal route is advantageous in facilitating the rapid onset of action and preventing first-pass metabolism (Zhang et al. 2016).

Certain other blood brain barrier crossing strategies have been researched; many of these are intrusive, including the blood brain barrier's osmotic opening. The trojan-horse technology, for example, which depends on the combination of drugs with genetically engineered proteins that could circumvent the blood brain barrier *via* receptor-mediated transport processes, has also been examined by other non-invasive methods. Such strategies are often rife with adverse effects, therefore more reliable, and less toxic methods are required to deliver medications to the brain to enhance the management of brain tumors (Busquets et al. 2015).

12.5 Polymeric Nanoparticles for Targeting Glioblastoma

Nanomedicine has equipped us with such a powerful candidate that can be used around the blood brain barrier to increase the absorption of drugs. It is because nanoparticles are capable of being loaded with drugs and functionalized with various ligands that allow the blood brain barrier targeting. By focusing the medication within or on the blood brain barrier surface, nanoparticles are suggested to carry out their operation in transmitting drugs through the blood brain barrier, resulting in a greater concentration gradient among brain and blood, facilitating drug's passive diffusion throughout the brain. An important advancement in nanotechnology is the polymeric nanoparticles delivery by active targeting through ligands mediated surface modification that attaches to a specific entity on the cancer cell surface or to some other cells inside the body (Holmes 2013; Re et al. 2012). Examples of such nanoparticles for treatment of malignant gliomas by targeting are shown in Table 12.1. Either for the intracellular or extracellular drugs delivery, actively targeted nanoparticles needs to be explored. If directed to intracellular sites, nanoparticles are most successful (Mahmoud et al. 2020). Nanoparticles provide the potential to diffuse through the improved permeability and retention effect into the leaky vasculature of tumor tissues (Wicki et al. 2015). Three key tasks can be achieved for successful treatment of glioblastoma multiforme: (i) to enhance blood brain barrier crossing ability of chemotherapeutic agents, to infiltrate into brain tissue and deliver the therapeutic concentrations in the tumor tissue, (ii) to prevent or eliminate adverse effects, and (iii) to preserve therapeutic drug concentrations at the tumor site, to extend its half-life and to prevent rapid clearance (Wicki et al. 2015).

Polymeric nanoparticles are known as colloidal nanoparticles of submicronic size that are employed as drug carriers in which drugs are either are attached to the surface or encapsulated inside the core. Several types of polymers, like poly (butylcyanoacrylate), poly (lactic acid), poly (glycolic acid), poly (ε-caprolactone), poly (amino acids), and poly (lactic-co-glycolic acid); that are used in the formulation of nanoparticles. Owing to low toxicity profile and biocompatible properties, poly (lactic-co-glycolic acid), poly (lactic acid) and poly (glycolic acid) are by far the most commonly used polymers in the brain targeted drug delivery. They convert into lactic acid and glycolic acid, which join the pathway of kreb's cycle, whereby their metabolites are extracted from the body as carbon dioxide and water. Polymeric

Table 12.1 Polymer nanoparticles developed as targeted drug delivery system for management of brain tumors malignant gliomas

Polymer type	Drug loaded	Particle size (nm)	Targeting strategy	Targeted site	Reference
PLGA	Dil	90	Transferrin	Transferrin receptors (proteins)	Chang et al. (2009)
PMLA	Antisense oligonucleotides	25	mAbs antisense oligonucleotides	Laminin-411	Ding et al. (2010)
PEG-PCL	Paclitaxel	<100	Angiopep	LRP	Xin et al. (2011)
PEG-PLGA	Coumarin 6	125	Peptide (12 amino-acid)	Peptides	Li et al. (2011)
PEG-PCL	Paclitaxel rhodamine	90	Angiopep	LRP	Xin et al. (2012)
PLGA	Methotrexate	85	Transferrin	Transferrin receptors	Jain et al. (2015)
PEG-PLGA	Doxorubicin	100–300	Endogenous tripeptide thiol (glutathione)	Glutathione transporters	Geldenhuys et al. (2015)
PLGA	Loperamide	100	mAbs (8D3)	Transferrin receptors	Fornaguera et al. (2015)
PLGA	Curcumin	100	Magnetic guidance peptide (T7)	Transferrin receptors	Cui et al. (2016)
PLGA	Doxorubicin	120	Poloxamer 188	LRP	Malinovskaya et al. (2017)
PEG-PLGA	Temozolomide	19	mAbs (OX26)	Transferrin receptors	Ramalho et al. (2018)
PLGA	Paclitaxel	230–255	Arginylglycylaspartic acid (RGD) SPIO	αvβ3integrin	Ganipineni et al. (2019)

PLGA poly (lactic-co-glycolic acid), *PMLA* poly (β-L-malic acid), *PEG-PCL* poly (ethylene glycol)-poly (ε-caprolactone) copolymers, *PEG-PLGA* poly (ethylene glycol)–poly (lactic acid-co-glycolic acid), *mAbs* monoclonal antibodies, *SPIO* superparamagnetic iron oxide, *LRP* lipoprotein receptor-related protein, *RGD* arginylglycylaspartic acid

nanoparticles have benefits over traditional nanoparticles, like enhanced kinetics of drug release, improved drugs compatibility, no phospholipid-like oxidation problems, and enhanced shelf life. An acknowledgment of the crystallinity, stability, and molecular weight of the polymers, and also the drug's physicochemical properties, is needed to successfully synthesize polymeric nanoparticles for brain drug delivery (Mahmoud et al. 2020).

Kreuter et al. formulated polymeric nanoparticles to deliver drugs to the brain. Blood brain barrier penetration of dalargin was substantially improved by formulating it into nanoparticles employing poly (butyl cyanoacrylate). In 2001, the same p80 coated dalargin-loaded poly (butyl cyanoacrylate) nanoparticles were used by Kreuter et al. to increase brain tissue penetration. This nanoparticle composition has been used to inject certain drugs into the brain, including loperamide as well as

doxorubicin (Kreuter 2001). Calvo et al. formulated nanoparticles of poly (ethylene glycol) – (poly (hexadecyl cyanoacrylate)) that showed significantly better brain buildup according to the p80 formulation, which might be attributable to passive diffusion or macrophage intake (Calvo et al. 2001). On the surface of nanoparticles, the poly (ethylene glycol) coating density influenced the degree to which they circumvent the blood brain barrier (Mahmoud et al. 2020).

For brain distribution of chemotherapeutic drugs, a multitude of distinct nanoparticle compositions were examined. In subsequent years, a major focus has been paid to nanoformulations for the glioblastoma multiforme management. The usage of nanoparticles for brain delivery can improve the probability of drugs crossing the blood brain barrier whilst minimizing unspecific aggregation in certain tissues. For instance, if correlated with free gadolinium, gadolinium-loaded nanoparticles improved the level of gadolinium by 100 folds. With the optimization of entrapment efficiency, drug loading, and drug release profile, the development of nanoparticles has increased in the last couple of years.

Advances in the stealth capabilities of nanoparticles have also strengthened their protection against protein agglutination in the blood, enabling them to avoid blood cleaning from the reticuloendothelial system. Ligand-modified surface nanoparticles have been documented to allow the imaging of nanoparticles for brain tumors as well. In an attempt to safeguard nanoparticles from plasma protein binding and reticulo endothelial system uptake, PEGylation approach for nanoparticles has been commonly utilized. Nanoparticles provide the brain with a non-invasive drug delivery means. To be much more efficient with decreased toxicity, nanoparticles for brain targeting needs to fulfill some significant tasks. The criteria involves nanocarriers to be biodegradable, non-toxic, no blood aggregation, greater encapsulation efficiency, extended circulation time, and the ability to cross the blood brain barrier (Tian et al. 2014).

Paclitaxel is a chemotherapeutic agent that was in a nanoparticle formulation composed of poly (lactic-co-glycolic acid). The entrapment allowed for the repossession of paclitaxel (toxic chemotherapeutic agent) only within the nanoparticles before the enhanced permeation and retention effect, which reduced systemic toxicity and entered the tumor tissue. Though enhanced permeation and retention effect is extremely advantageous for nanoparticulate drug delivery, this passive mechanism helps the nanocarriers to penetrate the glioblastoma multiforme tissue. Polymeric nanoparticles which do not penetrate cancerous tissues tends to retain in the liver, kidney, and spleen reticuloendothelial tissue (Mehrotra and Tripathi 2015; Pérez-Martínez et al. 2011). Convection-enhanced delivery is a tool to preserve a pressure gradient throughout interstitial infusions. It has been used to improve the paclitaxel loaded polymeric nanoparticles delivery to the brain parenchymal cells. It has been demonstrated that convection-enhanced delivery greatly enhances the distribution of small and large molecules inside the brain (Michael et al. 2018; Zhou et al. 2013).

Enhanced cytotoxicity of paclitaxel – loaded nanoparticles was reported when a ligand specific to the transmembrane human epidermal growth factor receptor 2;

extracellular domain was employed as compared to non-targeted nanoparticles. This increase in cytotoxicity was due to enhanced cellular absorption of the targeted nanoparticles. Due to the drawbacks associated with the use of multi-ligands in a single nanosystem for tumor cells targeting, many researchers have chosen to employ a single ligand. Multi-ligands affect the release of drugs as well as the mobility of nanoparticles. Also, the targeting efficacy of the nanoparticles is often seen to be decreased by interaction amongst ligands and/or competitive binding. It is understood that transferrin receptors as well as low-density protein-related lipoprotein receptor are over-expressed in glioma cells. Using binding the angiopep and anti-transferrin ligands to its exterior surface, these two receptors were targeted by polymeric nanoparticles to reach glioma cells. The most frequently described receptor-mediated transport mechanism is the transferrin receptor that ensures successful cellular uptake.

Using an *in vitro* blood brain barrier model, Chang et al. showed that the transferring coated poly (lactic-co-glycolic acid) nanoparticles showed 20-folds improvement in poly (lactic-co-glycolic acid) uptake relative to uncoated polymeric nanoparticles. The absorption of transferrin- poly (lactic-co-glycolic acid) nanoparticles by the blood brain barrier occurred by endocytosis pathway. The main drawback with transferrin as a ligand is its competition with endogenous transferrin for receptor binding. It may contribute to a decrease in cellular uptake by the tumor cells. An antibody directed against transferrin was used as an alternative ligand to the endogenous transferrin because it binds to the epitope of transferrin receptor, which is located at a higher position as compared to the transferrin binding position. Consequently, even if they do not interact with the transferrin intake process, nanoparticles get less binding rivalry. This improves their cellular uptake and hence their therapeutic efficacy. To improve brain uptake, various antibodies like 8D3 (both anti-mouse TfR mAbs), OX26 (anti-rat TfR mAbs), and R17–217 are being established. Rmalho et al. have produced temozolomide – mediated receptor loaded poly (lactic-co-glycolic acid) nanoparticles functionalized with OX26 mAbs for glioblastoma multiforme treatment. Especially the cellular internalization of OX26 mAbs nanoparticles was greatly improved as compared to the poly (lactic-co-glycolic acid) nanoparticles with no mAbs (Mahmoud et al. 2020).

Another technique for improving the blood brain barrier's absorption of nanoparticles is polymer coating that enhances cellular uptake process. Kreuter showed that *i.v.* injected doxorubicin-loaded p80-coated nanoparticles had a 40% more cure rate in rats with intracranially transplanted glioblastoma multiformes. Albeit not thoroughly elucidated, he hypothesized that endocytosis by the endothelial cells lining the brain blood capillaries may be the underlying mechanism for transporting the nanoparticles around the blood brain barrier. The coating of p80 nanoparticles resulted in the surface adsorption of apolipoprotein E from blood plasma on them. The nanosized particles then imitated low-density lipoprotein particles and were thus able to communicate with the low-density lipoprotein receptor, contributing to improved endothelial cell uptake (Kreuter 2001). The first polymeric nanoparticles for blood brain barrier absorption were studied in 1995 by Schröder et al. for hexapeptide dalargin-loaded nanoparticles by poly (butyl cyanoacrylate) nanoparticles

coated with p80 were noted. Wohlfart et al. showed that poly (lactic-co-glycolic acid) nanoparticles-coated with poloxamer 188 permitted the doxorubicin delivery at therapeutically efficient concentrations across blood brain barrier. Because of the poloxamer 188 coating, the reason for the transport through the blood brain barrier was hypothesized to be the adsorption of blood apolipoproteins (ApoE or ApoA-I) on the surface of nanoparticles. Manlioovskaya et al. showed that through clathrin-mediated endocytosis, the nanoparticles were taken up by human primary glioblastoma cells (U87). They also showed that doxorubicin was released from the nanoparticles through diffusion instead of intracellular degradation (Demeule et al. 2008). The research thus showed that poly (lactic-co-glycolic acid) nanoparticles coated with poloxamer 188 could increase the targeting of such chemotherapeutic drugs for brain tumors.

Angiopep is one more successful lipophorin-receptor ligand that is used for delivery of drugs to central nervous system (Demeule et al. 2008). In contrast to transferrin, the transcytosis potential and parenchymal aggregation of angiopep-2 is much higher. A series of research studies have verified the potential of angiopep to promote blood brain barrier absorption of polymeric nanoparticles (Mahmoud et al. 2020) for enhancing the paclitaxel delivery to glioma cells. Xin et al. formulated nanoparticles with dual-targeting approach. Angiopep-PEG-PCL nanoparticles, relative to non-targeted poly (ethylene glycol)-poly (ε-caprolactone) nanoparticles, were strongly endocytosed by human primary glioblastoma (U87) cells. These nanoparticles in 3D glioma spheroids displayed a greater amount of penetration, distribution, and aggregation as well as enhanced therapeutic effectiveness when in U87 tumor-carrying mice (Xin et al. 2012).

12.6 Peptide-Receptor as a Dual-Targeting Drug Delivery Approach

The use of receptors present on tumor cells for the targeting of nanomedicines is one technique to improve glioblastoma multiforme management. One such example is the low-density lipoprotein receptor (cell-surface receptor) that is expressed by blood brain barrier cells and over-expressed by glioblastoma cells. Angiopep-2, a 19 amino acid peptide that specifically binds to the low-density lipoprotein receptor, has been shown to improve the blood brain barrier delivery of wide chemotherapy agents when evaluated in both in vitro and in vivo models (Pitorre et al. 2020).

Xin et al. investigated the concept of developing dual-targeted angiopep-2 modified nanoparticles. The restructured nanoparticles need to first traverse the blood brain barrier and then target the tumor cells (Xin et al. 2011). Angiopep-2-conjugated poly (ethylene glycol)-poly (ε-caprolactone) nanoparticles were fabricated by coupling of angiopep-2 and maleimide- poly (ethylene glycol)-poly (ε-caprolactone) copolymer. Paclitaxel (PTX) was used as a model drug in the said system. The encapsulation ratio and angiopep-2-paclitaxel loading coefficient decreased without

a targeting ligand relative to paclitaxel loaded nanoparticles. The formulations were evaluated in nude mice implanted with intracranial tumor U87 MG upon intravenous injection. The findings indicated that angiopep-2-paclitaxel nanoparticles aggregation was much greater than paclitaxel nanoparticles in the brain of tumor bearing mice. The finding was supported by an *ex vivo* assessment of the expurgated tissues (liver, heart, kidney, spleen, and lung) that showed selective brain tumor deposition by the targeted nanoparticles (Pitorre et al. 2020). In the presence of low-density lipoprotein receptors in both tumor cells and blood brain barrier, differences in the absorption of angiopep-2-conjugated nanoparticles may be associated with peptide-induced infiltration as compared to non-conjugated nanoparticles. Similar authors examined the bioavailability of angiopep-2-poly (ethylene glycol)- paclitaxel nanoparticles utilizing a three dimensional glioma cell culture model. Angiopep-2-oly (ethylene glycol)-paclitaxel nanoparticles transcytosis through blood brain barrier cells shadowed by tumor cell endocytosis was shown by low-density lipoprotein receptor recognition, verifying the dual-targeting approach (Xin et al. 2012).

The evaluation of anti-tumor effectiveness was done *in vivo* in U87 MG tumor-bearing mouse model. In contrast to the control group treated with saline, tumor inhibition levels were 20.5%, 36.1%, and 65.6%, while mice were given poly (ethylene glycol)-paclitaxel nanoparticles; angiopep-2-poly (ethylene glycol)-paclitaxel nanoparticles or taxol respectively. Furthermore, the median survival time was 37 days for mice treated with angiopep-2-poly (ethylene glycol)-paclitaxel nanoparticles, which was substantially higher as compared to the poly (ethylene glycol)-paclitaxel nanoparticles or taxol treated mice. Altogether, the findings indicated the potential of the dual-targeting method using angiopep-2 conjugated nanoparticles. Besides, after *i.v.* infusion of conjugated non-loaded nanoparticles (100 mg/kg/day) over a week, acute toxicity was not observed in the liver, hematological system, brain and kidney parenchyma (Xin et al. 2011).

12.7 Dual-Targeting of Both Glioma and Neovascular Cells

Of all solid tumors, glioblastoma multiforme is one of the most studied one, and neovascularization has a major role in glioma development (Pitorre et al. 2020). Zhang et al. have established an interesting dual-targeting strategy by developing nanoparticles to target neovascular cells while delivering paclitaxel to control tumor cells. It has been shown that Enhanced green fluorescent protein (EGFP-EGF1), a fusion protein, binds tissue factor uniquely to neovascular and tumor cells. Poly (ethylene glycol)-poly (lactic acid) nanoparticles in the size range of 105 nm was formulated by emulsion-solvent evaporation process, which was evaluated in cells that express tissue factor. An improved *in vivo* absorption of functionalized nanoparticles in extravascular and neovascular tumor cells was observed 4 h after intravenous administration relative to non-functionalized nanoparticles. Also, the median survival time for control animals with functionalized nanoparticles was longer (41 days) compared to the non-functionalized nanoparticles treated animals

(21–27 days), taxol (13 days), and saline (14 days) (Mei et al. 2010; Zhang et al. 2014).

12.8 Aptamer-Peptide Conjugates as a Dual-Targeting Delivery System

Gao et al. designed a targeted delivery method capable of crossing the blood brain barrier. Poly (ethylene glycol)-polycaprolactone nanoparticles loaded with docetaxel were formulated using the emulsion solvent evaporation method and TGNYKALHPHNG (TGN), a 12 amino acid peptide and an aptamer (AS1411) was grafted to the surface of the nanoparticles to boost uptake across the blood brain barrier and target tumor cells, respectively (Gao et al. 2012, 2014). Utilizing mouse brain endothelial cells, the nanoparticles *in vitro* tumor-targeting efficacy was investigated. In contrast with the AS1411- nanoparticles and non-grafted nanoparticles, nanoparticles grafted with both TGN and AS1411 showed a greater brain uptake, which indicated a TGN mediated uptake of nanoparticles *via* blood brain barrier. Nanoparticles modified with TGN were identified in tumor cells as well as in healthy brain tissue, whereas TGN and AS1411 modified NPs both were primarily found within the glioblastoma multiforme cells. The outcomes of the nanoparticles uptake into the glioblastoma multiforme verified the formulation's dual-targeting efficiency. The enhanced *in vivo* effect of the dual-targeting approach *i.e.* mice bearing the tumor treated with AS1411-TGN nanoparticles was shown to have an enhanced survival time by 36 days comparable to TGN- nanoparticles (31 days) or AS1411-nanoparticles (30 days) treated mice (Pitorre et al. 2020).

12.9 Routes of Administration of Nanoparticles in the Treatment of Malignant Gliomas

For nanoparticles engineered to manage brain tumors, there have been three major routes of administration: (i) direct brain delivery (ii) direct systemic brain delivery and (iii) indirect systemic brain delivery. Direct brain delivery ensures nanoparticles injection directly into the brain, which bypasses the blood brain barrier. Convection-enhanced delivery was used specifically in brain tissue to infuse a nanoparticle suspension. Convection-enhanced delivery was used by Lollo et al. to deliver paclitaxel loaded lipid nanocapsules into mice brain. The findings indicated that for lipid nanocapsule-treated mice, the total survival period was substantially higher than that of free paclitaxel-treated mice (Allard et al. 2010; Lollo et al. 2015). Fourniols et al. reported the direct injection of polymerizable hydrogel containing micelles loaded with temozolomide into the brain using a syringe through an incision drilled into the skull. The temozolomide - loaded injection micelles were well tolerated and the hydrogel improved the drug release profile. The key drawbacks of direct brain

delivery included the contamination risk and the necessity to regulate essential factors like osmolarity and pH, which can lead to serious brain injury if not optimized (Huynh et al. 2012).

Specific systemic brain delivery involves the administration of nanoparticles directly *via* the carotid artery into the bloodstream, which are transferred to the brain, eliminating the rest of the systemic circulation. Compared to convection-enhanced delivery, this approach has demonstrated enhanced existence with a decreased risk of brain injury. Huynh et al., using both direct systemic brain delivery and convection-enhanced delivery in glioblastoma multiforme-inflicted rats, administered the nanoparticles loaded with ferrociphenol. Compared to convection-enhanced delivery community's survival of 24 days, direct systemic delivery provided a survival time period of 28 days. The findings showed that direct systemic delivery relative to direct brain delivery may provide a small improvement in survival time spans (Huynh et al. 2011, 2012).

Indirect systemic delivery is required for the further introduction of nanoparticles into systemic circulation through administration routes requiring absorption, such as nasal, oral, peritoneal and topical administration. Non-invasiveness and patient compliance are the main benefits of oral administration. Two different curcumin preparations (nanoparticles and plain suspension) were orally administered to rats and were evaluated in rat intestinal model *ex vivo*. The observations revealed that the nanoparticles bioavailability was 12 times higher compared to the single suspension of the neat drug. Intraperitoneal administration is commonly used as an alternative technique for the administration of the medication into peritoneal tissue. It can be used for delivering massive doses and in situations when a vein for direct systemic delivery is difficult to locate (Verreault et al. 2015).

12.10 Challenges Related to Nanotherapy of Malignant Gliomas

12.10.1 Reticulo Endothelial System

The mononuclear phagocyte system, often referred to as reticulo endothelial system, has cellular and non-cellular components. The administered nanosystems are often recognized by the reticulo endothelial system leading to an induction of cytokine cascade that causes inflammation and the circulating phagocytes may induce the removal of nanoparticles. Besides, macromolecules like proteins and lipids binds to the nanoparticles surface creating a biological corona that is identified and discharged from the bloodstream by the immune system. Surface modified nanoparticles are not recognized by the reticulo endothelial system, which helps in overcoming the said challenge and enables their presence in the bloodstream for extended durations. Surface modification is achieved by using zwitter

ionic ligands such as glutathione, PEGylation, or cysteine. In research conducted by Choi et al., the results inferred the fact that the use of neutral dihydrolipoic acid – connected polyethylene glycol; or zwitter ionic (cysteine) coating material for quantum dot coating prohibited serum protein adsorption and inhibited renal clearance. An *in vivo* study indicated that the use of PEGylated human serum albumin nanoparticles loaded with paclitaxel achieved extended systemic circulation by more than 96 h and improved tumor aggregation leading to improved anti-cancer efficacy and extended animal life expectancy (Mahmoud et al. 2020).

12.10.2 Renal System

The biggest challenge faced by the nanoparticles the systemic circulation is the renal clearance. Nanoparticles greater than 8 nm in size may find difficulties crossing the glomerular filtration barrier. Besides, cationic nanoparticles of 2–6 nm would show greater renal clearance than anionic or neutral nanoparticles of similar size because the glomerular basement membrane is negatively charged (von Roemeling et al. 2017). The shape of a nanoparticle could also influence renal clearance. Improved clearance of rod-shaped nanoparticles with a diameter of 0.8–1.2 nm were reported by Ruggiero et al. (Ruggiero et al. 2010). Optimization strategies may be employed by formulation scientists to design biodegradable nanoparticles that may be resistant to renal clearance. Nevertheless, before entering their target site, this may lead to premature release of the therapeutic drugs (Mahmoud et al. 2020).

12.10.3 Blood Brain Barrier

The blood brain barrier comprises of tight junctions that restricts access of nanoparticles into the brain. Nanoparticles with conjugated ligands, were readily internalized by blood brain barrier through the receptor-mediated endocytosis (Mahmoud et al. 2020).

12.10.4 Pathophysiological Barriers in Cancer

Nature of cancer, its location, stage, and patient's traits are the important characteristics that affect the composition and structure of tumor extracellular matrix and its vasculature (von Roemeling et al. 2017). These properties stand as major hurdle in achieving suitable penetration of the nanoparticles in the solid tumors. Delivery of drugs to the tumor cells involves the transport of nanoparticles through blood

vessels, crossing the interstitial space to reach the tumor site. This delivery is affected by the morphological differentiations between the tumor and normal cells and/or tissues. The abnormal tumor tissue environment leads to leaky vessels, abnormal blood flow, abnormal lymphatic vessels and vascular hyperpermeability. All of these factors contribute to interstitial hypertension, thereby hindering the diffusion process. Two major strategies have been extensively utilized to enhance the drug delivery, namely normalization of tumor vasculature by using antiangiogenic agents that repairs the imbalance between overexpressed proangiogenic and antiangiogenic factors in tumor tissues, and second is normalization of tumor matrix that is based on degradation of collagen and glycosaminoglycan to improve the nanoparticles penetration (Alexandrakis et al. 2004; Batchelor et al. 2007; Blanco et al. 2015; Boucher et al. 1990; Jain 2005; Mahmoud et al. 2020). Smart nanoparticles are being fabricated which can react to environmental conditions and enable better bioavailability for therapy (Mahmoud et al. 2020).

12.10.5 Multidrug Resistance

Multidrug resistance entails drug release outside the cells, either inherited or acquired from long-term drug exposure, causing a reduction in efficacy and concentration of drugs within the cell lumen. Cancer cells can be resistant to some chemotherapeutic agent viz. taxanes, anthracyclines, and vinca alkaloids, which when ejected by cancer cells causes increased toxicity to healthy cells (Szakács et al. 2006). Multidrug resistance probably occurs from overexpressed P-glycoprotein which is an ATP-binding cassette transporter (present in brain, liver and placenta) that functions as efflux pump with an ability of binding several hydrophobic drugs and also plays a role of protecting vital organs from toxins (Aller et al. 2009; Gottesman et al. 2002). Other multidrug resistance associated proteins includes multidrug resistance – associated protein-1 and the breast cancer resistance protein (Fletcher et al. 2010). Efflux pump inhibitors such as verapamil (covera) and cyclosporine have been investigated and are emerging as first-generation antagonists (Dean et al. 2005). Addressing multidrug resistance in cancer has involved the exploitation of nanoparticles drug delivery systems in encapsulating chemotherapy drugs. Liposomes and nanoparticles encapsulating doxorubicin and verapamil have been formulated for the targeted inhibition of P-glycoprotein (Wu et al. 2007).

12.11 Conclusion

Malignant gliomas are some of the most violent tumor types, which do not react to most traditional chemotherapy and radiation therapies. This is actually due to the blood brain barrier's selective nature, which prohibits most particles, particularly therapeutics, from accessing the brain. In addition, traditional glioma management

techniques only enable patients to live for a certain time period while dealing with harmful adverse effects that arise primarily from the invasiveness of treatment methods. Nanodrug delivery system is a non-invasive and versatile therapy area that enables the development of nanometer-size materials to serve as drug delivery systems. Such engineered nanoparticles only targets the over-expressed receptors on tumor tissues while sparing normal tissues, leading to reduced adverse effects. The positive pre-clinical data has formed the base for the suitable application of nanosystems in the clinical usage. FDA approval has been obtained for the application of nanoparticles for the intravenous route, which offers advantages for the management of metastasized tumors. Thanks to their biocompatible and biodegradable actions within the human body and the limitless shapes and features that can be manipulated into, polymeric nanoparticles are attracting further interest in the malignant glioma treatment. As previously discussed in this analysis, polymeric nanoparticles can be especially beneficial once PEGylated. Even more, efforts are required to optimize the scale up techniques, drug loading ability and drug release pattern, considering the physiological obstacles and various physicochemical properties of drugs that may impede their performance.

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Chapter 13
Polymeric Nanoparticles to Target Lung Cancer

Ashish Garg

13.1 Introduction

Lung cancer, which is more common in men, is the primary malignancy that causes elevated cancer-related mortality rates (Padhi et al. 2018). In 2018, it was estimated that lung cancer accounted for 1,54,050 deaths worldwide (Siegel et al. 2019; Bray et al. 2018). Long term tobacco smoking was evidenced to be the origin of cancer (85%) and at the time of diagnosis, it has been identified that lung cancer was metastasized to the other tissues of the body in many patients. 1.8 million new cases of lung cancer were recorded worldwide in the year 2012, of which 58% were identified in developing countries (Torre et al. 2015). In 2015, The American Cancer Society disclosed a report stating that an estimate 221,200 new cases and 158,040 death cases were reported due to lung cancer in United States (Bray et al. 2018). Among the lung cancer patients, 85% were diagnosed with non-small cell lung cancer while the rest were with small cell lung cancer. Early detection, diagnosis and surgical resection of the tumor increase the survival rate of the lung cancer patients (Rudokas et al. 2016; Miller et al. 2016).

The routine treatment modalities used in the treatment of cancer often results in poor survival outcome. Of all the therapies employed in the treatment of cancer, chemotherapy is the most effective and widely used technique for lung cancer. In chemotherapy, the therapeutic effectiveness is impeded by the availability of low drug concentration in the tumor tissue. To provide sufficient amount of drug at the tumor site, repeated administration of high doses of anticancer drugs are needed, which results in manifestation of untoward adverse side effects, the most common being toxicity to the surrounding healthy tissues (Cohen et al. 2015; Patnaik et al.

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2021). Under such circumstances, it is essential and urgent to develop the novel therapeutic modalities for effective treatment of lung cancer. To minimize the development of multi drug resistance, many attempts have been pioneered using small interfering RNAs combined with hydrophobic drugs, hybrid drug with a combination of hydrophilic and hydrophobic parts etc. (Xiong et al. 2011). Many efforts are being put forward in developing potent drug carrier systems encapsulating anticancer drugs that selectively deliver the drug at the targeted site (Shen et al. 2016). Moreover, surface of these drug carrier systems are covalently linked with tumor specific targeting motifs viz., peptides, antibodies, aptamers and proteins to enhance targeting capability (Zhang et al. 2012; Codony-Servat et al. 2018; Mukherjee et al. 2019).

Theranostics has proved to be more effective in treating lung cancer. Nanomedicine uses high efficacy nanocarriers which are attached with imaging and therapeutic drugs. The consent of nanoparticles, which are well suited for diagnosis and drug delivery, has led to the manifestation of personalized medicine in recent years.

Few essential aspects that need to be considered in designing various nanocarriers include (Cong et al. 2018; Chi et al. 2017; Muthuraj et al. 2016; Mukherjee et al. 2017):

(i) Selection of potent drug – small drug molecule to large peptide or nucleic acid
(ii) Choosing suitable carrier
(iii) Selection of appropriate drug delivery system
(iv) Suitable imaging agent

Exchange of gases takes place between air and blood in the lungs. Nearly, 95% of the blood is purified by expelling carbon dioxide and by intake of oxygen. Lung cancer or bronchogenic cancer occurs in the epithelial cells. In 1982, world health organization classified lung cancer into four categories, namely adenocarcinoma, squamous cell carcinoma, large cell carcinoma and small cell cancer. These are further grouped into (i) small cell lung carcinoma and (ii) non-small cell lung cancer. Small cell lung cancer is fatal and is very difficult to cure as compared to non-small cell lung cancer. Small cell lung cancer is mainly caused due to tobacco chewing, consumption of arsenic contaminated water and aspiration of gases. Bronchogenic cancer is developed in the inner walls of the bronchi. Primary stage symptoms include respiratory infections, hoarse throats, difficulty in breathing and chest pain. An advance secondary stage symptom includes fatigue, jaundice, cough with blood laden sputum and difficulty in swallowing. Stages of lung cancer are depicted in Fig. 13.1.

13.1.1 Pathology of Lung Cancer

Lungs are very complex organs with more than 50 cm^2 alveolar membrane that supports the effective passage of gases. In large airway, ciliary action clears the large inhaled particulates whereas antigens are eliminated by phagocytic cells.

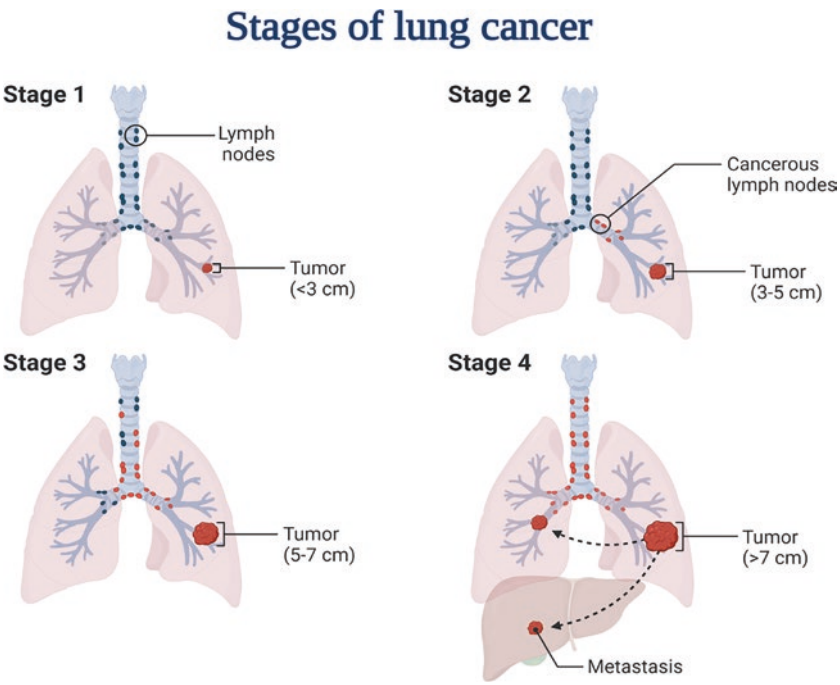

Fig. 13.1 Stages of lung cancer. Tumors in stage I are tiny and have not invaded lymph nodes. Stage I cancers are 3 cm or smaller. Stage IIA cancers have not migrated to lymph nodes. Stage III – the disease has spread to adjacent structures and is classified as stage IIIA, IIIB, or IIIC depending on tumor size. Stage IV – the cancer has spread to other places of the body or to fluid surrounding the lung or heart. In addition to the adrenal glands, lung cancer often extends to the brain, bone, and many other organs. Created by BioRender.com

Cells of the bronchi walls undergo accumulation and adapts to micro environmental conditions, resulting in altered balance of cell division and death, leading to cancer precipitation. Exposure to tobacco smoking for a long time often leads to damage of chronic obstructive lung pulmonary disease and emphysema. Smoking results in various morphological changes in the epithelium of bronchi which progresses to basal cell hyperplasis, metaplasis and severe dysplasia which ends up in carcinoma. In adenocarcinoma, the lungs are severely damaged and its sub-types are very dominant in non-smokers who are exposed to very low carcinogen levels (Travis et al. 1996). The advancement of adenocarcinoma is characterized by the formation of atypical adenomatous hyperplasia (pre-malignant lesions). The only distinguishing pathological characteristic that differentiates squamous cell carcinoma from adenocarcinoma is the presence of poorly differentiated tumours (Thummissen et al. 2014).

13.2 Modalities for the Treatment of Lung Cancer and Limitations

Based on the tumor size and its location, lung cancer can be treated with photodynamic therapy, surgery, target specific therapy, chemotherapy and radiation therapy.

13.2.1 Photodynamic Therapy

Porfimer sodium is the first-generation photosensitizer which utilizes porphyrin, texaphyrin and chlorine as organic photo-sensitive agents for the diagnosis of lung cancer. Photosensitizers are first incorporated at specific sites in the patient body. Exposure to specific wavelength of light leads to excitation from ground state to singlet state then to triplicate state. The singlet oxygen is highly reactive and readily destroys tumor cells (Dolmans et al. 2003).

13.2.2 Surgery

Patients with heart and lung diseases are exempted from the surgical sectioning of tumors. Surgery is recommended for non-small cell lung cancer patients who have stage-I and II forms. The techniques employed in surgical removal of lung cancer includes (a) lobectomy – removal of lung lobe, (b) wedge resection - removal of mass harmful tissues of the lung, (c) pneumonectomy – removal of lungs. Complete eradication is not possible but 10–35% of lung cancers can be cured by surgery. Surgery is the only modality in treating early stage cancer, whereas, however lung cancer surgery is a complex procedure and has many related serious side effects (Rani et al. 2012).

13.2.3 Chemotherapy

List of drugs approved by United State food and drug administration for the diagnosis of lung cancer are presented in Table 13.1. Lack of target specificity is the main drawback of chemotherapeutics for treating lung cancer. Many limitations have been observed in case of oral and IV administration of anticancer drugs, which includes degradation of drug in the stomach, modification of the drug molecule due to the processes of metabolism in the liver, lack of specificity resulting in toxic side effects to the surrounding healthy tissues (Schmid 2005).

Even though much progress has been evidenced in treatment of non-small cell lung cancer, only 5% survival rate has been recorded for last five years. In addition,

Table 13.1 Drugs approved by the United State food and drug administration for treatment of lung cancer disease

Sl. No.	Drugs	Utilized for type of lung cancer	Mode of action	References
1	Abraxane	Non small cell lung cancer	Proficiently inhibit the folic acid reductase cause disruption of synthesis mechanism of DNA leads to cell death	https://www.cancer.gov/about-cancer/treatment/drugs/lung
2	Afinitor	Small cell lung cancer	Alteration in the mammalian target of rapamycin pathway	https://www.cancer.gov/about-cancer/treatment/drugs/lung
3	Avastin	Non small cell lung cancer	Effectively bind with vascular endothelial growth factor leads to inhibit angiogenesis	Mukherji (2010)
4	Carboplatin	Non small cell lung cancer	Modified structure of DNA leads to inhibit synthesis mechanism of DNA	Sousa et al. (2014)
5	Docetaxel	Non small cell lung cancer	Inhibit/modifies the gene expression of bcl-2 & bcl-xl genes, and also inhibit the depolarization of microtubules	Lenz (2006)
6	Doxorubicin	Small cell lung cancer	Inhibit DNA synthesis and alter the biosynthesis pathway	Jackson (2003)
7	Gefitinib	Non small cell lung cancer	Inhibit endothelial growth factor receptor – tyrosin kinase domain	Lenz (2006)
8	Folex	Small cell lung cancer	Inhibit DNA, RNA and protein synthesis	https://www.drugs.com/mmx/folex.html
9	Methotrexate	Small cell lung cancer	Inhibit dihydrofolate reductase	Tian (2007)
10	Topotecan hydrochloride	Small cell lung cancer	Inhibit the cellular growth during S-phase of cell cycle, leads to inhibit the synthesis pathway	Palchaudhuri and Hergenrother (2007)

identification and detection of lung cancer at early stage in very difficult. Patients (approximately 75%) with lung cancer exhibit prominent symptoms, but by the time of diagnosis, patients are found to be in advance stage of the disease. Chemotherapy and radiation therapy were found to be very effective at the early stage treatment of non-small cell lung cancer. Molecular heterogeneity has been evidenced in the same cancer sub-types through robust sequencing techniques and with the studies associated with the genome. Based on the cell of origin, heterogeneity was observed in primary tumor and metastatic tumor and even within the cells of particular tumor. Development of adaptive resistance by the cancerous cells is the major limitations and hence poses a challenging feature in the development of effective modality for the diagnosis of the disease (Jamal-Hanjani et al. 2017). A new strategy has to be developed that focuses on patient specificity and molecular specificities in treatment of lung cancer.

13.2.4 Radiation Therapy

Radiation therapy utilizes X-Rays/ionizing radiation of Radium (^{228}Ra), Iridium (^{192}Ir), Phosphorus (^{32}P) and Cobalt (^{60}Co). Radiation therapy is of two types (a) brachytherapy – radioactive source in pellets is placed close to cancer cells and (b) tele-therapy – source is placed outside the body (Irradiate). Decrease in the white blood cells count and incidence of fatigue are the major side effects of this therapy. Patients who are sensitive to this therapy can also develop nausea, skin irritation and vomiting (Rani et al. 2012).

13.3 Advances in Drug Delivery Systems for the Diagnosis of Lung Cancer

Nanotechnology is an advanced technology in the area of biomedical science and it has been employed in the amelioration of cancer and various forms of diseases such as diabetes, cardiovascular diseases, infectious diseases etc. (DiSanto et al. 2015; Yae and Dai 2018). Due the advantage of its nano size, scientists and researchers have developed nanoscale drug delivery systems for entrapping chemotherapeutic molecules suitable for the treatment of lung cancer. Chemotherapeutics are delivered to the target tumor site by a number of nanovehicles such as polymeric nanoparticles, bio-nanoparticles, liposomes, solid lipid nanoparticles, nanoemulsions, metal nanoparticles etc. (Behera et al. 2020a, b; Hassan et al. 2021). The use of polymeric nanoparticles has an added advantage of enhanced permeability and retention effect which helps in specific accumulation of nanoparticles in the tumor cells (Matsumura and Maeda 1986; Padhi and Behera 2020). The surface of the nanoparticles can be suitably functionalized with suitable ligands for enhancing the targeting capability (Mukherjee et al. 2016). These are biocompatible and remains in the circulation for a prolonged period of time and this may be a probable reason for which this technique holds n edge over the available conventional therapeutic treatments. In addition, nanoparticles embody multifunctional attributes to be employed for diagnostics, imaging and therapeutics, sensing which makes it an apt modality for usage in various biomedical applications. Figure 13.2 depicts the application of polymeric nanoparticle system for lung cancer treatment.

13.4 Polymer-Based Nanoparticulate System for the Management of Lung Cancer

Polymeric nanoparticles, on the other hand, can be prepared either by nanoprecipitation or a double emulsion method via self-assembly of biodegradable amphiphilic block-copolymers with varying hydrophobicities between blocks and are suitable

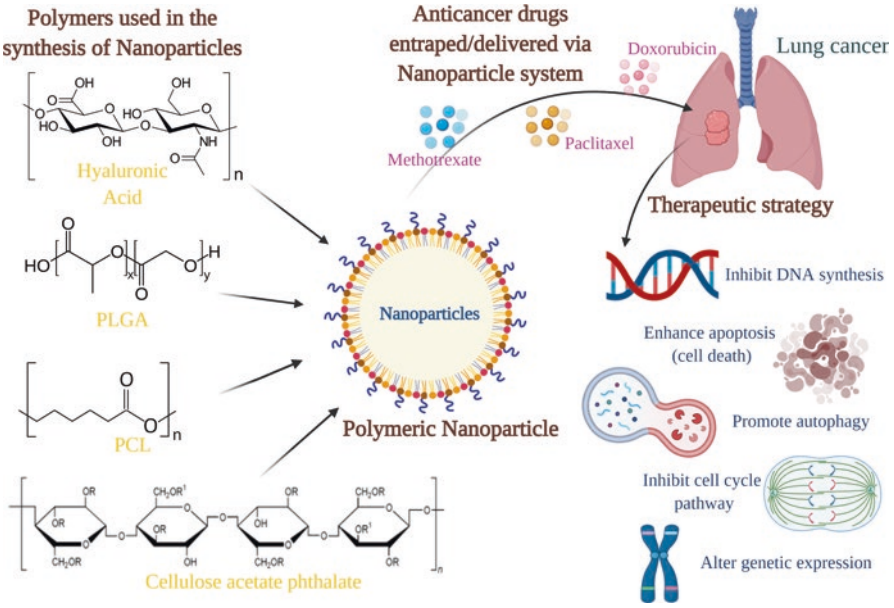

Fig. 13.2 Application of polymeric nanoparticle systems for lung cancer treatment. The polymeric nanoparticles are usually constructed by using variety of polymers such as Hyaluronic acid, poly (lactic- co – glycolic acid), polycaprolactone and cellulose acetate phthalate. These nanosystems have tendency to encapsulate anticancer drugs (methotrexate, paclitaxel, doxorubicin) and release inside the tumor to exhibit anticancer potential via variety of application like, promote autophagy, cell cycle inhibition, alter gene expression and via inhibition of DNA synthesis. Created by BioRender.com

for systemic administration (Verma et al. 2017). The core-shell structure of polymeric nanoparticles facilitates encapsulation of hydrophobic drugs, extension of circulation time, and sustained drug release. Their surfaces can also be decorated for targeted drug delivery (Torchilin 2007). For instance, Genexol-PM is a formulation of paclitaxel and poly (D,L-lactide)-b-poly(ethylene glycol)-methoxy, which is already marketed for metastatic breast cancer therapy in Korea and other European countries (Kim et al. 2004; Ahn et al. 2014). Here are few examples mentioned below which are used for the treatment of lung cancer using polymeric nanoparticles.

13.4.1 Poly(Lactic-Co-Glycolic Acid) Based Nanoparticles

The modified poly(lactic-co-glycolic acid) nanoparticles which showed effective tumor suppression in A549 lung cancer cells that is approved by United States food and drug administration. The benefits of use of poly(lactic-co-glycolic acid) are its circulation time, target delivery and less renal clearance of the drug molecules (Chan et al. 2009; Padhi et al. 2015).

In 2018, Zhang reported that all-trans retinoic acid- poly(lactic-co-glycolic acid) nanoparticles are able to restrict the multiplication and apoptosis of cancer initiating cells and cancer cells. Experimental evidences have proved the capability of retinoic acid to exhibit toxic effect on $CD133^{+}$ lung cancer initiating cells (Zhang et al. 2018).

In a study, administration of disulfiram in combination with cisplatin and vinorelbine to patients with diagnosed non small cell lung cancer in phase IIb showed prolonged survival rates. (Najlah et al. 2017). Moreover, low dose of disulfiram-loaded poly(lactic-co-glycolic acid) nanoparticles with oral administration of copper exhibited restricted growth of tumor and decreased metastasis in the xenograft of mouse lung cancer model.

Geng et al., have developed and discovered the effect of 20 (R) – ginsenoside Rg3 on Lewis mice with lung cancer and to explore the mechanisms of Rg3 – poly(lactic-co-glycolic acid) nanoparticles *in vivo,* and by the result obtained, it was concluded that the nanoparticles can suppress the expression of vascular endothelium growth factor in lewis mice cancerous lungs, thus indirectly contributing to their anti-tumor effects and to alleviating the general condition of the mice (Geng et al. 2016). Zhou et al., developed Salinomycin sodium hybrid lipid-polymer nanoparticles with differentiation cluster-133 and epidermal growth factor receptor antibody for the simultaneous treatment of lung cancer initiating cells and cancer cells, and suggested that nanoparticles had the best efficacy in inhibiting tumor growth relative to control agents in lung cancer-bearing mice and may represent the effective treatment for lung cancer (Zhou et al. 2018).

Vaidya et al. developed a nanoparticle strategy to overcome acquired resistance to erlotinib in non-small cell lung cancer. To induce erlotinib on poly(lactic-co-glycolic acid) biodegradable nanoparticles. Erlotinib-cyclodextrin formulated with β-cyclodextrin sulfobutyl ether, which was in turn loaded into the core of poly(lactic-co-glycolic acid) nanoparticles using polymeric solvent evaporation. And the data suggested that the prepared system improved the therapeutic efficacy against erlotinib-resistant lung cancer using modified nanoparticles erlotinib formulas (Vaidya et al. 2019).

Upadhyay et al., formulated PEGylated poly(lactic-co-glycolic acid) thymoquinone nanoparticles for better delivery of thymoquinone to lung cancer cells and suggest that nanoparticles adorned with transferrin successfully paired two RNA interference (iRNA) pathways to potentiate the cascade of death by apoptosis in very lethal lung cancer cells and also limits the migration of these cells without imparting any significant toxicity, which occurs in widely used chemotherapy combinations (Upadhyay et al. 2019).

Chemo-radiotherapy with paclitaxel and cisplatin is part of the standard of care for patients with locally advanced non-small cell lung cancer. Tian and his the colleague developed biocompatible nanoparticles for the simultaneous delivery of cytotoxic chemotherapeutic agents that can limit off-target tissue toxicity and improve therapeutic efficacy, and suggested that using a nanoparticles pre-delivery strategy for this common chemo-radiotherapy regimen could improve clinical reactions in cancer patients (Tian et al. 2017). He et al. developed folic acid-modified poly (ethylene glycol) - poly(lactic-co-glycolic acid) around cisplatin and

encapsulate paclitaxel for lung cancer. These results implied that nanoparticles could act as effective carriers of cisplatin and paclitaxel and that the simultaneous delivery of cisplatin and paclitaxel by nanoparticles resulted in more potent antitumor cause results than the combination of free drugs or nanoparticles loaded with individual drugs (He et al. 2016).

13.4.2 Polylactic Acid Based Nanoparticles

Yang et al., establishing lung cancer stem cells targeting polylactic acid encapsulated docetaxel nanoparticles for antimetastatic therapy and the data showed that, the strategy for metastatic treatment of lung cancer is effective, and this will offer a potential therapeutic approach to the management of metastatic lung cancer (Yang et al. 2015). Jiang et al. developed a nanoparticle formulation from commercial d-α-tocopheryl polyethylene glycol 1000 succinate (Jiang et al. 2013). In conclusion, the nanoparticles could improve cellular uptake and cytotoxicity, which revealed a possible application of oral chemotherapy for lung cancer. Liang et al., developed docetaxel encapsulated polylactic acid nanoparticles to prepare docetaxel targeted nanoparticles (Liang et al. 2015). The cell uptake and biodistribution indicated that the excellent antitumor efficacy of nanoparticles was attributed to both increased drug and cell uptake accumulation. To our knowledge, this is the first report on the establishment of a targeted delivery system small cell lung cancer that offers a therapeutic alternative potential for therapy purpose (Jiang et al. 2013).

13.4.3 Cellulose Acetate Phthalate-Based Nanoparticles

Garg et al. designed heparin modified polycaprolactone core shell type nanoparticles of usnic acid. The heparin modified polycaprolactone conjugated usnic acid nanoparticles showed prolonged drug release. The heparin modified polycaprolactone conjugated usnic acid nanoparticles showed better cytotoxicity in lungs cancer cells (A549) (Garg et al. 2018).

13.4.4 Polycaprolactone-Based Nanoparticles

Ming et al., studied to investigate the biologic effects of internal irradiation and the therapeutic effectiveness of ^{131}I-labeled arginine-glycine-aspartate-bovine serum albumin-polycaprolactone in murine lung cancer models and suggested that the nanoparticles has excellent cellular binding in vitro in a non-small cell lung cancer xenograft model. Moreover, the system was assessed as an imaging specialist and is a fascinating contender for focusing on treatments with regards to the non-little cell cellular

breakdown in the lungs xenograft model (Ming et al. 2017). Asadi et al. (2018) created superparamagnetic iron oxide nanoparticles and afterward usage of these nanoparticles for exemplification of anticancer medication 5-fluorouracil. Accordingly, these nanoparticles have continued delivery and can apply for disease treatment.

Wang et al., created methoxy poly (ethylene glycol) - poly(caprolactone) micelles stacked with curcumin and doxorubicin and suggest that the information likewise demonstrates that synthesized micelles might be a viable therapy system for disease later on (Wang et al. 2013). Garg et al., additionally used the polycaprolactone as polymer framework for epitomize the medication framework and move the medication into cellular breakdown in the lungs cell through heparin interceded focusing towards anaplastic lymphoma kinase receptors of lung cancer cells (A549) and the information acquired from the studies, it was presumed that the nanoparticle framework indicated powerful and potential focusing towards disease cell and should likewise be used in future for *in vivo* study (Garg et al. 2017).

13.4.5 Hyaluronic Acid Nanoparticles

Hyaluronic acid is one of the generally used polymers in medication conveyance framework for assortment of infection. Naringenin is notable for its chemopreventive properties since antiquated occasions however come up short on a fitting conveyance transporter. For this a gathering of analyst (Parashar et al. 2018), created naringenin stacked poly caprolactone nanoparticles enlivened with hyaluronic acid by using self-amassing layer by layer method, and proposed that the created definition has a promising potential as a helpful and chemopreventive specialist against urethane-instigated lung carcinoma in pale skinned person wistar rodents.

Kumar et al. combined a novel hyaluronic acid dihydroartemisinin form in which the hydroxyl gathering of dihydroartemisinin was covalently connected to carboxylic gathering of hyaluronic acid. The form was effectively described utilizing ^{1}H-NMR and fourier transform infrared spectroscopy. Definitively, present discoveries exhibit hyaluronic acid forms can be utilized to improve the remedial results of anticancer medications (Kumar et al. 2019). Almutairi et al. built up a novel delivery procedure by using hyaluronic acid and chitosan complexation to expand the half-life and movement of drug molecule. Subsequently, the writer investigated the favorable to apoptotic and cytotoxic impacts of nanoparticles against lung cancer cells (A549) and hepatic cancer cells (HepG2 and Huh-7) cell lines and these discoveries exhibit a promising medication conveyance framework to help alleviate drug opposition in cellular breakdown in the lungs (Almutairi et al. 2019).

Tumor-associated macrophages procure a supportive of tumor (M2) aggregate, which advances tumor development, angiogenesis, and metastasis. Certain microRNAs for example, miR-125b, can reinvent tumor-associated macrophages into an antitumor/favorable to incendiary (M1) aggregate. Parayath et al. used CD44 focusing on hyaluronic acid poly (ethylenimine) based nanoparticles typifying miR-125b, and indicated macrophage-explicit conveyance and transfection upon

intraperitoneal organization and these investigations show that nanoparticles can effectively transfect tumor-associated macrophages in lung tissues of both gullible mice and a lung cancer mouse model (Parayath et al. 2018).

Parashar et al., created cellulose acetate phthalate stacked hyaluronic acid nanoparticles using layer by layer procedure to accomplish improved and exact conveyance just as target particularity. The standout helpful capability of nanoparticles was supported from the consequences of receptive oxygen species and mitochondrial film intervened apoptosis. A more noteworthy medication collection in tumor tissue as uncovered from biodistribution examines confirmations focusing on capability of nanoparticles in urethane-initiated lung carcinoma (Parashar et al. 2019). Selenium nanoparticles was connected with hyaluronic acid was set up by Zou et al., to get ready tumor-focused on conveyance vehicle stacked with paclitaxel to create functionalized selenium nanoparticles. Furthermore, found that the selenium nanoparticles indicated more prominent *in vivo* antitumor action (Zou et al. 2019).

Chang et al. created, hyaluronic acid ceramide-based nanoparticles are ideal focusing on transporters for cellular breakdown in the lungs, and reasoned that the mix treatment of photodynamic therapy and chemotherapy, and hyaluronic acid ceramide nanoparticle-based focused on conveyance improved the impacts of photodynamic therapy in cellular breakdown in the lungs in mice (Chang et al. 2016). Novel tumor-focusing on zirconium phosphate nanoparticles altered with hyaluronic acid were created by Li et al., to build a tumor-focusing on paclitaxel conveyance framework for potential cellular breakdown in the lungs treatment (Li et al. 2017). Kim et al., build zinc oxide nanoparticles are utilized in present day malignancy treatment dependent on their particular objective, viability, low poisonousness and biocompatibility and propose that the likely anticancer potential of nanoparticles (Kim et al. 2019).

A redox-sensitive nanocarrier framework was created by Song et al., for tumor-focused on medication conveyance and adequate medication arrival of the chemotherapeutic specialist paclitaxel for improved cellular breakdown in the lungs treatment and recommended that nanoparticles exhibited upgraded antitumor viability and improved security of paclitaxel (Song et al. 2018).

Zhang et al., arranged hyaluronic acid -chitosan nanoparticles stacked with cyanine 3- marked small interfering RNA, focused on treatment for non small cell lung cancer communicating via CD44 receptor (Zhang et al. 2019). Liu et al., utilized tumor-explicit CD44 focused on, hyaluronidase - degradable hyaluronic acid and little measured, renal-clearable, red emanation, cationic ox-like serum egg whites secured gold nanocluster to effectively build size-reducible nano platform and reasoned that the framework give a technique that completely fulfilled the worries in medication conveyance to tumor for improved antitumor impact (Liu et al. 2018). Gong et al., arranged hyaluronic acid changed doxorubicin conjugated ferric oxide nanoparticles for explicit conveyance of ferric oxide nanoparticles to CD44 – positive 4 T1 tumor cells and tumor related macrophages (Gong et al. 2019).

The manufactured novel focused on nanoparticles for conveyance of anticancer chemotherapeutics, involved self-gathering maillard response based forms of

hyaluronic acid and cow-like serum egg whites. Hyaluronic acid filled in as the hydrophilic portion, and as the ligand for effectively focusing on malignant growth cells overexpressing CD44 (Edelman et al. 2017). Wu et al. created hyaluronic acid covered poly(lactic-co-glycolic acid) nanoparticulate with docetaxel is especially strong and can viably target and smother orthotropic human cellular breakdown in the lungs (Wu et al. 2017).

13.4.6 Other

Garg et al. formulated usnic acid loaded heparin modified gellan gum nanoparticles and suggested that nanoparticles displayed a sustained release of usnic acid as compared to gellan gum nanoparticles. Usnic acid loaded nanoparticles exhibited an enormous cytotoxic potential against lung cancer cells and the overall anticancer efficacy and rate of internalization deciphered the higher anticancer potential of nanoparticles (Garg et al. 2019).

13.5 Benefits of Drug Delivery Systems for the Management of Lung Cancer

Nanoparticles have attracted attention of nano-biomedical researchers due to their smaller size which has a huge amount of energy and allows the particle to adsorb and carry the hydrophilic and hydrophobic macromolecules/drug component to the target site (Padhi et al. 2020). Nanoparticles are structured size nanomaterials ranging from 1 to 100 nm in size and offers several advantages such as specific targeted drug delivery, potency to improves stability and eradicate toxicity, capability for active and passive drug targeting, controlled release release pattern (Sivarajakumar et al. 2018). The nanoparticles have promising applications in drug delivery as it is often employed for site specific targeting approaches via active or passive mode, which yields better therapeutic outcomes with reduced off-target side effects (Feng et al. 2008; Behera and Padhi 2020).

A plethora of polymeric nanoparticles encapsulating chemotherapeutic drugs are used for lung cancer targeting (Alivisators, 1996), and the targeting phenomenon of nanoparticle system against human lung cancer cell is depicted in Fig. 13.3. In this, the author demonstrates the potential features of heparin nanoparticles for site specific delivery of usnic acid against lung cancer cells and also demonstrates that the hepatotoxic nature of usnic acid can be minimized by encapsulating usnic acid inside the heparin based nanoparticles (Fig. 13.3 (i)); whereas in the Fig. 13.3 (ii), the targeting proficiency and the mechanism of drug molecule inside the tumor cell was clearly demonstrated to inhibit the DNA synthesis (Garg et al. 2019).

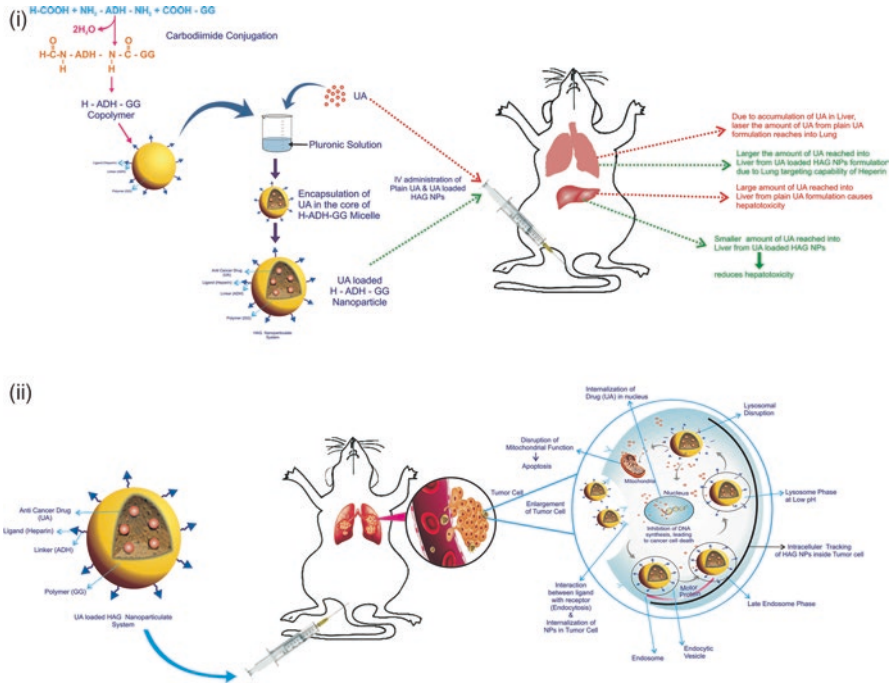

Fig. 13.3 Targeting strategy of polymeric nanoparticle system against human lung cancer cell line (A549). The structure explains the synthesis of heparin anchored gallen gum nanoparticles in section (i), the pluronic acts as surfactants and involve in the synthesis of nanoparticle formulation. The nanoparticle system encapsulates usnic acid as a anticancer drug. Section (i) also demonstrate the hepatotoxic effect of usnic acid, when directly administrated to animal, whereas the usnic acid loaded nanoparticles may reduce the toxic effect of nanoparticles. Section (ii) explains the cellular transportation of nanoparticle system inside the lung cancer cells and explains the treatment strategy of nanoparticle system. Reused with the permission of Elsevier – License No. 4957371327831, 27 November 2020 (Garg et al. 2019)

13.6 Recent Patents and Clinical Trials on Drug Delivery Systems for Lung Cancer

Advances in nanoparticle designing and formulations are related to numerous applications in the detection and treatment of malignant growth. The development of lung cancer nanotheranostics, with an accentuation on clinical use is limited due to associated drawbacks. The reproducible synthesis of several nanoplatforms with composite structures and its scale-up produces significant complications. Though, coupling therapy with diagnosis together into a single theranostics platform should provide significant advantages but the imaging and tumor targeting components might have different pharmacokinetics and dynamics and can mount a significant manufacturing challenge. Weighing the potential benefits to possible adverse health risk assessment of lung cancer nanotheranostics needs to be considered based on

Table 13.2 Completed and ongoing clinical trials on nanoparticle based drug delivery for lung cancer management

Sl. No.	Study	Phase: Ongoing & completed	References (Clinical Trial Government Identifier No.)
1	Study to determine the safety and efficacy of BIND-014 (docetaxel nanoparticles for injectable suspension) as second-line therapy to patients with non-small cell lung cancer	Phase 2	NCT01792479
2	Neoadjuvant chemotherapy of nanoparticle albumin-bound paclitaxel in lung cancer	Phase 2	NCT02016209
3	BIND-014 (docetaxel nanoparticles for injectable suspension) as second-line therapy for patients with KRAS positive or squamous cell non-small cell lung cancer	Phase 2	NCT02283320
4	Neoadjuvant chemotherapy of nanoparticle albumin-bound paclitaxel/carboplatin vs. paclitaxel / carboplatin in stage II B and IIIA squamous cell carcinoma of the lung	Phase 2	NCT01872403
5	Carboplatin and paclitaxel albumin-stabilized nanoparticle formulation followed by radiation therapy and erlotinib in treating patients with stage III non-small cell lung cancer that cannot be removed by surgery	Phase 2	NCT00553462
6	Paclitaxel albumin-stabilized nanoparticle formulation and carboplatin in treating patients with stage IIIB, stage IV, or recurrent non-small cell lung cancer	Phase 2	NCT00729612
7	Paclitaxel albumin - stabilized nanoparticle formulation in treating patients with previously treated advanced non-small cell lung cancer	Phase 2	NCT01620190
8	TUSC2-nanoparticles and erlotinib in stage IV lung cancer	Phase 1 & 2	NCT01455389
9	Trial of EP0057, a nanoparticle camptothecin with olaparib in people with relapsed/refractory small cell lung cancer	Phase 1 & 2	NCT02769962

scientifically sound, evidence-based and well-controlled clinical studies. The clinical trials (completed and ongoing) and patents for the management of cancer specifically lung cancer are depicted in Tables 13.2 and 13.3 respectively.

13.7 Conclusion

Nanomaterials have enormous advantage and are currently being exploited for multiple biomedical applications. Nanostructures, because of their novel attributes can regularly beat solvency and strength issues through surface change/wrappings.

Table 13.3 Patents on nanoparticle-based drug delivery for cancer management

Sl. No.	Study	Patent No.	References
1	Controlled release cyclodextrin-based polymer (CDP) systems for the delivery of therapeutic peptides.	US20120302505	Fetzer et al. (2012)
2	Nanoparticle composed of diblock copolymer of poly (ethylene glycol) and poly (lactic acid) or poly (ethylene glycol) and poly (lactic – co - glycolic acid)	US8246968B2	Zale et al. (2012)
3	Nanoparticles encapsulated with multiple agents including targeting agents.	US20130315831	Shi et al. (2013)
4	Method for synthesizing curcumin loaded (in a hydrophobic core) polymer-based nanoparticles with a hydrophilic shell with one or more chemotherapeutic agents.	US20130330412A1	Maitra et al. (2013)
5	Methods for killing cancer cells by exposing the cells to cancer drug based on antibody, attached with gold, copper, iron oxide, silver, liposome or polystyrene, nanoparticles, and irradiation with ultrasound for treating the non-malignant and malignant epithelial cells of lung	US20140335156A1	Kosheleva et al. (2014)
6	Treating liver cancer and inhibition of metastasis with nanoparticles targeting chemokine receptor type-4 (CXCR4).	US9415011B1	Chen et al. (2016)
7	Therapeutic nanoparticle that includes an active agent or therapeutic agent, e.g. vinorelbine or vincristine or pharmaceutically acceptable salts thereof, and one, two, or three biocompatible polymers.	US9351933B2	Zale et al. (2016)
8	A nanoparticle complex based on gelatin is discussed for targeted delivery of small interfering RNA for silencing specific gene cancer cells.	US9415060B2	Kim et al. (2016)
9	Synthetic or an isolated targeting peptide, comprising a sequence of amino acid, wherein the targeting peptide is active in, binding to a cell of human lung cancer but not a normal cell.	US9387257B2	Wu et al. (2016)
10	Discussing the nanoparticles of polyester having properties suited for encapsulating fluorescent dyes together with therapeutic drugs, resulting in theranostic particles, useful in therapeutic treatments and diagnostic methods of cancer.	US9555008B2	Perez et al. (2017)
11	Methods of preparation of an activated polymeric nanoparticle and its composition have been discussed for targeted drug delivery. The composition consists of a biocompatible polymer and an amphiphilic stabilizing agent.	US9555011B2	Braden et al. (2017)

The incorporated methodology of attaching ligands, drugs, biomolecules, and imaging modalities into a functionalized nanoparticle empowers medication conveyance and diagnostics. Another perspective that guides is the higher payload and the high surface area owing to their nano size range. However, a ton of unsolved bottlenecks

still exists with the usage of polymeric nanoparticles which majorly includes scale-up issues, efficient manufacturing and altered pharmacokinetics of the medication and so on. Extra issues with nanotoxicity and administration routes need to be solved out to confer targeted delivery of the drugs in the lungs. In spite of the fact that nanotechnology has accomplished incredible walks, yet at the same time it seems not to be utilized to maximal effect in different malignancies, especially lungs.

With the appearance of more grounded sequencing, immunohistochemistry and proteomic strategies, a superior comprehension of the components of malignant growth and distinguishing proof of new authoritative biomarkers are in transit. The combination of the relative multitude of advances in these unified fields may change the fate of therapeutics for a definitive destruction of disease in the coming times.

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Chapter 14
Polymer-Based Nanoplatforms for Targeting Breast Cancer

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14.1 Introduction

Breast cancer remains the utmost feared disease among women with the highest mortality rate. It impacts about 2.1 million lives each year (DeSantis et al. 2019). Researchers speculate that soon breast cancer will be the major disease of concern in the health care system. Clinicians describe or classify by staging or grading systems. The systematic classification helps to find suitable or available treatment options along with the forecasting of treatment strategies (Harwansh and Deshmukh 2020; Tade and Patil 2020). The molecular as well as histological indications

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suggest three categories of breast cancer: (1) breast cancer expressing estrogen or progesterone receptors known as ER-positive or PR positive, (2) breast cancer expressing human epidermal receptor 2 known as HER2 positive and (3) triple-negative breast cancer that is devoid of estrogen or progesterone receptors (Phipps et al. 2011).

Though specific reasons that result in the cancer initiation is not clear, the gene sequencing and cellular analysis is still a ray of hope in the path of advanced material based research. Genetic sequencing, cellular expression and cellular targeting models are now being utilized at *in vitro in vivo* (IVIV) levels. In the recent years, many early diagnostic tools such as uPA/PAI-1, Oncotype DX, MammaPrint and antibody – drug conjugates have been discovered and they have made a significant contribution in clinical outcomes as well as patient survival rates (Barzaman et al. 2020).

14.1.1 Breast Cancer Types and Cellular Targets

Breast cancer is a highly diverse disease, comprising of genetically and epigenetically distinct clinical characteristics. Generally, it can be classified according to histopathology, grades, stages, receptor status or other different characteristics. Mostly, histopathological and WHO classification is envisaged for further assessments (Tavassoli 2003). A large amount of current data on breast cancer is generated from *in vitro-in vivo* experiments using breast cancer cell lines. Cell lines are still used as crude models for the study of nanomaterials in which they give the portrayals of typical therapy. This forms the basis of foremost study, which ultimately reflects the future challenges that can be countered at cellular levels.

The most commonly used breast cancer cells express subtypes such as estrogen receptor, progesterone receptor and Human epidermal growth factor receptor 2. A majority of cancer cells overexpress these receptors except triple-negative breast cancer cells. Despite this, gene expression profiling is also used to categorize breast cancer cells. Triple negative breast cancer cells results in poor prognosis due to the lack of these receptors (Dai et al. 2017; Rosa et al. 2014). Many approaches are designed to minimize drug resistance and receptor targeting due to the failure of traditional therapy (Gupta et al. 2018). Generalized histological classification of breast cancer is summarized in Fig. 14.1.

14.1.2 Types of Polymeric Materials and Nanoplatforms

Nanotechnology gifted materials with advanced functionality has overwhelmed the horizons of research and changed the perspective of scientists (Di Sia 2014; Padhi et al. 2020). Progress in polymer chemistry has resulted in remarkable growth in the field. The ability to tune and monitor the properties of polymeric materials makes

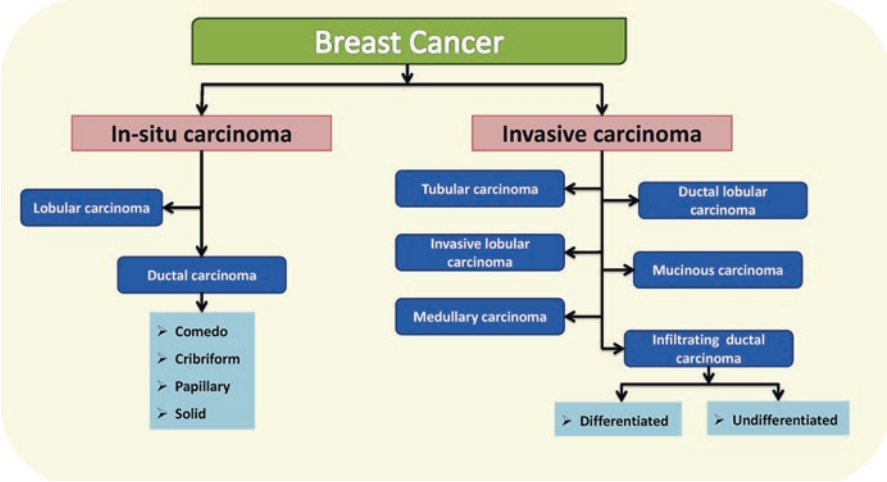

Fig. 14.1 Histological classification of breast cancer. Typically, breast cancer tumor exists as localized or metastatic forms. Localized form is known as in-situ carcinoma and metastatic forms is known as invasive carcinoma. In-situ breast carcinoma is further classified into lobular and ductal carcinoma. Similarly invasive breast carcinoma is classified into tubular, ductal lobular, invasive lobular, mucinous, medullary and infiltrating ductal carcinoma according to histology of the metastatic breast cancer

them a viable delivery option for different biomedical applications (Agarwal et al. 2019; Padhi et al. 2015). Though many sources and routes of polymeric materials are available, the natural and semi-synthetic materials have remained a prime focus for researchers. Various synthesis and purification strategies are employed for polymeric materials procured from natural resources. Besides, novel natural materials and typical grafting methods are being studied by researchers worldwide. Post-synthetic modification and surface-functionalization protocols for the polymeric materials are also being investigated.

The route of administration is the key factor to be considered before designing polymer based nanoplatforms in drug delivery. Delivery or design consideration is a prerequisite for the targeted treatment therapy, and this could be achieved by assessing different aspects of polymeric materials (Han et al. 2018, Xu et al. 2014). The physicochemical attributes of polymeric materials changes according to the chemical nature, hence a critical design consideration is prerequisite. In this context, suitable custom modifications or surface modification protocols need to be designed, which further helps to add tailor-made properties to specific polymeric materials. A common pathway involving the selection of an ideal polymeric material based therapeutic platform, from its origin to multiple applications is illustrated in Fig. 14.2.

Fig. 14.2 Synthesis and applications of polymeric nanomaterial. This scheme depicts the origin of polymers and how the polymeric nanocarriers can be prepared. The synthesized nanoparticles then can be used in various drug delivery platforms

Out of the pool of nanocarriers employed for the successful delivery of chemotherapeutics, polymeric nanoparticles have garnered wide attention of scientists (Padhi et al. 2018). There is a lack of categorized or classified accounts on the polymeric nanoparticles but the origin and their functional use reflects their applications in various fields. The polymers used in the biomedical arena are generally biodegradable, derived from natural materials or accomplished with chemosynthetic modification in their native structure. Both the category has been a common axiom for the investigators for drug delivery and supportive use in biomedicine.

14.1.3 Current Trends

Several polymeric nanoplatform based targeted therapies for breast cancer are on the horizon with promising outcomes which would lead to shifting the epitome of breast cancer treatment away from traditional therapy towards aiming of tumor biology. Nonetheless, genomic complexity, tumor heterogeneity and clonal progression are the major impediments to the success of precision therapy of cancer (Han et al. 2018).

A very few polymer based nanoformulations are approved by the Food and drug administration or European medicines agency in breast cancer treatment. These include PEGylated liposomes like Doxil®, Onpattro® lipid carrier, CosmoFer® (launched in market for anaemia) and other viral liposomes vectors for hepatitis. Other polymeric nanoparticles based formulation in clinical evaluations such as ThermoDox® for doxorubicin, mitoxantrone hydrochloride liposome, merrimack, human epidermal receptor 2 – targeted liposomal PEGylated doxorubicin and Cynviloq IG-001 (paclitaxel polymeric micelle) (Anselmo and Mitragotri 2015). Research outcomes on polymeric nanoparticles are typically limited to their physicochemical attributes and toxicity issues (Padhi et al. 2020). In addition, interaction of nanoparticles with cells or tumor microenvironment, biomolecules in the body, perfusion rate of organs or tissue, might result in the changes of nanoparticle behaviour (Ashfaq et al. 2017).

14.2 Strategies for Choosing Polymeric Nanomaterials

Polymeric nanoparticles based drug delivery is mushrooming rapidly. Various studies have reported around 50 different types of polymer based materials, which are presently being exploited for clinical usage (Anselmo and Mitragotri 2015). The urge to design and customize strategies for drug delivery is a prerequisite. Though cellular or animal models have been developed for the therapeutic effectiveness assessment and targeted study, there surfaces a need to develop solid strategies to deploy polymeric nanoparticles in clinical settings. Considering the complex nature of cancer, the amalgamation of polymeric nanoparticles with advanced functional materials such as grapheme quantum dots and fluorescent dyes is necessary for better treatment (Jagtap et al. 2020; Soares et al. 2020).

Unceasing investigations in nanotechnology has been established many monumental findings and contributed to the advancement of diagnosis and treatment sectors. The overall expertise in the fundamentals of cellular biology of cancer has helped to identify cellular mechanisms and morphologies, resulting in benefit to the research scholars working on specific delivery platforms (Agarwal et al. 2019). Generally, scale-up and clinical translations of polymeric materials depend on the selection of specific material, its surface properties and delivery routes, which remains the major point of concern. The polymeric nanoparticles based drug

targeting platforms revolves around the paradigms of investigational drugs. Besides, the on-going clinical trial outputs might help in evaluating and establishing current clinical demands that are not achieved through traditional drug delivery approaches (Bennet and Kim 2014).

14.3 Polymeric Nanoplatforms for Cargo Delivery to Tumor Cells

14.3.1 Polymeric Nanoparticles

Polymeric nanoparticles are colloidal particles that are solid in nature and with particle size in between 10 nm to 1 μm. According to the formulation methods, polymeric nanoparticles can be divided into two categories: nanospheres and nanocapsules (Crucho and Barros 2017). Nanospheres have a matrix structure that contains homogeneously dispersed drug, while in nanocapsules the drug is enclosed by a polymeric membrane (Bhatia 2016). Polymeric nanoparticles are composed of innumerable categories of polymers and it is used for the fabrication of nanospheres or nanocapsules (Calzoni et al. 2019). Biodegradable polymers are widely used in biomedical applications (Ahlawat et al. 2018) such as the treatment of neurodegenerative (Calzoni et al. 2019), brain concomitant diseases (Carroll et al. 2010), and cancer (Malik and Mukherjee 2018; Khuroo et al. 2014).

Polymeric micelles loaded with zileuton were found to be effective in anticancer therapy. Pluronic F127 is a polymer with well-known features and validated leads for the synthesis of drug delivery systems was used to encapsulate zileuton. The technique of thin-film hydration was used to synthesize polymeric micelles captured in their hydrophobic inner core with zileuton. Two cancer stem cells models *viz.* breast cancer cell lines (MDA-MB-231 and MCF-7) have been used for the study. Authors observed the IC_{50} breast cancer cell line (MCF-7) = 292 μM, IC_{50} (MDA-MB-231) = 461 μM with no such toxicity (*in vivo*), when the most feasible dose found to be 15 kg/mg. Encapsulated zileuton decreased the number of cancer stem cells in the tumor and blocked the circulating tumor cells in the bloodstream and metastatic spread effectively (Gener et al. 2020). Hyaluronic acid mediated tumor targeting with pH-sensitive amphiphilic polymeric nanoparticles were fabricated by Chen et al., for combined delivery of the drug ethambutol and tumor necrosis factor-related apoptosis-inducing ligand plasmid for synergistic anticancer efficacy. Using the amphiphilic polymers polyethylenimine – poly [(1, 6-hexanediol)-diacrylate-β-5-hydroxyamylamine], which was synthesized through michael addition polymerization, these pH-sensitive amphiphilic polymeric nanoparticles were produced. The particular linkage between hyaluronic acid and CD444 (highly expressed on breast cancer cells MDA-MB-231) resulted in higher drug penetration in MDA-MB-231 cells compared with MCF-7 (non triple negative Breast cancer cells) with lower CD44 expression. In addition, polyethylenimine -poly [(1,

6-hexanediol)-diacrylate-β-5-hydroxyamylamine] material showed higher cytotoxic and pro-apoptotic effects in MDA-MB-231 cells compared to free drug and tumor necrosis factor-related apoptosis-inducing ligand plasmid – loaded nanoparticles due to caspase 3/7 activation, increased levels of reactive oxygen species and inhibition of apoptosis-related protein expressions. Co-delivery of ethambutol and tumor necrosis factor-related apoptosis-inducing ligand plasmid by hyaluronic acidconjugated pH sensitive polymeric nanoparticles ominously enhanced cytotoxicity and apoptosis due to the expansion of the caspase 3/7 activity. These results showed that polyethylenimine – poly [(1, 6-hexanediol)-diacrylate-β-5-hydroxyamylamine] framework had increased cytotoxic and pro-apoptotic effects against MDA-MB-231 cells and showed a high potential for triple negative breast cancer therapy (Xu et al. 2020a).

Novel polymeric nanoparticles were prepared by free radical mechanism using *Artemisia absinthium* extract. Further, study was performed against breast cell lines (MCF-7 and MDA MB-231) to examine the possible target responsible for cytotoxicity. The cytotoxicity effects of *A. absinthium* extract loaded polymeric nanoparticles were studied using apoptosis assay, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, cell cycle study and carboxy fluorescein diacetate succinimidyl ester proliferation assay (Mughees et al. 2020). Phenylboronic acid: poly (ethylene glycol)-poly ε-caprolactone and acetylated chondroitin sulfate-protoporphyri polymers were blended by solvent evaporation method. Doxorubicin -loaded phenylboronic acid: poly (ethylene glycol)-poly ε-caprolactone micelles and R837-loaded micelles were fabricated and sprinkled in tetrahydrofuran. These micelles were further used for targeted delivery of not only R837 but also drug doxorubicin to Tamoxifen and tumor cells via *intramuscular* and intravenous injection. This study reported an excellent *in vivo* antitumor effect with highest tumor inhibition rate of about 85% and the highest survival rate of mice up to 80% (Wei et al. 2019).

Recently, PEGylated nanoparticles made up of poly (D, L-lactic-co-glycolic acid)- hydroxymethyl glycolic acid were reported for the targeted delivery of saporin within the cytosol of human epidermal growth factor receptor 2 expressing cancer cells. The mutual use of saporin loaded 11A4-nanoparticles and photochemical internalization inhibited the cell proliferation and diminished cell viability by apoptosis. The combined influence of targeting nanoshell on nanoparticles with photochemical internalization are active means to appreciate discerning uptake and cytotoxicity of saporin loaded nanoparticles (Martínez-Jothar et al. 2019). Epirubicin loaded polymeric micelles having a pH-triggered drug release characteristics against axillary lymph node metastasis of triple negative breast cancer was fabricated. Therapeutic effect of epirubicin micelles against axillary lymph node metastasis of breast cancer was assessed along with association of drug release behavior using a metastatic tumor model. Enhancement in the drug delivery of epirubicin micelles and the anticancer effect was found to be dose dependent. This confirms the usefulness of pH-responsive nature of epirubicin micelles for the specific management of axillary lymph node metastasis in breast cancer (Chida et al. 2018).

In recent times, superparamagnetic iron oxide nanoparticles have gained great deal of consideration for their use in magnetically-guided drug delivery for cancer therapeutics owing to their excellent chemical stability, biodegradability and superparamagnetism (Kandasamy et al. 2019). Calcitriol-loaded polymeric nanoparticles with different polymer to oil ratio were presented by Nicolas et al., anti-proliferative and cytotoxic activities of prepared calcitriol polymeric nanoparticles was evaluated *in vitro* using human breast adenocarcinoma cells (MCF-7) and exhibited calcitriol-induced cell growth inhibition was thoroughly related to its release kinetics (Nicolas et al. 2018). Polymersome structure for the controlled release of methotrexate as an anticancer drug has been formulated with the goal of refining the drug's loading efficiency in polymersomes along with the achievement of proficient control over the drug release rate from nanocarriers. Methotrexate was encapsulated into 12 ± 0.09% loading power polymersomes and 45.5 ± 0.41% encapsulation performance (Nosrati et al. 2019). As drug delivery systems, polymeric hydrogels greatly characterise hydrophilic and biocompatible devices commonly used in the pharmaceutical industry. The benefit of swelling capacity control can be utilised to decrease dose-dependent adverse side effects due to the need to consume significant quantities of water to retain the desired volume of water (Scrivano et al. 2019). Fluorous polymeric micelle that is covalently conjugated with doxorubicin via a hydrazone linkage for pH responsiveness has been developed by Wallat and co-workers. The selective deposition of the drug at the tumor site or within tumor cells and mitigates dose-limiting toxicity via passive targeting for multiple tumor models had been proved (Wallat et al. 2018).

In a recent study, charge switchable nanoparticles were prepared that showed improved diffusion and cellular uptake for combined delivery of doxorubicin and lapatinib. 2, 3-dimethylmaleic anhydride PEG poly-L-lysine doxorubicin or lapatinib nanoparticles for synergistically eradicating breast cancer were prepared, which not only can converse their surface charge in cancer tissue microenvironment, but also precisely discharge the encapsulated drugs specifically into cancer cells. The encouraging physicochemical properties of these nanoparticles were conducive to their stable circulation in the blood and selective accumulation in the cancer tissue (Guo et al. 2020).

14.3.2 Polymer-Based Nanocomposites

An important group of materials with different physicochemical properties is polymer-based nanocomposites which do not have access to individual components that function independently. Polymer-based nanocomposites are a polymer matrix containing a uniform nanoscale distribution of organic or inorganic fillers (around 10–100 nm in dimension) produced by physical mixing or chemical polymerization techniques (Hossain and Hoque 2018). As a class of materials with extraordinary characteristics, polymer-based nanocomposites are implemented. The primary difficult aspect of polymer-based nanocomposites is the complex interfacial regions

between the nanoparticles and polymer matrices (Dhillon and Kumar 2018; Holt 2016).

For targeted delivery to solid tumor cells, biodegradable and tumor-microenvironmental sensitive polymers have been synthesized. The N-(2-Hydroxypropyl) methacrylamide monomer was linked to doxorubicin through pH-responsive hydrazone attachment to form N-(2-Hydroxypropyl) methacrylamide-doxorubicin, which was further rejoined with free N-(2-Hydroxypropyl) methacrylamide using methoxy poly (ethylene glycol)$_2$–4,4-azobis-(4-cyanopentanoic acid) as a macro-initiator in the fundamental polymerization reaction (Bobde et al. 2020). Lipid polymer hybrid nanoparticles loaded with docetaxel had been fabricated for precise delivery of the chemotherapy agents for breast cancer therapy. Lipid polymer hybrid nanoparticles have recently appeared as an auspicious drug delivery system to overcome their shortcomings such as low solubility, high dose toxicity, unspecific dissemination to the undesired site, limited half-life in the bloodstream (Jadon and Sharma 2019). Nanoparticles of either poly (L-lactide-co-glycolic) acid or poly (ethylene glycol) and superparamagnetic iron oxide were hydrothermally fabricated and captured in a poly (lactic-co-glycolic acid) – poly (ethylene glycol) layer with the docetaxel by an adapted emulsion evaporation process for appropriate delivery of the drug to targeted breast cancer cells. The study recorded a prevailing uptake of breast cancer cells (MCF-7) over a 0.5 h incubation period, indicating equal cytotoxicity against MCF-7 cells (Panda et al. 2019).

14.3.3 Polymeric Nanoplexes

A nanoplex is made up of a cationic or an anionic drug bound to an oppositely charged substance (polyelectrolyte) to form a drug nanoparticle complex (Kadam et al. 2015). The yield of nanoplex is higher compared to other nanostructures and its preparation methods are easy (Empedocles et al. 2015). Depending on the solubility, the drugs are dissolved to form a cation or anion in an acidic or simple aqueous solution (Hofmann and Mysels 1992). Amphoteric drugs produce cations when they are solubilized in aqueous acetic acid solutions when pH of the solution is less than the drug pKa while acidic drugs form anions when dissolved in aqueous potassium hydroxide solutions (pH of the solution is more than the drug pKa) (Avdeef et al. 2016). Subsequently, the ionized drug solution is added to an aqueous polyelectrolyte solution that is oppositely charged, where the drug-polyelectrolyte electrostatic interaction takes place resulting in the formation of a resolvable drug polyelectrolyte composite (Cheow et al. 2014).

Polysaccharide-based nanocomplexes have been considered as a promising strategy for chemotherapy and photodynamic therapy. A sequential self-assembly process was used to prepare polysaccharide-based nanocomplexes from aldehyde-functionalized hyaluronic acid and hydroxyethyl chitosan. The nanocomplexes were chemically conjugated to doxorubicin and pro-photosensitizer

5-aminolevulinic acid using a schiff base linkage and their surface was decorated with an anti- human epidermal growth factor receptor 2 antibody as a targeting moiety. The polysaccharide nanocomplexes showed cytocompatibility in breast cancer cells (MCF-7); however, in the acidic microenvironment, doxorubicin and aminolevulinic nanocomplexes exhibited negative: positive charge reversing mechanism and rapid drug release. In addition, the coating of nanocomplexes with anti-human epidermal growth factor receptor 2 antibody as a targeting moiety considerably enhanced its cellular uptake through endocytosis mediated by the human epidermal growth factor receptor −2 receptor (Wang et al. 2019).

14.3.4 Surface-Modified Nanoplatforms

Surface modification strategies provide the added advantages of design and fabrication of specific targeted delivery systems. Addition or attachment of different surface functionalities on cargo particles could help in site-specific delivery of the active materials (Behera and Padhi 2020). There are several aspects involved in surface modification such as physical entrapment and surface immobilization technique. These methods comprise of a non-specific surface adsorption of the biologic moiety or a chemical compound followed by chemical crosslinking (Padhi and Behera 2020). Similarly, electrostatic attraction implies ion-ion or ion-dipole interactions. In the case of biomolecules based surface modifications strategy, specific biological interfaces involve prior immobilization of the precise binding sites. Chemical bonding facilitates specific chemical reactions on explicit functional groups (e.g. –hydroxyl, carboxyl and amine, etc.), which are needed to be pre-reacted to activate these sites for particular bond formation (Kumar Pramod et al. 2019a; Patel et al. 2019; ud Din et al. 2017).

14.3.5 Antibodies and Immunological Agents

Human epidermal growth factor receptor overexpression is an indication of human epidermal growth factor receptor positive tumor cells. Poly (lactic-co-glycolic acid) encapsulated tamoxifen with co-polymer polyvinyl-pyrrolidone as a stabilizer was conjugated with human epidermal growth factor receptor -antibody for the site-specific delivery of tamoxifen against MCF-7 cells. Human epidermal growth factor receptor-conjugated polymeric nanoparticles exhibited notable improvement in anti-tumor activity. The cytotoxicity studies and flow cytometry analysis indicated an excellent biocompatibility as well as the internalization capability of polymeric nanoparticles. Additionally, the c-Jun N-terminal kinase facilitated caspase-dependent apoptotic signaling path also confirmed apoptotic cell death. An *in vivo* mice model showed substantial tumor suppression, which concluded the use of these surface-modified polymeric nanoparticles in cancer treatment (Vivek et al. 2014). Tamoxifen

and its related cancer immunotherapy use is a favorable approach that involves shifting the immunosuppressive tumor microenvironment with the imiquimod for improved cancer therapy. Imiquimod possesses low water solubility, which limits its applications. Wei et al. developed dual-targeting polymer micelles that can deliver separately imiquimod and doxorubicin to tamoxifen cells to overcome the problem of solubility. The drug-loaded micelles were directly injected into the 4 T1 cells tumor-induced mice. After injection, the selective accumulation showed superb tumor suppression. The imiquimod released and bound to the lysosomal membrane via toll-like receptor (TLR)-7 receptor ultimately resulted in the production of immune response as indicated by the tumor suppression. This novel strategy put forth a new path involving chemo-immunotherapy against breast cancer (Wei et al. 2019).

The use of immunological agents with novel delivery platforms offers site-specific delivery options. A dual-acting immunostimulatory prodrug carrier (poly (ethylene glycol) $_{2K}$/Fmoc-1-methyl tryptophan) for 1-methyl tryptophan (indoleamine 2,3-dioxygenase inhibitor) for simultaneous delivery of doxorubicin and studied in 4 T1 cells. Doxorubicin loaded polymeric carrier showed marked cell proliferation inhibition and enhanced apoptosis. The overall mechanism resulted in the dropping of kynurenine production. Decreased tryptophan/kynurenine ratio in blood as well as in tumor tissue promoted effector $CD4^+$ and $CD8^+$ T cells as a result it caused reduced regulatory T cells expression. When tested in 4 T1 tumor-bearing mice, the doxorubicin-poly(ethylene glycol) $_{2K}$/Fmoc-1-methyl tryptophan polymeric nanoparticles showed significant potential as an immunotherapy agent in cancer (Lan et al. 2020).

14.3.6 Aptamers

The aptamer is short single-stranded oligonucleotide comprising of about 10–100 nucleotides (nt), which binds its targets with great affinity and specificity (Ray et al. 2013). An isolated DNA aptamer with excellent affinity and specificity towards human epidermal growth factor receptor-2 was coated on a chitosan graft pluronic polymeric nanoparticles loaded paclitaxel introduced for breast cancer treatment. This model platform demonstrated an efficient delivery of paclitaxel to the specific tumor cells (Nguyen et al. 2016).

A novel approach demonstrating the drug distribution to the tumor was established using heat shock protein 70 (Hsp70) bounded with covalently 8-mer peptide aptamer. In addition, doxorubicin also conjugated with prepared polymeric nanoparticles. The synthesis of poly (ethylene glycol methacrylate) -co- N-(tert-butoxycarbonyl)-N′-(6-methacrylamidohexanoyl)hydrazine -co- ethylene glycol dimethacrylate -co- cyanine-5 methacrylamide was carried out with amalgamating N-(tert-butoxycarbonyl)-N′-(6-methacrylamidohexanoyl) hydrazine monomer using reversible addition−fragmentation chain-transfer agent 4 – cyano-(dodecyl sulfanyl thiocarbonyl) sulfanyl pentanoic propyne. Subsequently, it was prepared and loaded with doxorubicin. Hyperbranched polymer-3 and hyperbranched

polymer −5 were attached to an aptamer. Synthesized polymeric nanoparticles showed a significant accumulation within xenograft tumors to that of the non-targeted analogue. Besides, it showed a controlled release of doxorubicin *in vitro,* which was accomplished by the pH-sensitive hydrazone bonding with high stability at pH 7.4. These reported novel polymeric nanoparticles based platform could be a pavement for the targeted delivery for breast cancer (Zhao et al. 2017).

14.3.7 Small Interfering RNA and Messenger RNA

Small interfering RNA based targeted delivery system has become popular because of its role in protecting the cell from foreign mRNA attacks (Chiu and Rana 2002). To attain effective targeted delivery of siRNA in a tumor cell or in its inner environment remains the prime focus for researchers for achieving clinical translation. Small interfering RNA has the potential to target all types of proteins. Studies demonstrated its effectiveness when compared to the use of an antibody. The tumor microenvironment and its related factors affect the site-specific action of small interfering RNA and thus need an extensive investigation (Ngamcherdtrakul et al. 2016). Asadi et al. developed lipid-polymer hybrid nanoparticles for delivery of small interfering RNA and insulin-like growth factor type I silencing, which was further demonstrated in MCF7 cells. Insulin-like growth factor type I involves cell proliferation and migration regarding the apoptosis process. Fabricated poly lactic-polyethylene glycol nanoparticles showed significant biocompatibility and cellular penetrability. This lipid-polymer hybrid nanoparticles-based platform could serve as a proficient moiety for the delivery of small interfering RNA (Asadi et al. 2018). A similar platform-based small interfering RNA for the silencing of X-box-binding protein 1, which involves breast cancer chemoresistance was reported by Zhang and co-workers. The human epidermal growth factor receptor 2-targeted nanoparticles were synthesized using a stepwise bottom-up approach. The fabricated X-box-binding protein 1 nanoparticles showed highly specific binding to the tumor cells and successful tumor suppression. The intravenous administration of prepared RNA nanoparticles showed good distribution and X-box-binding protein 1 deletion with inhibited cell proliferation (Zhang et al. 2020). Typical strategies for the small interfering RNA delivery through different routes have been illustrated in Fig. 14.3.

An interesting study involving the combination of aptamer with small interfering RNA was demonstrated in receptor tyrosine-protein kinase positive MCF-7 cancer cells. The whole system is named aptamer-protamine-nanoparticles, which is composed of small interfering RNA (oncogene) surviving small interfering RNA to sense the inhibitory potential of aptamer-protamine-nanoparticles to inhibit tumor growth by silencing the targeted gene. The *in vitro* study indicated a specific targeting potential of aptamer-protamine-nanoparticles in MCF-7 cells as well as the *in vivo mice* tumor suppression confirmed the precise growth inhibition (Xu et al. 2020b). Tedious procedure and costly surface modification strategies limit the use of miRNA in tumor-targeted delivery. Xu et al. developed a strategy for co-delivery of miRNA (tRNA-mir-34a) with doxorubicin via amphiphilic copolymer-based

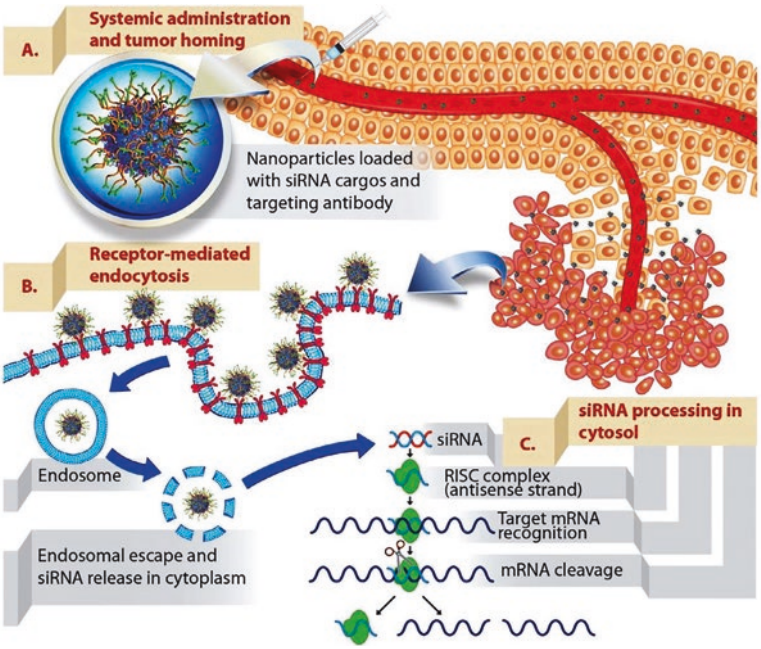

Fig. 14.3 Fate of small interfering RNA delivered intravenously to the tumor. (**a**) Nanoparticles produced as small interfering RNA carriers must be able to shield small interfering RNA from blood breakdown and extend their blood residence duration (for example, mesoporous silica nanoparticles surface functionalized with polymer and targeting antibody). The enhanced permeability and retention effect will increase the likelihood of small interfering RNA – nanoparticles to collect in malignancies. (**b**) Nanoparticles are taken up to cancer cells by endocytosis, an important step in the development of tumour stem cells. When small interfering RNA are endocytosized, they must escape to the cytosol, where they will act. Small interfering RNA is then processed by intracellular machinery to deliver information about which genes to target. Reprinted from Ngamcherdtrakul et al. (2016). Copyright 2016, with permission from Elsevier. *siRNA* small interfering RNA, *mRNA* messenger RNA, *RISC* RNA-induced silencing

nanomicelles. These conjugates not only showed down-regulation of the target gene but also synergized the antitumor efficacy of doxorubicin. The system showed outstanding results in 4 T1 breast cancer cells (Xu et al. 2019).

14.3.8 Miscellaneous

New amphiphilic linear-dendritic polymeric hybrids amphiphilic linear-dendritic polymeric hybrids based fluorescent-labeled nanocarrier was introduced for co-delivery of triptolide and doxorubicin. The assembly of the macromolecular structure was achieved in a structure control manner using cholesterol and dendritic block co-polymer with polyethylene glycol. This customary modification offered precise control over the hydrophobic/hydrophilic ratio. The use of doxorubicin-loaded amphiphilic linear-dendritic polymeric hybrids in MCF-7 and

resistant-MCF-7 showed a substantial decrease in drug resistance by 90% and up to 50%, respectively (Andrén et al. 2017). Recently, highly biocompatible molecularly engineered antitumor drug-loaded nanomicelles investigated the delivery to CD44 overexpressed tumor cells. These nanomicelles were fabricated with carbodiimide chemistry approach using hyaluronic acid and further conjugated to niclosamide. The formation of nanomicelles was confirmed using microscopy. The nanomicelles revealed the effective membrane and cytoplasmic targeting potential in MDA-MB-231 cells lines. Further, the targeting potential was studied in HT1080 tumor xenograft mice model, the niclosamide – hyaluronic acid showed promising tumor suppression (Jain et al. 2020).

Cholic acids as a drug targeting spacer were explored for the targeted delivery of Paclitaxel via poly (bis (carboxyphenoxy) phosphazene) – Poly 51 (di allyl dimethyl ammonium chloride) polymeric micelles. Generally, Farnesoid X receptor is over-expressed in breast cancer cells. These micelles form a gel-like structure at the body temperature, which helps in binding to specific tumor sites. The log-time retention of gel prolonged the drug release. H2A histone family member X assay demonstrated a genetic damage in the cell and efficient cellular uptake. This study indicated an efficient strategy for site-specific delivery in cancer treatment (Mehnath et al. 2018). A novel core/shell chitosan and hyaluronan hybrid nanoparticles have been fabricated for the tumor targeted delivery. The hybrid consisted of a core with a drug and the shell of cysteine cross-linked via by disulfide bonds. The confocal imaging confirmed the successful endocytosis of the drug in MCF-7 and MDA-MB-453 cells. It was noted that the polymer hybrid not only improved the drug loading but also exhibited greater stability and intracellular drug delivery via glutathione-hyaluronic acid multiple stimuli, which makes it a superior candidate for drug delivery in cancer (Wu et al. 2018).

A variety of polymeric materials and novel strategies regarding their multi-functional use have been explored in recent times. The major advantages of polymeric nanoparticles are attributed to their biocompatibility, tailor-made property and biodegradability. There is still a need to emphasize and study various aspects of polymeric nanoparticles to reach a significant milestone in cancer treatment (Table 14.1).

14.4 Polymeric Nanoparticles and Nanomaterials-Based Theranostic Strategies

The multifaceted nature of breast cancer demands a multifunctional motif, though it is not possible to fabricate such materials with multifunctional properties (Thorat and Bauer 2020). The typical attributes of the tumor microenvironment such as hypoxia, altered vasculature, intratumoral pressure and pH obstruct the drug penetration. The traditional drug delivery system fails to offer both diagnosis and therapy (theranostic) for single material composition. Hence, there is a prerequisite to

Table 14.1 Polymeric nanoplatform (PNP)-based tumor specific cargo delivery

Sr. No.	Anti cancer agent	Polymeric nanoparticles	Synthesis method	Cell lines	References
Polymeric nanoparticles					
1	Zileuton™	Pluronic F127 polymeric micelles	Thin-film hydration technique	MDA-MB-231-ALDH1A1:, MCF7-ALDH1A1:	Gener et al. (2020)
2	Embelin	Amphiphilic polymers, polyethylenimine modified Poly(β-amino ester)	Michael addition polymerization	MDA-MB-231 cells	Xu et al. (2020b)
3	Artemisia absinthium	*Artemisia absinthium* extract loaded polymeric nanoparticles	Free radical mechanism	MCF-7, MDA MB-231	Mughees et al.(2020)
4	Doxorubicin, immunomodulator imiquimod (R837)	chemotherapeutic micelle -dox-orubicin and immune stimulating micelle (ACP-R837)	Solvent evaporation method	RAW264.7, 4 T1	Wei et al. (2019)
5	Saporin	PEGylated poly(D,L-lactic-co-glycolic-co-hydroxymethyl glycolic acid)	Copolymerization	(HER2 positive), ATCC HTB-30), MDA-MB-231 (HER2 negative, ATCC CRM-HTB-26)	Martínez-Jothar et al. (2019)
6	Epirubicin	Drug loaded polymeric micelles	Copolymerization	(MDA-MB-231-luc-D3H2LN)	Chida et al. (2018)
7	Curcumin and Verapamil	multifunctional magnetic-polymeric nanoparticles	Thermolysis	–	Kandasamy et al. (2019)
8	Calcitriol	Calcitriol-loaded polymeric nanoparticles	Nanoprecipitation	MCF-7	Nicolas et al. (2018)
9	Methotrexate	Mono methoxy poly (ethylene glycol)-poly (ε – caprolactone) methoxy poly (ethylene glycol)-polycaprolactone di block copolymers	Ring opening polymerization	MCF-7	Nosrati et al. (2019)
10	Sunitinib	Molecularly imprinted polymer	Free radical bulk polymerization	MCF-7	Scrivano et al. (2019)

(continued)

Table 14.1 (continued)

Sr. No.	Anti cancer agent	Polymeric nanoparticles	Synthesis method	Cell lines	References
11	Doxorubicin	pH-responsive doxorubicin fluorous polymer	Copolymerization	(A2780), (OVCAR3), (ID8-B7) (MDA-MB-231)	Wallat et al. (2018)
12	Doxorubicin and lapatinib	2,3-dimethylmaleic-anhydride-poly(ethylene glycol)-ε-poly-l-lysine-doxorubicin /lapatinib polymeric nanoplatform	Nanoprecipitation	MCF-7	Guo et al. (2020)
Polymer-based nanocomposites					
13	doxorubicin	N-(2-hydroxypropyl) methacrylamide copolymer-doxorubicin)-b-polyethylene glycol	Copolymerization	MCF-7, 4 T1	Bobde et al. (2020)
14	Docetaxel	Docetaxel encapsulated polymeric nanoparticles.	Nanoprecipitation	MDA-MB-231	Jadon and Sharma (2019)
15	Docetaxel	Engineered polymeric iron oxide nanoparticles	Hydrothermal reaction	MCF-7	Panda et al. (2019)
Polymeric nanoplexes					
16	doxorubicin	Multifunctional polysaccharide-based nanocomplexes	Nanoprecipitation	MDA-MB-453 and MDA-MB-435	Wang et al. (2019)

developing a multifunctional system that could provide 'all-in-one' type functionalities. In this line, several endeavors have been made to overcome this issue, especially in polymer-based platforms with different nanomaterials have been envisaged (ud Din et al. 2017). Polymeric materials such as dendrimers (Ray et al. 2018), lipid-based polymer vesicles (Luk et al. 2012), polymer nanocomposites and other stimuli-responsive nanocarriers were investigated for the theranostic approaches (Chapman et al. 2013; Mi 2020). Polylactic acid is a biodegradable polymeric carrier is extensively investigated in combination with different nanomaterials. Polymeric nanoparticles for targeting cell surface markers were developed by Liu et al., for enhanced ultrasound imaging (Liu et al. 2007).

Photothermal therapy was acclaimed by investigators due to its great specificity, low side-effect, and efficacy (Xu et al. 2014). The major advantage of the theranostic strategy that is 'see and treat' was effectively utilized with polyacrylamide polymer-based drug-loaded nanocarrier. The polyacrylamide nanoparticles were attached with rhodamine. Cyanine dye was then loaded in the nanocarrier with F3 peptide-cysteine by reaction between amino groups of nanoparticles and sulfosuccinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate. The *in vitro* targeting studies in MDA-MB-435 cells showed that polymeric nanoparticles can be effectively transported to tumor cells. The incubation of multifunctional nanocarriers into tumor cells provided powerful fluorescence imaging signals. Irradiation of the photosensitizing drug integrated into the nanoparticles, with the light of the appropriate wavelength, causes substantial but specific disruption to the saturated tumor cells, but only within the illuminated zones (Li et al. 2017).

Surface treated superparamagnetic nanoparticles were studied comprehensively in the last two decades for hyperthermia in breast cancer with or without polymeric materials. Surface-coated superparamagnetic nanoparticles with polyethylene glycol mounted onto reduced graphene oxide nanosheets were introduced for magnetic hyperthermia in breast cancer cells. It was found that reduced graphene oxide acts as a propagation ground for the spatially scattered nano-sites around which ferrihydrite seeds accumulate to ultimately transform them into immobilized superparamagnetic nanoparticles. The PEGylated superparamagnetic nanoparticles-reduced graphene oxide was found to be biocompatible compared to PEGylated superparamagnetic nanoparticles. This affirmed no significant cell apoptosis or other destructive damage to cells. On the contrary, a dose-dependent cytotoxicity response was noted in both nanocomposites. Magnetothermal studies noted that composite induced local heating (specific absorption rate value 1760 ± 97 W/g) of about 43 ± 0.3 °C. The composite showed a substantial decrease in breast tumor cells (MCF-7) to about 78 ± 10% upon application of an external magnetic field. Summing up, reduced graphene oxide and polyethylene glycol moieties have a synergistic effect in the improvement of magnetothermal properties of superparamagnetic nanoparticles (Alhasan et al. 2019).

The chemo-photothermal approach provides all in one type of benefits, which in turn is realized in cancer treatments. Feng et al. synthesized nanocages for the simultaneous delivery of erlotinib and doxorubicin, based on endogenous and exogenous stimuli-responsive nanocarriers. The nanocages (gold nanocomposites) were

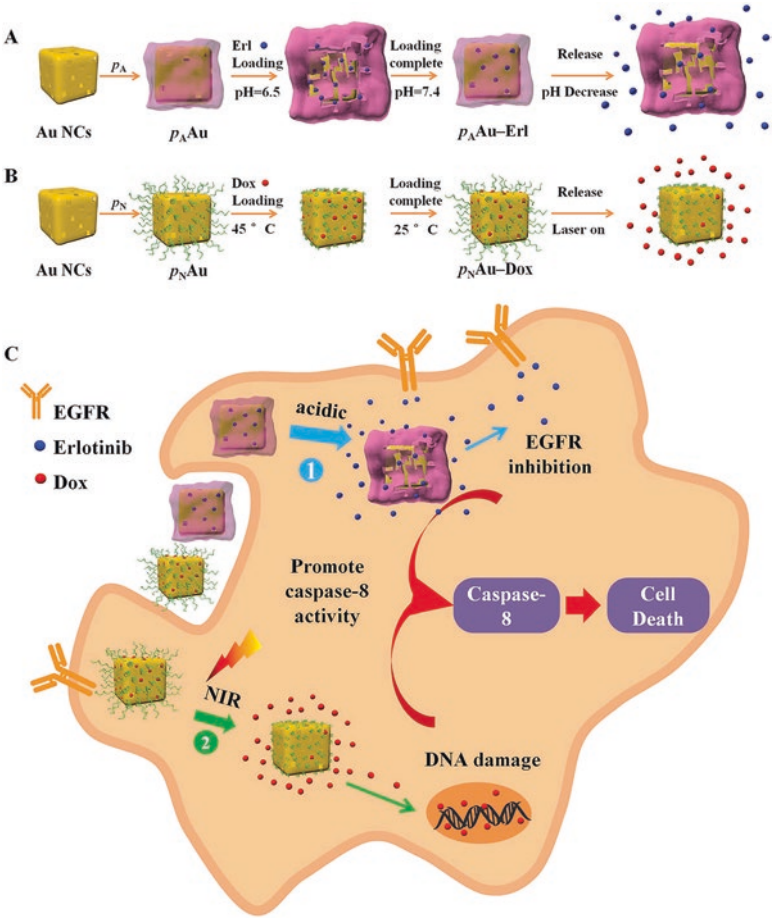

Fig. 14.4 Fabrication and internalization of gold nanocages by use of different polymeric material. Scheme A and B represents the fabrication of gold nanocages with erlotinib and doxorubicin. "C" represents the cellular fate of the prepared nanocages with near infra-red radiation. Gold conjugated polymeric erlotinib and doxorubicin get internalized and subsequently regulate the caspage −8 signalling leading to cell death. Reproduced from Feng et al. (2019). Copyright 2019, with permission from Elsevier

fabricated by galvanic replacement reaction with chloroauric acid. A typical process of nanocages formulation is depicted in Fig. 14.4.

These pH-responsive microcontainers with specific targetability were investigated in epidermal growth factor receptor-expressed cancer cells (MCF-7 and A431). Under the influence of near-infrared irradiation and tumor microenvironment, the activation of assembled nanocages selectively released the erlotinib and doxorubicin. Time phased release of both drugs and photothermal therapy promoted apoptotic signaling, leading to an improved tumor cell destruction in both MCF-7 (reduced epidermal growth factor receptor expression) and A431 (high epidermal

growth factor receptor expression) tumor cells. It provided controlled duration and drug exposure both in spatial and chronological order. This novel carrier system paved a new pathway for photo-chemotherapeutic strategy in cancer treatment (Feng et al. 2019).

The use of biomimetic agents was found to be decreased in exaggerated cellular response and increased specific targeting potential. Kumar et al. used infra-red780–1 loaded with MDA-MB-831 cell-membrane capped nanoparticles for targeting and bioimaging of brain metastatic breast cancer. An 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was used to assess the cytotoxic activity of doxorubicin-loaded and cell-membrane capped nanoparticles. The *in vivo* bioimaging potential and blood brain barrier crossing potential were assessed in mice by injecting 0.1 mg/mL/gm and was confirmed using vivo imaging system. Near-infrared fluorescent imaging (in nude mice) indicated the extended distribution of cell-membrane capped nanoparticles compared to uncoated nanoparticles. In addition, *ex-vivo* imaging showed a longer survival duration of cell-membrane capped nanoparticles (up to 48 h) in the brain relative to uncoated nanoparticles. However, these results indicate that cell-membrane capped nanoparticles hold a huge potential for targeted as well as guided fluorescence imaging (Kumar Piyush et al. 2019b).

Near infrared – II window is being explored in fluorescence imaging for its strong deep tissue penetration ability (Ding et al. 2020). Hu et al. prepared a gadolinium-chelated polymer conjugate for *in vivo* photoacoustic, magnetic resonance and near infrared – II imaging-guided tumor photothermal therapy. The structured 9, 9- dioctylfluorene-co-dithienylquinacridone by co-polymerization and polycondensation to endow conducting properties. Further, polycondensation was employed to coat the polyethylene glycol. The carboxyl functionalities of polymeric-fluorene-thiadiazoloquinoxaline- poly(ethylene glycol) were blended with gadolinium to form a chelate (polymeric-fluorene-thiadiazoloquinoxaline-poly(ethylene glycol)-gold nanoparticles). The *in vitro* behavior of polymeric-fluorene-thiadiazoloquinoxaline-poly(ethylene glycol)-gold nanoparticles was explored along with its *in vivo* multimodal imaging and anticancer efficacy in mice bearing 4 T1 cell tumors (Fig. 14.5). Polymeric-fluorene-thiadiazoloquinoxaline-poly(ethylene glycol)-gold nanoparticles exhibited exceptional chemical stability and good biocompatibility. After 24 h of systemic administration of polymeric-fluorene-thiadiazoloquinoxaline-poly(ethylene glycol)-gold nanoparticles, the tumor locations of mice showed noticeable enhancement in photoacoustic intensity. Moreover, substantial enhancement in the magnetic resonance and near infrared – II fluorescence intensity was noted post 24 h of polymeric-fluorene-thiadiazoloquinoxaline-poly (ethylene glycol)-gold nanoparticles administration to mice. This novel platform can be considered as a perfect gateway with adjoined traditional and advanced nanomaterials for the combined cancer chemo-photothermal therapy (Hu et al. 2019).

Future developments in polymeric nano theranostics would need to hopefully address issues such as discrepancies between drug loading and tracking, due to the combination and properties of anti-cancer drugs and tracking agents. However, we can be positive that biomedical research will overcome these issues to address the

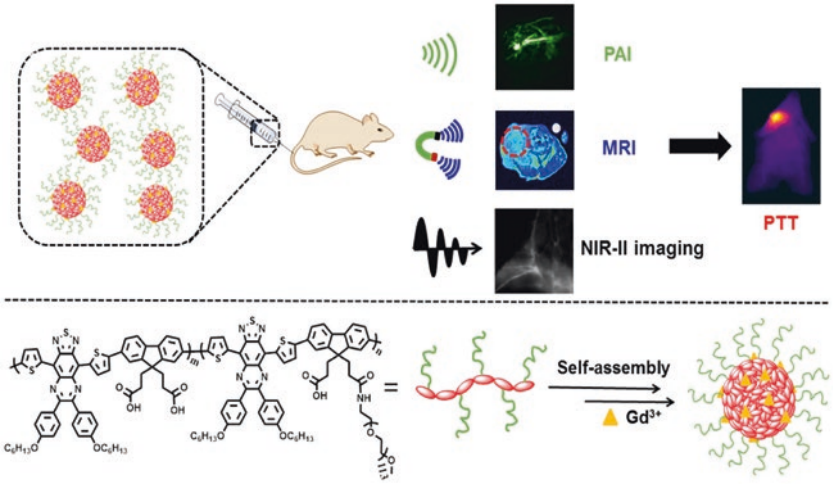

Fig. 14.5 High-resolution multimodal imaging-guided cancer photothermal therapy using polymeric-fluorene-thiadiazoloquinoxaline-poly(ethylene glycol))-gold nanoparticles. The scheme depicts the formulation and structure creating a conjugated polymer had a semiconducting spine composed of low-bandgap donor-acceptor which possessed strong near infra red absorption for photoacoustic imaging/photothermal therapy and long fluorescence emission to near infrared-II region for biological imaging. Reproduced from an open access article "Gadolinium-chelated conjugated polymer-based nanotheranostics for photoacoustic/magnetic resonance/NIR-II fluorescence imaging-guided cancer photothermal therapy" Hu et al. (2019). *PAI* photoacoustic imaging, *NIR-II* near-infrared II, *MRI* magnetic resonance imaging, *PTT* photothermal therapy, *Gd* gadolinium

technical challenges for realistic clinical applications. Imaging techniques using nano-formulations can also allow advancement from the "anatomical level" to the "molecular level." These consequential advanced therapies may lead to cancer cures caused by particular gene mutations (Indoria et al. 2020; Table 14.2).

14.5 Toxicological Perspectives of Polymeric Nanoplatforms

The market demands of anti-cancer entrapped polymeric nanoparticles based formulations have promoted the high throughput research efforts that are focused on the fabrication and development of refined nanomedicines, which in turn may get into the clinic shortly (Jaymand 2019). However, polymeric nanoparticles based agents present many problems, which need critical evaluation before they enter the market. Nature of polymeric material, its origin and methods of preparation result in altered physicochemical properties, which show some extent of incompatibility to the body. The particle size-shape, surface charge and the functional agent present on the surface of polymeric nanoparticles have a significant role in toxicity. Multiple cellular events such as reactive oxygen species formation, alteration in cell

Table 14.2 Theranostic strategies based on polymeric nanoplatforms

Sl. No.	Polymeric material	Anticancer agent/Drugs	Fabrication approach	Cellular/animal models	Application	Reference
1.	Polylactic acid	Herceptin	Surface conjugation	MDA-MB-231 and SkBr3	Targeted ultrasound imaging	Liu et al. (2007)
2.	Polyacrylamide	F3-cys and cyanine dye	Amine-functionalization	MDA-MB-435	Tumor targeting, fluorescence imaging, and photodynamic therapy	Wang et al. (2012)
3.	Ferrimagnetic polyethylene glycol-poly(2-hexoxy-2-oxo-1,3,2-dioxaphospholane	–	–	MDA-MB-231 tumor mice	Photothermal therapy and photoacoustic imaging	Li et al. (2017)
4.	Polyethylene glycol-coated superparamagnetic iron oxide nanoparticles	–	PEGylation	MCF-7	Superparamagnetic-thermal therapy	Alhasan et al. (2019)
5.	N-isopropyl-acrylamide-co-acrylamide	Erlotinib and doxorubicin	Heating and reversible addition–fragmentation chain transfer polymerization	MCF-7 and A431c	Photothermal therapy and bioimaging	Feng et al. (2019)
6.	m-polyethyleneglycole-poly (lactic-co-glycolic acid)	Doxorubicin, IR 780	–	MDA-MB-831, mice	Image-guided therapy	Kumar Piyush et al. (2019b)
7.	Polymeric-fluorene-thiadiazoloquinoxaline-polyethylene glycol-gold nanoparticles	Gadolinium	Grafting-on method	4T1 neoplastic mice model	Photoacoustic-magnetic resonance/second near infrared imaging and guided photothermal therapy	Hu et al. (2019)
8.	Hyaluronic acid and hydroxyethyl chitosan	doxorubicin	Aldehyde-functionalization and assembly	MCF-7 cells	Chemotherapy and photodynamic therapy	Wang et al. (2019)

membrane properties, damage to cellular components and apoptotic events can result from polymeric nanoparticles (Sukhanova et al. 2018). Most of the polymeric nanoparticles are not acknowledged by the body defense systems, resulting in reduced metabolism and significant accumulation of polymeric nanoparticles in organs and tissues. Investigations suggested that the typical surface treatment and the size of the polymeric nanoparticles is critical factor, which can be explored to modify and decrease cellular toxicity (Zhou et al. 2020).

Recently, pentaerythritol and dipentaerythritol cored dendrimers star polymer-based carrier loaded with doxorubicin has been explored in the treatment of breast cancer. It exhibited dose-dependent cytotoxic response in MCF-7 and MCF-10a cells. However, the cytotoxicity of these polymeric nanoparticles nanocarriers was noted less as compared to that of the free doxorubicin (Wong et al. 2020). Behdarvand et al. has confirmed the reduced toxicity of Tam with the use of Polylactic acid/1,2-dipalmitoyl-sn-glycerol-3-phosphoethanolamine-N [methoxy (polyethyleneglycol)-2000] as polymeric nanocarriers in MCF-7 cancer cells. The expression of cellular (p53 and p21) genes with the cell cycle are upregulated by nanocapsules, ultimately showing the arrest at the G1 phase (Behdarvand et al. 2020). The nanocapsules loaded with curcumin were evaluated for their anticancer activity and cytotoxic response in breast cancer cell line (MDA-MB-231) cells. The confocal laser imaging showed a higher division rate and apoptotic response due to an increase in reactive oxygen species for curcumin-loaded nanocapsules as compared to the plain drug (Wang et al. 2018). Regardless of polymeric material properties, more emphasis can be added on the route of administration its relevance to augmenting toxicity. Though the mainstream *in vitro* toxicity studies is carried out on cellular or animal models, their different behavior in the living system can be expected (Sukhanova et al. 2018). Summing up, there are on-going *in vivo* and clinical investigations to understand the implications of polymeric nanoparticles based therapeutics. Fortunately, many of these have been passed for food and drug administration approval and some are waiting for dispatch in clinical use. The major factor obstructing the application of polymeric nanoparticles based on the novel formulation in humans is the uncertain interactions. For instance, polymeric nanoparticles based formulation passes all the cellular, *in vitro – in vivo* correlation levels but fails in the clinical stage. Therefore, a foundational demonstration remains as a prerequisite for recognizing the attributes of polymeric nanoparticles for deployment in the clinical trial.

14.6 Conclusion

Polymeric nanoparticles-based nanotheranostics is a forthcoming budding platform used for therapeutic delivery and a promising real-time tool for the treatment and diagnosis of cancer and other life-threatening diseases. In this prospect, different multifunctional polymeric nano-formulations such as polymer-based superparamagnetic iron oxide nanoparticles, magnetic resonance imaging and near-infrared

triggered polymeric nanoparticles; loaded with drugs and fluorescent dyes have been introduced by many researchers for theranostics purposes. The amalgamation of multi-modal nano-formulations offers a great promise for the near future applications in clinical trials, which may help clinicians to predict and glimpse actual drug accumulation and movement at the tumor sites. Ultimately, this platform might allow the time-course monitoring of tumor growth/inhibition in patients. Polymeric nanoparticles have received attention to address these problems and overcome the technological challenges for practical clinical applications. However, additional investigations may lead to a better understanding of the design of polymeric nanoparticles -based theranostic systems to accomplish more targeting attributes and higher anticancer efficacy. This rapidly developing platform is still in its nascent stage. Regardless of various obstacles, polymeric nanoparticles – based theranostic strategies would show a promising trend for imminent nanomedicine and cancer therapy.

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Chapter 15
pH-Sensitive Polymeric Nanoparticles for Cancer Treatment

Anindita Behera and Santwana Padhi

15.1 Introduction

As the second leading cause of death after cardiovascular diseases, the growing prevalence of various forms of cancer is now becoming a significant global public health problem (Akhavan et al. 2014). Cancer treatment is possible in many circumstances and, if diagnosed early enough, can also aid in its complete eradication. Despite pioneering efforts in the field of nanotechnology to detect cancer at the single-cell level, cancer is not identified until distant body parts have been affected. The associated side effects of the chemotherapeutics can severely restrict their usage, since these cytotoxic agents damage the surrounding normal healthy cells at the same time as they destroy cancerous cells (Patnaik et al. 2021; Padhi et al. 2015; Verma et al. 2017).

Advances in the field of nanotechnology have had a significant impact on multiple scientific domains (Padhi et al. 2018; Behera et al. 2020b; Khuroo et al. 2014). Various nanocarriers, such as liposomes, nanoemulsion, metallic and polymeric nanoparticles, are progressively being used in medical applications, such as the delivery of macromolecules as therapeutic agents (Padhi et al. 2020; Behera et al. 2020c; Hassan et al. 2021; Padhi et al. 2020). It is undeniably a challenging task,

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given that the appropriate drug delivery system must be both site-specific and time-release monitored. Heat, solvent polarity, ionic strength, biomolecule activity, or the effects of electric/magnetic fields or light are all examples of external or internal stimuli that can cause regulated drug release.

Sometimes the tumor microenvironment become stimuli sensitive as there may be change in the intracellular and extracellular pH, redox potential and temperature in the tumor cells as compared to surrounding normal cells. Hence the treatment of solid tumors may be selectively targeted by different approaches of active delivery of anticancer drugs by formulating stimuli responsive formulations (Behera and Padhi 2020). Nanoformulations of pH sensitive formulations have been used extensively for targeted delivery of anticancer drugs for effective and efficacious therapeutic activity.

The difference between the solid tumor cells and the normal cells lies in the level of nutrition and metabolic environment. The supply of required nutrition and oxygen are often inadequate among the growing tumor cells. Hence the energy requirement of the tumor cells depends on anaerobic glycolysis due to increased concentration of pyruvic acid and hypoxia in the tumor environment, which produces large amount of lactic acid and lesser amount of adenosine triphosphate (ATP) as energy. During growth of tumor, requirement of large amount of energy is required by the tumors as compared to the normal cells as tumor produces high amount of carbon dioxide and lactate. The carbon dioxide produced is released to the extracellular region making the tumor environment acidic (Hao et al. 2018). As per Warburg et al., tumor cells utilize glycolysis rather than oxidative phosphorylation to attain the required energy level, even in the presence of oxygen. Anaerobic glycolysis has been considered as the major reason for the extracellular acidity of tumor tissues (pH_e) (Gerweck and Seetharaman 1996). Most of the malignant cells possess 0.3–0.7 units lesser pH as compared to surrounding normal cells. In addition, the intracellular pH (pH_i) of tumor cells is slightly higher than the corresponding normal cells. Difference of intracellular pH is found to be approximately 0.1 unit or less than that of surrounding normal cells which is considered to be not significant (Hao et al. 2018). The balance of extracellular and intracellular pH is balanced by the presence of numerous membrane proteins (carbonic anhydrase enzymes, CA2, CA9 and CA12), passive diffusion and active membrane transporters like anion exchangers (SLC4A1, SLC4A2 and SLC4A3), proton transporter vacuolar ATPase, monocarboxylate transporters (MCT1, MCT2, MCT3, and MCT4), sodium ion based chloride/bicarbonate exchanger (SLC4A8) and sodium potassium exchanger 1 (SLC9A1) as represented in Fig. 15.1 (Supuran 2010).

Carbonic anhydrase enzymes like CA2, CA9 and CA12 regulates the concentration of bicarbonate and proton in the tumor microenvironment and affects the tumor cell survival and proliferation (Mboge et al. 2018). Anion exchangers like SLC4A1, SLC4A2, and SLC4A3 help in exchange of intracellular bicarbonate ion and chloride concentration and releases the bicarbonate out of the cell into extracellular space and regulates the intracellular pH (Alper 2006). The vacuolar ATPase is a family of proton pumps that are involved in hydrolysis of ATP and transport the

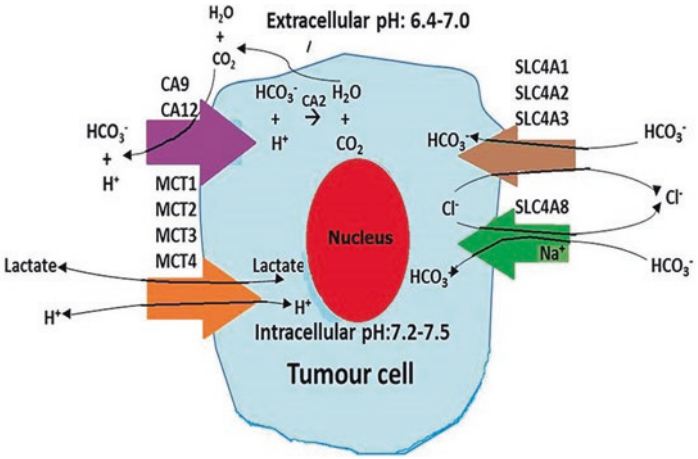

Fig. 15.1 Regulation of extracellular and intracellular pH of tumor cells. The pH in the tumor microenvironment is regulated by presence of numerous membrane proteins (carbonic anhydrase enzymes, CA2, CA9 and CA12), passive diffusion and active membrane transporters like anion exchangers (SLC4A1, SLC4A2 and SLC4A3), proton transporter vacuolar ATPase, monocarboxylate transporters (MCT1, MCT2, MCT3, and MCT4), sodium ion based chloride/bicarbonate exchanger (SLC4A8) and sodium potassium exchanger 1 (SLC9A1). HCO_3^- – bicarbonate ion, H^+ – proton, H_2O – water, CO_2 – carbon dioxide, Cl^- – chloride ion, Na^+ – sodium ion

proton into the intracellular matrix through plasma membrane. The vacuolar ATPase regulates cellular processes in cancer like wingless-related integration site, notch and mammalian target of rapamycin signaling which are required for survival of cancer cells in highly acidic environment (Stransky et al. 2016). Lactate ion acts as a major fuel for oxidative cancer cells and monocarboxylate transporters that helps in exchange of lactate ion across the plasma membrane. The monocarboxylate transporters 1, 2, 3 and 4 transport the lactates and other monocarboxylates both ways across the cell membrane and helps in the process of angiogenesis and metastasis in cancer cells (Payen et al. 2020). SLC4A8 transports the sodium ion gated chloride ion out of tumor cell and simultaneously imports the bicarbonate ion into the tumor cells, thus affecting both extracellular and intracellular pH (Damaghi et al. 2013). Sodium/hydrogen exchanger (NHE1) also known by SLC9A1 is mainly involved in the efflux of proton which maintains the intracellular alkaline pH (Guan et al. 2018).

For target specific delivery of anticancer drugs utilizing polymeric nanocarriers, various stimuli responsive nanoparticles have been designed and have demonstrated potential therapeutic applications (Tang et al. 2019). In response to different stimuli like intracellular and extracellular pH, temperature and redox potential of tumor cells can change the physical and chemical properties of polymeric nanocarriers.

This chapter reviews the application of fabricated polymeric nanoparticles delivering the anticancer drugs in response to change in pH of tumor cells.

15.2 Mechanism of Action of pH-Sensitive Polymeric Nanoparticles

In normal tissues, the extracellular pH is 7.4 and the intracellular pH is 7.2. But in most of the tumor cells there is reversal of pH gradient i.e. the intracellular pH becomes higher than extracellular pH, which implies the fact that the extracellular pH of tumor cells is lesser than surrounding normal cells (Engin et al. 1995; van Sluis et al. 1999). The primary cause of decrease of extracellular pH is the higher rate of glycolysis in cancer cells in both aerobic and anaerobic conditions (Stubbs et al. 2000). The cellular organelles become more sensitive to acidic pH and sequester the anticancer drugs resulting in multidrug resistance and decreased therapeutic efficacy of chemotherapeutic agents (Belhoussine et al. 1998). Most of the anticancer drugs easily get ionized, hence the extracellular and intracellular pH are the critical factors which affect the partition and distribution of the drugs into the tumor cells (Simon 1999). The extracellular pH is generally responsible for triggering different signaling events which can be targeted for active targeting and delivery of anticancer drugs in response to extracellular pH.

15.3 Designing of pH-Sensitive Polymeric Nanoparticles

pH responsive polymeric nanoparticles can be designed by modification of surface chemistry by using suitable polymers which changes the hydrophilicity or charge of the nanodelivery system in response to change in pH of tumor microenvironment (Deirram et al. 2019). The change in properties of nanoparticles enhances the cellular uptake and controlled release of the cargo at the targeted tumor microenvironment. The design of pH sensitive nanoparticles can be attained by any one of the following three strategies

(i) By using the charge shifting polymers
(ii) By using acid labile linkers as pendant functionality
(iii) By using acid linkers to produce cross-linked particles

The above strategies are being used successfully for delivering the anticancer drugs.

15.3.1 Charge Shifting Polymers

Polymer building blocks are used to alter charge and hydrophilicity in accordance with the change in pH of the tumor microenvironment. Modulation of these stated properties causes rearrangement, swelling and disassembly of the nanoparticles to deliver the entrapped drug. Charge shifting of polymeric nanoparticles depends on the pKa value and type of the polymer used in relation to pH of the medium. Cationic

polymers gets changed to positively charged hydrophilic moiety when the pH of the medium decreases. Similarly, anionic polymers get changed to negatively charge hydrophobic moiety as the pH of the medium decreases. The detailed mechanism of the charge transfer of the polymers in polymeric nanoparticle is enumerated below (Deirram et al. 2019).

(i) Change in charge from hydrophobic to hydrophilic with a decrease in pH: When the pH decreases below the pKa value of cationic polymers, they attain hydrophilicity from hydrophobicity. The polymers containing amino groups accept a proton and become hydrophilic, when the pH of the environment decreases from the pKa value of the polymer. Using this technique, drug delivery systems are formulated with an amphiphilic block copolymer in which the pH responsive part is hydrophobic in the pH of the systemic circulation (nearly 7.4) in a self assembled structure. In solid tumor sites, with the decrease in pH, the block becomes hydrophilic causing the solubilization and disassembly of the polymeric nanoparticles containing the anticancer drug (Liang et al. 2014).
(ii) Change in charge from hydrophilicity to hydrophobicity with decrease in pH: Polymers derived from acid monomers like poly (methacrylic acid), poly (aspartic acid) and sulfonamide derived polymers become hydrophobic, in response to decrease in pH of the surrounding cellular environment (Pickett et al. 2018; Han et al. 2017).

15.3.2 Acid Labile Linkers as Pendant Functionality

Targeting the pH responsive polymeric nanoparticles to the solid tumors requires the designing of drug delivery systems in such a way that the drug loaded polymeric nanoparticles remains stable in body fluids but becomes unstable due to acidic environment around the cancer cells. The polymeric nanoparticles can be linked covalently to pH sensitive linker groups which remain stable in neutral pH of the systemic circulation, but once they get internalized inside the tumor microenvironment, they disassemble due to decease in pH and deliver the drug to the targeted tumor cells (Pang et al. 2016). Various linkages used for the formulation of pH sensitive polymeric nanoparticles are hydrazone, imine, ortho ester, acetal or ketal, cis-aconityl group and β-thiopropionate groups.

(i) Hydrazone linker group: Hydrazone linker is the most preferred linkage utilized in the formulation of pH sensitive drug delivery system. The hydrazone linkage is stable at systemic pH of 7.4 and undergoes hydrolysis slowly. When the formulated polymeric nanoparticles reach the tumor site, the decreased pH of 5–6 leads to the increased hydrolysis of the hydrazone linkage and deliver the drug to the tumor site. The utilization of hydrazone linker results in the high drug loading capacity, easy methods of preparation of polymer precursor with specific drug release with high antitumor activity (Chytil et al. 2014).

(ii) Imine linker group: Imine linker group is acid labile linker group which dissociates at low pH to keto and amine group (Wang et al. 2014).
(iii) Cis-aconityl (Maleic acid amides) linker group: Cis-aconityl linker group is a derivative of maleic acid amides which is generally used for linkage of (N-(2-hydroxypropyl) methacrylamide), poly (amidoamine), polyvinyl alcohol), poly (L-lactide)-poly (ethylene glycol) copolymers (Chytil et al. 2006; Zhu et al. 2018; Hu et al. 2009).
(iv) Acetal or ketal linker group: The acetal and ketal linkers are converted from hydrophobic to hydrophilic moiety with a decrease in pH of the surrounding (Bachelder et al. 2008).
(v) Ortho ester linker groups: These are the pH responsive linker groups which degrade into potential biocompatible forms to deliver the anticancer drugs at tumor sites (Huang et al. 2009).
(vi) pH cleavable groups with polymers: The linker groups act as pendant groups to deliver the drug by changing their surface chemistry. Some of these linkers like poly (ortho esters) are used for gene delivery as it increases the transfection efficiency and decreases the cytotoxicity. Other acid sensitive linkers like hydrazone, acetal, imine, ortho ester, maleic acid amide functional groups are developed to deliver the drugs. But considerations for the same include the stability of the polymer-acid labile linker groups which needs to be stable at physiological pH for preventing the non-specific delivery of the drugs. Mostly the acid sensitive polymeric nanoparticles are targeted to deliver the drugs at pH 5.0 in the tumor cells (Deirram et al. 2019).

15.3.3 Acid Linkers to Produce Cross-Linked Particles

Delivery of drugs by polymeric nanoparticles system faces the greatest challenge with the stability of the nanoparticle, as the polymeric systems with acid sensitivity can change the properties of the nanoparticles in biological systems. Hence crosslinking is one of the strategies used for overcoming the problem of stability by covalent or non-covalent interactions (Li et al. 2014). The ideal crosslinking should be reversible to deliver the drugs when they reach the target site of tumor. pH sensitive crosslinking can be designed for efficient designing of polymeric nanoparticles. The interactions can be achieved by following mechanisms:

(i) *Host – guest recognition*: In this type of interaction, two or more ions or molecules interact with each other by non-covalent bonds like hydrogen bonds, ionic bonds, van der Waals forces and hydrophobic interactions (Datz et al. 2018).
(ii) *Using metal ions*: In this, metal-phenolic networks are used for crosslinking the drugs with polymers as these metals-phenolic are universal and negligibly cytotoxic (Besford et al. 2018).

15.4 Characteristics of Polymers Used for pH-Sensitive Polymeric Nanoparticles

The application of chemotherapeutics in the treatments for solid tumors have been associated with a lot of limitations the major being non-specific cytotoxicity, low bioavailability, serious adverse effects etc. Years of research in nanoscale techniques has resulted in the generation of nanocarriers with highly specific delivery efficacy of multiple anticancer molecules at the intended site. Drug delivery by nanotechnology has prompted for selective, specific and controlled release of drugs at the site of action either by passive targeting or active targeting (Padhi and Behera 2020). To overcome the limitations of the anticancer drug delivery, polymeric drug delivery systems have been used for enhanced therapeutic efficacy. These polymeric nanoparticles, specifically deliver the drugs at the required site of action in a controlled fashion by improving the therapeutic index of the enclosed drugs.

Poly (glycolic acid)), poly (D, L-lactic acid)) and poly (lactic-co-glycolic acid)) have been employed as biodegradable polymers in the fabrication of nanocarrier systems since 1960's as they can be degraded inside the biological system and produce natural byproducts like water and carbon dioxide which get eliminated easily from body (Kulkarni et al. 1971; Fredenberg et al. 2011). The polymers employed in the preparation of nanoparticles should have some other properties which must be taken into consideration during the formulation process. The most desired property remains the biodegradability profile of the polymer, which depends on their origin like natural or synthetic. Synthetic polymers are more preferred than natural as they have less variation in the properties during batch to batch manufacturing process and are less immunogenic (Marin et al. 2013). Biodegradation of the polymers is accomplished by enzymatic cleavage or hydrolysis. Release of drug from the matrix of polymer can be achieved by (Kamaly et al. 2016).

(i) Erosion of surface of the polymer matrix
(ii) Break down of chemical bonds at the surface or inside the bulk of the matrix
(iii) Diffusion of physically loaded drug

Apart from this, the number and chemistry of the monomers influences the biodegradation profile which in turn influences their toxicity and biocompatibility profile. The number of monomers present in a polymer is known as degree of polymerization which affects the physical and chemical properties of a polymer (Cowie and Arrighi 2008). Properties of polymers like molecular weight, polymer crystallinity, glass transition temperature (T_g) and solubility affects the rate and release of the drug from polymeric nanoparticles and also their biodegradation (Makadia and Siegel 2011). A brief description of ideal properties of polymers employed for polymeric nanoparticles are discussed below:

(i) Crystallinity of polymers: Crystallinity of a polymer is attributed to the fractional alignment of molecular chains of the polymer which affects the physical and chemical properties of the nanoparticles. Degree of crystallinity affects the strength, swelling capability, hydrolysis and rate of biodegradation of the poly-

mer (Kamaly et al. 2016). Release and delivery of a drug from the polymeric drug delivery system is greatly affected by the crystallinity. High crystallinity in low molecular weight polymeric systems delivers the drug slowly whereas the effect is less seen in high molecular weight polymers with high degree of porosity (Cowie and Arrighi 2008)

(ii) Glass transition temperature of polymers: Glass transition temperature of a polymer influences the crystallinity and physicochemical structures of a polymer. It is the temperature where the rubber like state of a polymer is changed to glasslike state and determined by differential scanning calorimetry (Cowie and Arrighi 2008). Below the glass transition temperature, the polymer exists in glass like state which has restricted mobility and slower diffusion rate whereas above the glass transition temperature, the polymer has rubber like state which has higher rate of mass transfer of drugs through water and matrices (Liechty et al. 2010). The efficacy of drug delivery system depends on the balance between the crystallinity and amorphous state as it influences the mechanical toughness to the polymer. Hence polymeric nanoparticles are prepared by use of copolymers containing both hydrophobic and hydrophilic parts (Kaushal et al. 2004).

(iii) Hydrophobic and hydrophilic property of a polymer: Hydrophobicity or hydrophilicity of a polymer determines the solubility of a polymeric drug delivery system. Increase in molecular weight with increase in branching of the backbone of a polymer increases the hydrophobic property of a polymer which decreases its solubility in water (Liechty et al. 2010). Hydrophobicity of the polymer controls the release of drug from the matrices by surface erosion. If there is balance between hydrophobic and hydrophilic functional groups in the skeleton of the polymer, release of drug occurs by degradation from within the polymeric system (Liechty et al. 2010)

(iv) Molecular weight of polymer: All the physical properties of a polymer like glass transition temperature, solubility, viscosity, crystallinity, mechanical strength, and degradation rate are greatly influenced by molecular weight. Low molecular weight polymers degrade rapidly for the delivery of a drug. Not only the physical properties but also the drug release profile from the polymeric Nanoparticles and the biological properties are equally affected by molecular weight (Knopp et al. 2015).

15.5 Application of pH-Sensitive Polymeric Nanoparticles in the Treatment of Cancer

15.5.1 Change of Hydrophobic Property to Hydrophilic Property by Transfer of Charge

Liang et al. synthesized chitosan-copper-curcumin nanoparticles, where the hydrophobic curcumin transferred the charge to copper (Cu^{2+}) forming a stable co-ordinate chitosan-copper-curcumin nanoparticles. The cytotoxicity study on human

non-small cell lung cancer cell lines (H1299) showed about 49% release of curcumin from the chitosan-copper-curcumin nanoparticles at tumor site at a pH 4.0 (Liang et al. 2020).

Wong et al. designed a core shell type polymeric nanoparticles responsive to the decreased pH in the endosomes of the tumor cells. The nanoparticles were made up from a homopolymer poly (2-diethyl amino) ethyl methacrylate) and a copolymer (poly (2-diethyl amino) ethyl methacrylate-b-poly (ethylene glycol). The core consisted of (poly (2-diethyl amino) ethyl methacrylate) which was capable of carrying the drug and the shell made up of the copolymer poly (2-diethyl amino) ethyl methacrylate-b-poly (ethylene glycol) provided stability of the nanoparticles in the physiological pH. But once the polymeric nanoparticles entered the cytosol, the pH sensitive nanoparticles caused the endosomal escape of the drugs (Wong et al. 2015).

Later, Kongkatigumjorn et al. worked further using (poly (2-diethyl amino) ethyl methacrylate) and a copolymer (poly (2-diethyl amino) ethyl methacrylate-b-poly (ethylene glycol) with other copolymers like (2-diethyl amino) ethyl methacrylate and 2-(diisopropylamino) ethyl methacrylate. In this study, the polymeric nanoparticles was synthesized utilizing the polymer (2-diethyl amino) ethyl methacrylate-b-poly (ethylene glycol) with different ratios of co-polymers (2-diethyl amino) ethyl methacrylate and 2-(diisopropylamino) ethyl methacrylate at ratios of 1:0, 3:1, 1:1, 1:3 and 0:1. Endosomal escape was investigated and the combination of 2-(diisopropylamino) ethyl methacrylate and 2-(diisopropylamino) ethyl methacrylate-b-2-(diisopropylamino) ethyl methacrylate (1:1) showed the highest association and stability at pH more than 7.2. The disassembly of the nanoparticles was found between pH 4.9 and 7.0. The variation in the composition and concentration of nanoparticles defined the cellular behavior with respect to change in pH of the physiological condition (Kongkatigumjorn et al. 2018).

Ellis et al. synthesized pH sensitive nanoparticles with a core-anchored multilayer shell of triblock copolymers. The triblock copolymer was prepared utilizing poly (oligo (ethylene glycol) methyl ether methacrylate), poly (2-(diisopropyl amino) ethyl methacrylate) and poly (2-(methacryloyloxy) ethyl phosphorylcholine). The core was composed of gold nanoparticles loaded with a highly toxic anticancer drug i.e. doxorubicin and coated with tri-polymer block which demonstrated significant colloidal stability. The shell was composed of pH sensitive amino groups which was completely protonated and aided in prevention of micellisation of the polymer. The efficacy of the formulation was evaluated on breast cancer cell lines (MCF-7). The formulation was found to be stable at pH 7.2 for more than 24 h and as the pH reduced to 4, the triblock polymeric shell got protonated, became hydrophilic releasing the entrapped doxorubicin at the site of cancerous tissues. Hence it can be inferred that tri-block polymeric nanoparticles may be suitable for co-delivery of hydrophilic and hydrophobic drugs (Ellis et al. 2017).

Ali Raja et al. prepared self assembled pH sensitive nanoparticles of core-shell type structure where the shell was prepared of amphiphilic polymer block

with a hydrophobic segment of N-acetyl histidine and a hydrophilic segment of arginine modified chitosan. The outer hydrophilic arginine modified chitosan aided in cellular uptake and the inner core N-acetyl histidine was held responsible for release of the drug doxorubicin with respect to the acidic environment of the tumor cells. The carrier to drug ratio of 3:1 showed the highest encapsulation efficiency of about 81% with loading efficiency of 21% and the average particle size of the formulation was found to be approximately 130 nm. The cytotoxicity study was conducted in human breast cancer cell lines (MCF-7) and its resistant variety (MCF-7/ADR). The drug loaded nanoparticles were found effective in both sensitive and resistant cells by dose and time dependent manner (Ali Raja et al. 2017).

Yuan et al. designed pH sensitive core shell type polymeric nanoparticles for the co-delivery of curcumin and doxorubicin. The polymeric triblock was designed with an inner hybrid core of b-poly (D, L-lactic-co-glycolic acid)-b-poly (L-glutamic acid) and monomethoxy poly (ethylene glycol). Co-delivery of curcumin and doxorubicin resulted in the pH stimulated release and reversed drug resistance with minimizing the *in vitro* toxicity. The drug loading efficiency in the nanoparticles was found to be 80.3% and 96.2% for curcumin and doxorubicin, respectively. The release kinetic showed that at acidic condition of tumor cells i.e. at pH 5.0, the curcumin (80%) was released at a faster rate whereas the doxorubicin (85%) showed a sustained release and reversed the drug resistance in MCF-7/ADR cells of human breast cancer (Yuan et al. 2018).

Similarly Zhang et al. designed polymeric nanoparticles for co-delivery of curcumin and doxorubicin with amphiphilic poly (β-aminoester) copolymer. The cytotoxicity of curcumin-doxorubicin nanoparticles was studied against human hepatic cancer cells (SMMC 7721) and human umbilical vein endothelial cells (HUVECs). In human hepatic cancer cells, curcumin-doxorubicin nanoparticles showed enhanced apoptosis by decreasing mitochondrial membrane potential. In human umbilical vein endothelial cells, the polymeric nanoparticles showed strong anti-angiogenic effect with inhibition of proliferation, migration, invasion and vascular endothelial growth factor pathway *in vitro* and *in vivo* (Zhang et al. 2017).

Xiong et al. synthesized tri-block copolymer with poly (ε-caprolactone)-poly (ethylene glycol)-poly (ε-caprolactone) and encapsulated paclitaxel and curcumin to develop pH sensitive nanoparticles. The tri-block copolymer consisted of a hydrophobic poly (ε-caprolactone) block and a hydrophilic poly (ethylene glycol) block and the entrapment efficiency was noted to be 92.6% and 90.3% for paclitaxel and curcumin, respectively with an average particles size of approximately 28 nm. The cytotoxicity effect of paclitaxel-curcumin nanoparticles was studied in breast cancer cells (MCF-7) and the efficacy of the co-delivered paclitaxel and curcumin showed an increased rate of apoptosis due to synergistic effect of both the drugs (Xiong et al. 2020).

15.5.2 Change of Hydrophilic Property to Hydrophobic Property by Transfer of Charge

Sunoqrot and Abujamous reported polymeric nanoparticles of quercetin with eudragit® S100 to deliver a pH sensitive drug delivery system to colon cancer cells (CT26 murine colon carcinoma cells). The colon targeting pH sensitive polymeric nanoparticles remained stable through the gastrointestinal tract environment and reached the colon where the drug was released at the pH of the colon by dissolution of polymer. The quercetin eudragit® S100 nanoparticles were evaluated for release at lower pH demonstrating the gastrointestinal tract environment, the methacrylic acid moieties in eudragit® S100 got protonated at low pH (1.2 and 4.5) making it insoluble and prevented the release of quercetin. But when the pH was enhanced to 7.2, the quercetin got released as the methacrylic acid moieties got ionized. Ionization of polymer caused electrostatic repulsion and swelling of the polymer and the disruption of polymer released about 92% of encapsulated quercetin (Sunoqrot and Abujamous 2019).

15.5.3 pH-Responsive Polymers with Acid Labile Linkages

(a) Hydrazones: Liao et al. designed hyaluronic acid hydrazone linked doxorubicin acid sensitive polymeric Nanoparticles. The amphiphilic polymeric-drug conjugates got self-assembled in the medium and spherical shaped core-shell type stable nanoparticles were produced. The *in-vitro* study conferred that the nanoparticles showed an endosomal or lysosomal pH responsive release pattern of the drug doxorubicin at pH 5. The *in vitro* cytotoxicity study on normal cells of mouse fibroblast cells and tumor cells of human cervical cancer cells (HeLa) revealed that the pH sensitive doxorubicin nanoparticles showed a dose dependent cytotoxic activity on tumor cells whereas they were non-toxic to normal cells. The hydrophilic polysaccharide polymer and the hydrophobic drug by hydrazone conjugation formed a stable pH sensitive delivery system (Liao et al. 2018). Liu et al. also designed the hyaluronic acid conjugated doxorubicin by a hydrazone linkage and coated it with a nanocore containing an anti-tumor immune regulator R848 (TLR7/8 agonist) encapsulated with poly (L-histidine) (PHIS/R848). The core shell type polymeric hyaluronic acid-doxorubicin-poly (L-histidine)-R848 nanoparticles showed both chemotherapeutic and immunoregulatory effect on breast cancer cells (MCF-7 cells). The poly (L-histidine) ionized at the pH of tumor microenvironment at pH 6 by conversion of hydrophobic to hydrophilic nature and released R848 to exhibit the immunoregulatory effect. Similarly, the hydrazone bond of hyaluronic acid-doxorubicin was ruptured at pH 5.5 and the doxorubicin got internalized into the over-expressed

CD44 cells of breast cancer by endocytosis and interfered the tumor cell growth. The polymeric hyaluronic acid-doxorubicin-poly (L-histidine)-R848 nanoparticles not only restricted the tumor cell growth by upgrading the tumor immunity but also killed the tumor cells (Liu et al. 2018).

Tao et al. co-delivered docetaxel and dihydroartemisinin with poly (ethylene glycol) to evaluate the anti-metastatic effect in metastatic breast cancer cells (4 T1 cells). The drug loading of docetaxel was increased up to 40%. A pH sensitive nanoparticle was synthesized by hydrazone bond with a mean size of 142.5 nm. The release of the two drugs was triggered at the acidic pH of the tumor and showed excellent anti-metastatic effect with IC_{50} value of 7 mg/ml along with arrest of cell cycle and suppression of movements of tumor cells and their invasion. The mechanism behind these effects was found to be downregulation of expression of protein kinase B, nuclear factor kappa light chain enhancer of activated B cells and matrix metalloproteinase-2. Docetaxel restricted tumor cell proliferation by downregulation of nuclear factor kappa light chain enhancer of activated B cells whereas dihydroartemisinin affected nuclear factor kappa light chain enhancer of activated B cells via protein kinase B/nuclear factor kappa light chain enhancer of activated B cells or nuclear factor kappa light chain enhancer of activated B cells/glucose transporter 1 pathway (Kim et al. 2012; Jiang et al. 2016). Both the drugs synergistically decreased the expression of GLUT 1and matrix metalloproteinase-2. Inhibition of matrix metalloproteinase-2 was responsible for reduction of matrix of cytoplasm and basement membrane and the expression of protein kinase B was responsible for tumor metastasis (Tao et al. 2018).

Zhao et al. also co-delivered two anticancer drugs i.e. doxorubicin and paclitaxel in a nano-in-nano polymer dendrimer hybrid nanoparticle system with an average particle size of 150 nm. The polymer-dendrimer hybrid nanoparticle was made up of a smaller poly (amidoamine) dendrimer nanoparticles entrapped within bigger poly (lactic-co-glycolic acid) nanoparticles and doxorubicin and paclitaxel are loaded in poly (amido amine) and poly (lactic-co-glycolic acid) respectively. The doxorubicin-paclitaxel-polymer dendrimer hybrid nanoparticles showed an efficient and controlled release at the lysosomal pH 5 with more bioavailability at the tumor site with decrease in premature drug release and undesirable side effects. The cytotoxicity study for the anticancer activity was studied in breast cancer cells (MDA-MB-468) and lung cancer cells (A549). The doxorubicin-paclitaxel-polymer dendrimer hybrid nanoparticles showed enhanced biocompatibility and synergistic anticancer activity due to increase in accumulation at the tumor site, controlled drug release and improved pharmacokinetic profile in the dual drug loaded nanoparticles. The higher drug loading and enhanced intracellular accumulation resulted in enhanced cytotoxicity (Zhao et al. 2017).

Zhang et al. designed pH sensitive polymeric nanoparticles of daunorubicin with a series of carboxymethyl chitosan with macromolecules of variable molecular weight by acid sensitive hydrazone linkage, which was prone to hydrolysis at pH 5–6 in cervical cancer cells (HeLa). The carboxymethyl chitosan-daunorubicin nanoparticles were found to be stable at physiological pH 6.5 and 7.4 and showed

cytotoxic activity at tumor microenvironment at pH 5.0. The IC_{50} of carboxymethyl chitosan-daunorubicin nanoparticles has increased in anticancer activity as compared to free daunorubicin by two fold (Zhang et al. 2016).

Fang et al. designed three different types of pH sensitive nanoparticles of doxorubicin by varying the polymers with different side chains like guanidinium group, an imidazole group, and a tertiary amine group. All three types of doxorubicin nanoparticles were prepared by hydrazone bonding and the size of the nanoparticles was about 80 nm. The imidazole-doxorubicin and guanidinium-doxorubicin nanoparticles showed enhanced intracellular uptake in ramos cells (Burkitt's lymphoma) as compared to amine-doxorubicin nanoparticles. This study ensued that the type of side chain in the polymeric nanoparticles influenced the extent of uptake by the tumor cells. The study by confocal microscopy demonstrated that imidazole-doxorubicin and guanidinium-doxorubicin nanoparticles were able to deliver doxorubicin to the nuclei of the tumor cell by overcoming the endosomal confinement. The IC_{50} of imidazole-doxorubicin and guanidinium-doxorubicin nanoparticles were found to be several orders higher as compared to free doxorubicin whereas the IC_{50} of amine-doxorubicin nanoparticles was not significantly different than free doxorubicin (Fang et al. 2012).

Jin et al. designed a glutathione sensitive and pH sensitive prodrug of cisplatin and doxorubicin conjugate. Doxorubicin and cisplatin were conjugated by hydrazone bond and glutathione sensitive linker for active targeting to lung cancer (A549) and cisplatin resistant lung cancer cells (A549). Lipid nanocarrier was selected for design of selective prodrug delivery of doxorubicin and cisplatin. The prodrug of cisplatin-doxorubicin nanocarrier showed about 80% tumor inhibition effect at pH 5 as compared to free drugs (Jin et al. 2020).

Pang et al. designed a core-shell type nanodelivery system for co-delivery of erlotinib and bevacizumab with a lipid-polymer hybrid nanoparticles for targeting and suppressing non small cells lungs cancer cells (A549 and H1975 cells). Lipid polymer hybrid nanoparticles contained a biodegradable polymeric core and an outer phospholipid layer as shell. In this study, hyaluronic acid modified with adipic acid hydrazide linked to poly (ethylene glycol) by an acid sensitive hydrazone bond formed the outer layer and both the drugs erlotinib and bevacizumab were enclosed into hydrophobic polymeric core consisting of polycaprolactone. The ratio of erlotinib and bevacizumab showed the best synergistic effect on A549 and H1975 cells. The pH responsive hyaluronic acid modified lipid-polymer hybrid nanoparticles showed increased cellular uptake (52.3%) as the expression of CD44 receptors helped in internalization of hyaluronic acid-erlotinib / bevacizumab-lipid-polymer hybrid nanoparticles and maximum drug delivery occurred at pH 5.5. Hyaluronic acid-erlotinib/bevacizumab-lipid-polymer hybrid nanoparticles showed about 80% reduction in tumor growth (Pang et al. 2020).

Bobde et al. designed polymeric nanoparticles targeting to extracellular and intracellular acidic environment of solid tumors. In this study, a hydroxypropyl methacrylamide based polymer (P6) was synthesized and linked to an anticancer drug doxorubicin by a pH sensitive hydrazone linkage to produce hydroxypropyl methacrylamide doxorubicin nanoparticles. The cellular uptake and cytotoxicity of

P6-doxorubicin nanoparticles was conducted in murine mammary carcinoma cell line (4 T1), and human breast cancer cell lines (MCF-7) and the release kinetics was studied at different pH like 7.4, 6.5 and 5.5. The drug release of P6-doxorubicin nanoparticles were found to be 86%, 80% and 13% at pH 5.5, 6.5 and 7.4, respectively at the end of 48 h. The cellular uptake was studied by both confocal microscopy and flow cytometry and it was found to be higher for P6-doxorubicin nanoparticles as compared to free doxorubicin. But the cytotoxicity of P6-doxorubicin nanoparticles was found to be lesser than free doxorubicin as the slower breakage of hydrazone bond might responsible for the slower release of free doxorubicin inside the tumor cells (Bobde et al. 2020).

Wallat et al. synthesized polymeric nanoparticles of doxorubicin with fluorous polymer by acid sensitive hydrazone bond by conjugating with tri fluoroethyl methacrylate and oligo (ethylene glycol) methyl ether methacrylate. The cytotoxicity activity of polymeric conjugate of doxorubicin was studied on breast (MDA-MB-231) and ovarian (A2780, OVCAR3 and ID8) cancer cell lines and found to have higher efficacy as anticancer agent. The azide end of the copolymer provided pH sensitivity to the nanoparticles which delivered the doxorubicin by an active targeting approach (Wallat et al. 2018).

Aryal et al. prepared polymer-cisplatin conjugate and studied the anticancer activity against human ovarian cancer cell lines A2780. A prodrug containing the cisplatin analogue Pt (IV) was conjugated with a polymeric system of poly (ethylene glycol) and poly (L-lactide). The prepared polymeric nanoparticles were designed by conjugating hydrazine terminated poly-(ethylene glycol)-*b*-poly (L-lactide) copolymer and a levulinic acid modified cisplatin analogue platinum (IV) prodrug. The limitations of cisplatin like low solubility and low bioavailability was resolved by improving the release kinetics from the prodrug conjugated polymeric nanoparticles with minimization of leaching during circulation in the blood (pH = 7.0). The nanoparticles upon reaching the tumor site got endocytosed into the tumor cells (pH = 5.0–6.0) and released the drug. The conjugation of the polymer and the dug was achieved with an acid sensitive hydrazone bond in a stoichiometric ratio of 2:1 resulting in a polymer-cisplatin prodrug conjugate. The cancer cells lines treated with polymer-cisplatin prodrug conjugate nanoparticles resulted in decrease of cellular viability of about 65% after 4 h of incubation (Aryal et al. 2010).

(b) Imine: Kong et al. designed polymeric nanoparticles of doxorubicin with an amphiphilic derivative of cellulose obtained by reaction of 2, 3-dialdehyde cellulose and amino compounds. The biocompatible polymer was synthesized by schiff reaction of 2, 3-dialdehyde cellulose with oleyl amine and aminoethyl rhodamine by imine bond formation. The release of doxorubicin from the polymeric nanoparticles was dependant on the pH of the tumor environment (pH = 4.0). The cytotoxicity study was conducted in lung cancer cells (A549) and the *in vitro* release was found to be 82.2% (Kong et al. 2020).

Guo et al. designed pH sensitive polymeric nanoparticles for co-delivery of doxorubicin and lapatinib against breast cancer cells (MCF-7). The polymeric block contained 2, 3-dimethylmaleic-anhydride-poly (ethylene glycol)-ε-poly-L-lysine

entrapping doxorubicin and lapatinib. The polymeric nanoparticles showed synergistic activity against breast cancer cells. For the conjugation of drugs with the polymeric system, doxorubicin was attached to the polymer side chain by an imine bond to produce poly (ethylene glycol)-ε-poly-L-lysine-doxorubicin conjugate. Ɛ-poly-L-lysine as a naturally biodegradable homopoly (amino-acid) was further subjected to amidation which produced 2,3-dimethylmaleic anhydride. 2, 3-dimethylmaleic anhydride shielded the positive charge of the nanoparticles to prolong circulation time of the drug in blood. After internalization, the cleavage of imine bond released the dual drug doxorubicin and lapatinib into the tumor cell microenvironment. The co-delivery of doxorubicin and lapatinib inhibited the cell proliferation in breast cancer cell lines (MCF-7) synergistically (Guo et al. 2020).

Cheng et al. designed polymeric nanoparticles of cisplatin by conjugation with aldehyde hyaluronic acid. The imine bond linked aldehyde hyaluronic acid and cisplatin produced aldehyde hyaluronic acid-cisplatin nanoparticles which showed sustained and pH sensitive release in embryonic cell lines (NIH-3T3) and cervical cancer cell lines (HeLa). The *in vitro* release of aldehyde hyaluronic acid-cisplatin nanoparticles showed about 51% release of cisplatin at pH 6.5 as compared to 11.5% at pH 7.4. Conjugation of cisplatin with aldehyde hyaluronic acid was responsible for cellular uptake of the nanoparticles as the tumor cell surface had overexpression of CD44 receptors and hyaluronic acid selectively gets attached to CD44 receptors and receptor mediated endocytosis was responsible for degradation and sustained release of cisplatin for targeted antitumor activity (Cheng et al. 2019).

Verma et al. designed a hydroxyapatite nanoparticle of doxorubicin. The hydroxyapatite was first functionalized by amino acid glycine to produce glycine-hydroxyapatite nanoparticles. The anticancer drug doxorubicin was bonded to glycine-hydroxyapatite nanoparticles via a pH sensitive covalent imine bond using a cross linker glutaraldehyde forming doxorubicin-glutaraldehyde-glycine-hydroxyapatite nanoparticles. *In vitro* drug release study showed that the doxorubicin-glutaraldehyde-glycine-hydroxyapatite nanoparticles achieved 10%, 15%, 35% and 48% release at pH of 7.4, 6.5, 5.5 & 4.0 respectively. Therefore it can be stated that the designed nanoparticles were suitable for delivery of doxorubicin in tumor microenvironment. The cytotoxicity study was conducted with WEHI-164 mouse fibrosarcoma cancer cells which confirmed the slow and sustained release of doxorubicin from doxorubicin-glutaraldehyde-glycine-hydroxyapatite nanoparticles due to breakdown of the imine linkage by reduction of pH (4.0) from physiological pH (7.4) (Verma et al. 2018).

Xie et al. synthesized a core-shell type self assembled pH sensitive polymeric nanoparticles for co-delivery of methotrexate and curcumin. Methotrexate was synthesized as a prodrug by coupling with 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[aldehyde(polyethylene glycol)-2000] by a pH sensitive imine bond via schiff's base reaction. The prodrug conjugates 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[aldehyde (poly ethylene glycol)-2000-imine-methotrexate got self assembled into micellar nanoparticles of methotrexate-imine-methotrexate in aqueous medium and encapsulated curcumin in the core by hydrophobic interactions. The methotrexate-imine-methotrexate-curcumin nanoparticles consisted of outer hydrophilic shell by

bishydroxyl poly (ethylene glycol) with methotrexate prodrug and inner hydrophobic core 1,2-distearoyl-sn-glycero-3-phosphoethanolamine/curcumin. The dynamic imine bond linking the 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[aldehyde (polyethylene glycol)-2000 and methotrexate was found to be stable in physiological pH and rapidly got degraded in acidic tumor microenvironment. Methotrexate-imine-methotrexate-curcumin nanoparticles was internalized by folate receptor mediated endocytosis and co-delivered methotrexate and curcumin at acidic environment by degradation of pH sensitive imine bond at pH 5.0. Methotrexate-imine-methotrexate-curcumin nanoparticles showed effective anticancer activity in breast cancer cell lines (MCF-7 cells) (Xie et al. 2018).

Similarly Li et al. designed prodrug of methotrexate-1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-poly (ethylene glycol)-CH=N-methotrexate) and conjugated with acid sensitive epirubicin-phospholipid complex to produce methotrexate-poly (ethylene glycol)-epirubicin-phospholipid complex nanoparticles. The *in vitro* kinetic study revealed that the significant difference in release of methotrexate and epirubicin at pH at 7.4 and 5.0. About 20% of epirubicin and 15% of methotrexate were released at pH 7.4. Whereas at pH 5.0, the release of epirubicin was about 75% and 60% for methotrexate over a duration of 24 h. Release of epirubicin from the nanoparticles was due to the isoelectric point of epirubicin and phospholipid complex. *In vivo* cellular uptake and cellular internalization was conducted in human cervical carcinoma cells (HeLa), human breast carcinoma cells (MCF-7), human lung carcinoma cells (A549) and mouse fibroblast cells (NIH-3T3). Anticancer activity of methotrexate-poly (ethylene glycol)-epirubicin-phospholipid complex nanoparticles was found stronger in HeLa and MCF-7 cells (folate receptor positive cell lines) than in A549 and NIH-3T3 cells (folate receptor negative cell lines). The stronger internalization in folate receptor positive cells was due to interaction of methotrexate with folate receptors and subsequent endocytosis delivered both the drugs into the endosomes or lysosomes and nucleus of tumor cells (Li et al. 2017).

Feng et al. designed pH sensitive polymeric prodrug for delivery of doxorubicin to cancer cells. Polysaccharide based polymer, dextran was chosen for conjugation of doxorubicin for preparation of an acid sensitive polymeric nanoparticles. Before conjugation with doxorubicin, dextran was converted into aldehyde form by an imine bond via schiff base reaction. The cytotoxicity of dextran-doxorubicin nanoparticles was conducted on mouse B16F10 melanoma cells. *In vitro* release kinetics was conducted at pH 7.4, 6.8. 5.0 and 4.0. The % of drug release of doxorubicin was found to be 11%, 35.8%, 75.1% and 96.0%, respectively. The increased release at pH 4.0 was due to break down of acid sensitive imine bond in tumor microenvironment. Dextran-doxorubicin showed a time dependent cytotoxicity towards murine melanoma cell lines (B16F10) (Feng et al. 2017).

(c) Cis-aconityl: Lin et al. designed a redox and pH sensitive self-assembling nanoparticles containing hyaluronic acid with an active targeting peptide luteinizing hormone releasing hormone conjugated with doxorubicin by a cis-aconityl linkage. The luteinizing hormone releasing hormone-hyaluronic acid-cis-

aconityl-doxorubicin nanoparticles showed specific internalization into the tumor cells by interaction of luteinizing hormone releasing hormone peptide and hyaluronic acid with tumor surface receptors. Cystamine ($Cys_{5.5}$) was conjugated for theranostic property of the nanoparticles. Acid or pH sensitive delivery of the luteinizing hormone releasing hormone-hyaluronic acid-cis-aconityl-doxorubicin nanoparticles at pH 5.0 was about 70% after 150 h. The cytotoxicity effect was conducted on human ovarian cancer cells (OVCAR-3) and the nanoparticles showed effective antitumor activity (Lin et al. 2016).

Song et al. developed a pH sensitive prodrug delivery system of doxorubicin in which PEGylated doxorubicin was conjugated with a peptide cysteine-arginine-glycine-aspartate-lysine (CRGDK) by an acid cleavable cis-aconityl bond. The prodrug nanoparticles specifically bound to neurophilin-1 receptors showed a release of 60% of doxorubicin by acid-triggered hydrolysis of cis-aconityl bond at pH 4.0 and disintegration of nanoparticles. Internalization of PEGylated-doxorubicin-CRGDK nanoparticles was achieved by receptor mediated endocytosis and cytotoxicity study was conducted on human liver carcinoma (HepG2), human breast cancer (MCF-7 and MDA-MB-231) and human cardiomyocyte (H2C9) cells. The PEGylated-doxorubicin-CRGDK nanoparticles showed appreciable cytotoxicity to HepG2, MCF-7 and MDA-MB-231 cells whereas lesser cytotoxicity was found in H2C9 cells (Song et al. 2015).

Lavignac et al. designed a polymeric nanoparticles of doxorubicin with poly(amido amine) with amino pendant groups (dansyl cadaverine) by a pH sensitive cis-aconityl spacer linkage. The poly (amido amine)-doxorubicin nanoparticles showed an increased release of doxorubicin at pH 5.0. The cytotoxicity study on murine melanoma B16F10 model showed better uptake and internalization with increased anticancer activity (Lavignac et al. 1998).

(d) Acetal: Zhao et al. designed pH sensitive polymeric nanoparticles of 10-hydroxyl camptothecin for targeted delivery to the acidic environment of colon cancer. Cinnamaldehyde was linked by an acetal bond to dextran to produce a polymer which could encapsulate 10-hydroxyl camptothecin. 10-hydroxyl camptothecin conjugated cinnamaldehyde nanoparticles were found to be potent anticancer moiety as it induced apoptosis and reduced the cancer cell proliferation by generation of intracellular reactive oxygen species after internalization. The cytotoxicity study in xenograft mouse models of HCT116 cells showed strong suppression of tumor growth and very less damage to kidneys were found indicating the safety of 10-hydroxyl camptothecin conjugated cinnamaldehyde nanoparticles. The *in vitro* release of 10-hydroxyl camptothecin conjugated cinnamaldehyde nanoparticles was found to be 45% and 90% at pH 7.4 and 5.0, respectively. The results confirmed the fact that the hydrolysis of acid sensitive acetal linkage in the polymer at lower pH released more 10-hydroxyl camptothecin inside the tumor cells (Zhao et al. 2019).

Yoo et al. conjugated cinnamaldehyde to maltodextrin by an acetal linkage to produce a polymeric prodrug and drug carrier. The cinnamaldehyde-maltodextrin was used to encapsulate camptothecin. The entrapment efficiency was about 80% and the in vitro release at pH 7.4 was found to be less than 40% after 48 h, whereas the release was about 80% at pH 5.5 within 24 h. The *in vitro* cytotoxicity study on SW620 showed better anticancer activity as compared to free camptothecin or cinnamaldehyde-maltodextrin due to the synergistic effect of camptothecin and the generation of intracellular oxidative stress by cinnamaldehyde-maltodextrin (Yoo et al. 2018).

(e) Ortho ester: Wang et al. designed a pH sensitive nanocarrier by crosslinking bromelain (Br) with an ortho ester-based crosslinking agent and encapsulated doxorubicin for targeted delivery to hepatocellular (HepG2 and H22) cancer cells. The *in vitro* release of bromelain-doxorubicin nanoparticles showed about 86% drug release at pH 5.5 after 120 h whereas the release was only 28% at pH 7.4 after 120 h. The decrease in tumor growth was about 62.5% after 7 days of treatment with bromelain-doxorubicin nanoparticles (Wang et al. 2020).

(f) β-thiopropionate: Pan et al. synthesized pH-sensitive poly (β-thiopropionate) nanoparticles with a supermagnetic core and folic acid conjugation (folic acid-doxorubicin-iron oxide nanoparticles) for targeted delivery of doxorubicin, for the treatment of folate receptor-overexpressed breast cancer. These polymeric nanoparticles could be used as a theranostic tool where the nanoparticles not only fulfilled the purpose of imaging but also released their payloads in response to an acidic tumor microenvironment of pH 5.0. The cytotoxicity study was conducted on three breast cancer cell lines MCF-7, BT549, and MD-MBA-231. The *in vitro* release kinetics at different pH were conducted and found to be approximately 32.5% and 75% at pH 7.4 and 5.0, respectively at 48 h. Folic acid-doxorubicin-iron oxide nanoparticles showed enhanced antitumor activity due to increased the cellular uptake by the overexpressed folate receptors in breast cancer cells and the magnetic effect of iron oxide suppressed the tumor growth (Pan et al. 2018).

Xu et al. designed a pH sensitive polymeric nanoparticles of camptothecin with a polymer scaffold P(HEO_2MA)-b-P(HEMA-DHLA). The polymer scaffold was prepared by polymerization of methacrylate derivative of lipoic acid (HEMA-LA) with poly (2-(2-hydroethoxy) ethyl methacrylate) (P(HEO_2MA)) and subsequent reduction of disulphide bonds in lipoic acid, produced dihydrolipoic acid (DHLA) as pendant groups. Camptothecin was coupled to the polymeric scaffold to produce a polymeric prodrug moiety, which later on self assembled via acid sensitive β-thiopropionate linkage. The polymeric prodrug of camptothecin showed similar cytotoxicity as free camptothecin to cervical cancer cell lines (HeLa). The prodrug of camptothecin polymeric nanoparticles released the drug at pH 5.0 within 72 h and the enhanced cellular uptake showed better tumor growth suppression as compared to free camptothecin (Xu et al. 2018).

15.5.4 Crosslinking

Li et al. designed pH sensitive crosslinked polymeric nanoparticles of cisplatin and doxorubicin in a prodrug form. The crosslinking of polysaccharides was achieved by linking the succinic acid decorated dextran and the nanoparticles were further loaded with doxorubicin. In aqueous medium, anionic dextran-succinic acid interacted electrically with cationic doxorubicin and formed crosslinked nanoparticles. *In situ* crosslinking of the polymeric crosslinked nanoparticles with cisplatin resulted in a co-delivery of two antitumor agents. Doxorubicin was released in a controlled manner in a pH dependent manner from the crosslinked nanoparticles with increased drug circulation time and tumor accumulation. The cisplatin prodrug polysaccharide based crosslinked nanoparticles was proved to be an efficient targeted delivery of doxorubicin in tumors (Li et al. 2014).

Yang et al. prepared a polymeric carrier by host-guest interaction of β-cyclodextrins and benzimidazole. The mechanism of targeted supramolecular prodrug complexes -based self-assemblies and supramolecular prodrug complexes -based self-assemblies were evaluated for cell proliferation, cellular uptake, cellular apoptosis and cytotoxicity. The pH sensitive self assemblies of lactobionic acid were prepared easily and delivered the doxorubicin from targeted supramolecular prodrug complexes with decrease in pH in a controlled manner in hepatocellular cancer (HepG2) cells. Doxorubicin was released at a faster rate as the pH of the medium decreased due to tumor microenvironment. The tumor cell proliferation was prevented by the self assembled nanoparticles by induction of cell apoptosis (Yang et al. 2019).

Lin et al. designed polymeric nanoparticles synthesized by host-guest recognition between camptothecin and three pH-sensitive acyclic cucurbit[n]uril. The designed camptothecin nanoparticles were tested in normal cells (human HEK-293 cell line) as well as three tumor cells (human hepatocellular (HepG2), human colorectal (HCT116), neuroblastoma (SH-SY5Y) cell lines). The cellular uptake and internalization led to increased antitumor activity of camptothecin as the polymeric carrier cucurbit[n]uril was responsible for enhanced water solubility of camptothecin (Lin et al. 2019).

15.6 Conclusion

Drug delivery to desired cells or tissues has already been demonstrated by the rapidly emerging field of pH responsive, polymeric nanocarrier systems. A deeper perception of biological differences between diseased and healthy cells as well as advances in nanomaterial technologies has led to remarkable improvement in the domain of pH responsive nanocarrier systems to enhance drug targetability and biodistribution. Due to the simultaneous inclusion of an active polymer matrix and a stimulus part, stimuli-responsive polymeric nanocarriers display synergistic

activity. Stimuli-responsive polymeric nanocarriers have been developed for different domains of biomedical applications. However there are some drawbacks that need to be tackled in the future which majorly includes the safety of the added excipients, toxicity profile, alterations of substituents in various disease conditions as well as inconsistencies in the *in vitro* studies. To ensure sufficient blood circulation and resist renal clearance as well as avoid capture by the reticulo endothelial system, numerous variables such as charge, size and surface functionalization of the polymeric nanostructures need to be optimized. Ultimately, major efforts are necessary to maintain the effective and widespread application of polymeric nanomaterials in drug delivery, imaging, and cancer diagnosis.

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Chapter 16
Polymeric Nanoplatforms for the Targeted Treatment of Prostate Cancer

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16.1 Introduction

Cancer is one of the world's principal public health problems (Fitzmaurice et al. 2015; Padhi and Behera 2020). It is the second-largest consequence of patient deaths and is accountable for seven million deaths annually (12.5% worldwide) (Orive et al. 2005; Siege et al. 2014). Followed by cardiovascular disease, cancer in the United States is the second most frequent cause of death with a total of 1,665,540 new cancer cases and 585,720 deaths in 2014 (Siege et al. 2014). More particularly, as per american cancer statistics evidence (2014), prostate cancer (233,000), female breast cancer (235,030), lungs/bronchus cancer (224,210), colon/rectum cancer (136,830) seems to be the most common forms of cancer (Salaam et al. 2018; Siege

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et al. 2014). By 2040, there will be an increase of 29.5 million new cancer cases a year, with 16.4 million cancer-related deaths. Worthy to mention that, about 43% of all cancers diagnosed in men in 2020 are prostate cancer, lung cancer, and colorectal cancer (Oh et al. 2019).

Prostate cancer is the common non-skin (Chhabra et al. 2018), the male reproductive system-associated cancer type that develops in the prostate gland. Additionally, it is the most frequent malignancy in men and accounts for 10% of the new estimated cases of cancer (Rawla 2019; Siege et al. 2014). In the United States (2020), prostate cancer accounted for 191,930 new cases and 33,330 (5.5%) death cases (Oh et al. 2019). As per the published report, the world's second most common male malignancy is prostate cancer, with 1,276,106 new diagnoses and 358,989 deaths (3.8% of all men cancer deaths) in 2018 (Rawla 2019). Although an estimated 2,293,818 new cases are to be reported by 2040, the mortality rate is small (1.05% increase) (Rawla 2019). It is well known that prostate cancer may be reasonably harmless or highly aggressive. In the case of aggressive prostate cancer, it soon spreads to lymph nodes and bone in particular. Furthermore, prostate cancer is categorized and further staged for aggressiveness and the exact extent to which it spreads. Herein, stages A and B are restricted to the prostate gland, whereas, stage C is outside the gland, but locally only, and stage D is expanded into lymph nodes and distant sites (Ast 2003).

Prostate cancer pathogenesis has relied heavily on androgen receptor signaling, a ligand-dependent transcription factor (Chhabra et al. 2018). In general, prostate cancer is again largely considered an old-age (average age 66 years) disease because of the more frequent occurrence in older individuals (Rawla 2019; Siege et al. 2014). Prostate cancer may also be associated with age, as it influences 80% of males over 80 years. Based on the age group, the percent of new cases of prostate cancer have been depicted in Fig. 16.1. Prostate cancer risk also includes inheritance, race, alcohol intake, obesity, sexual behavior, ultraviolet radiation exposure, diet, gender, inflammatory diseases, etc. (Chiam et al. 2014). It should be noticed that the prostate cancer prevalence of african-american men in contrast with white men is higher (Panigrahi et al. 2019). The explanations for this imbalance are believed to be social, environmental, and genetic variations (Rawla 2019).

Prostate cancer diagnosis and treatment were linked to depressing moral systems, nervousness, psychological distress, and reduced well-being efficiency (Mohamed et al. 2012), often linked to physical symptoms viz. erectile dysfunction, fecal leakage diseases, and pathetic urinary stream (Johansson et al. 2009). The conventional treatment methods for prostate cancer are surgery (i.e. resection of tumors), chemotherapy, and radiation therapy (Nilsson et al. 2004). Notably, this technique can be used alone or in combination to produce realistically positive outcomes. One of the best ways to cure metastatic diseases (known as cancer) is to undergo chemotherapy using antimetabolites or alkylating agents (Salaam et al. 2018). Regardless of the impressive advantages of chemotherapy, remarkable toxicity, reduced drug intake, overtime tolerance to drugs in the cancer cells and minimal patient-specific therapies are some of the foremost chemotherapy concerns. Unfortunately, toxicity is a factual concern, because chemotherapy will target cells

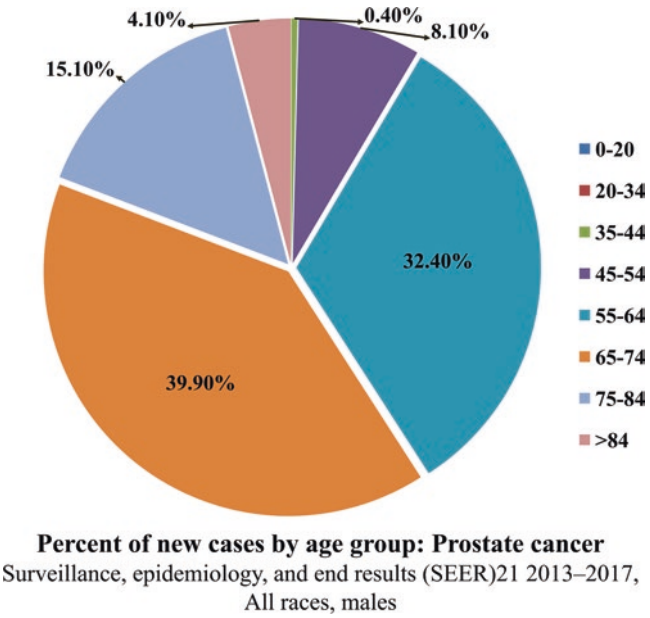

Fig. 16.1 Prostate cancer percent new cases by age group from 2013–2017 (all races of male population). The figure represents the percentage of male patients suffering from prostate cancer and classified according to age group

without specificity that divide hurriedly and annihilate perfectly healthy, non-cancerous cells (hair and immune cells) of the human body (Schirrmacher 2019). Consequently, researchers spotlight mostly on tailored delivery mechanisms, with selectively terminating cancer cells and creating mostly patient-specific treatment regimes.

In this book chapter, the fundaments of revolutionary polymeric nanoparticles and their ground-breaking applications to prostate cancer have been elaborated in detail. Furthermore, it gives a comprehensive insight into targeting strategies and types of polymeric nanoparticles that have been reported to prostate cancer treatment. It discussed the recent advances in polymeric nanoparticles and the application section of these polymeric nanoparticles. Notwithstanding this, current challenges and future-outlook of polymeric nanoparticles for prostate cancer therapy have also been discussed in brief. Overall, this chapter gives detailed insight into the pathway and type of polymeric nanoparticles for prostate cancer treatment, which may persuade their future applications in biomedical fields.

Nanotechnology has a huge perspective for revolutionizing precise detection and efficient treatment of cancer (Padhi et al. 2018). Over the recent four decades, comprehensive work has been performed in the field of prostate cancer therapy by researchers. Even with advances in science and technology, obstacles and major problems in prostate cancer care are drug resistance against anticancer drugs,

difficult stem cell targeting, side effects of anticancer actives, low therapeutic window of anticancer drug, lack of tools, and cost as a critical factor for cancer diagnosis, etc. As we know, the most modern therapies for prostate cancer are viz. radiation therapy, chemotherapy, surgery, more recently enrolled immunotherapy as well as immune regulation therapy that frequently destroys healthy cells and triggers severe side effects, hence proved to be ineffective for certain patient populations (von Roemeling et al. 2017). Besides this, the maximum allowed dose of anticancer actives is also limited (Agarwal et al. 2019).

Often large doses of drugs are needed to prevent widespread cancer in different tissues and bodies that are economically unfeasible and create extreme toxicity (Padhi et al. 2020). Concisely, its un-specificity in cancerous cells is the most unifying trait that restricts anticancer drug delivery to a tumor mass. Unfortunately, an anticancer agent cannot differentiate between healthy cells and cancer cells. Therefore it functions together with cancer cells in healthy cells. The high tissue distribution and the accumulation of anticancer drugs in other organs is another consideration in cancer treatment. Consequently, a higher number of doses are to be given to exploit the therapeutic reaction and consequently results in severe side effects and toxicity (Gad et al. 2016). On the other hand, physiochemical property includes poor solubility of anticancer drugs which accounts for lower bioavailability that affects the overall performance of active. On the contrary, the high solubility of anticancer candidates is accounted for speedy elimination from the patient's body (Arya et al. 2019).

Despite the revolutionary merits of engaged anticancer therapies, the defeat of cancer treatment at an advanced point of prostate cancer, and the huge expansion of multiple drug resistance is the foremost barrier (Espinosa-Cano et al. 2018). Additionally, the existence of intra-tumor heterogeneity is an additional concern to the aggressive prostate cancer expansion. Unfortunately, in most cases, prostate cancer reoccurrences have been observed after the complete cancer treatment. Overall, nanocarrier-mediated co-delivery is the major objective of groundbreaking and successful anti-cancer chemotherapy (Arya et al. 2019; Parveen and Sahoo 2008; Padhi et al. 2015). Another prostate cancer treatment strategy includes prodrugs due to admirable stability at biological fluids (viz. blood) and it is less toxic than its activated form. Interestingly, it exhibits low toxicity due to the activation of the product within the targeted prostate cancer tumor microenvironment (Ast 2003). In another pioneering work, prostate cancer tumor-related peptidase enzymes (prostate-specific antigen) have been reported in the activation of prodrugs. Unfortunately, it shows the one-third metabolism of the prodrug into an active form (DeFeo-Jones et al. 2000).

Recently, targeted treatments for prostate cancer have been reported by various studies that concentrate on focusing on anomalies but there are also some limits. Unfortunately, hormone therapies do not respond to androgen-independent prostate cancer. In addition, immunotherapy is much costly than the other techniques and has lower success rates because of less assurance of the related improvement in immune performance. Furthermore, anticancer molecules used in particular for prostate cancer are poorly bioavailable and are insufficient to use. Consequently, an

appropriate drug-delivery vehicle is desired as a modification substratum to bind target ligands/receptors (Salaam et al. 2018). Novel chemotherapies are tremendously implemented in new advances that can improve the abilities of medications by hitting cancer cells either passively or actively (Parveen and Sahoo 2008; Behera and Padhi 2020). In that, passive targeting utilizes the tendency of cancer cells to collect anticancer-loaded nanocarriers with the enhanced permeability and retention effect (Agarwal et al. 2019; Khuroo et al. 2014). The molecules-conjugated nanostructures that entangle the prostate cancer antigen or appropriate receptor overexpressed to cancer cells become actively targeted approach (Peer et al. 2007).

Recently, nanocarrier frameworks can also be divided into three groups based on the manner chemotherapeutic/anticancer agent is targeted at cancer cells and their specific physicochemical properties (Agarwal et al. 2019). In brief, the first nanocarrier frameworks consist of simple nanoparticles in the matrix and nanocapsules in the reservoir form (Jiashi et al. 2009). Furthermore, the second series of the nanostructure is laminate by types of polymers that exhibit over-expressed cancer cell-specific binding sites (Peer et al. 2007). In recent years, polymeric nanoparticles linked to preferred ligands, are widely used for detection in the three generation series. Recently reported publications to support the fact that this two generations series of remarkable polymeric nanoparticles target the prostate tumor through an active targeting approach (Agarwal et al. 2019; Peer et al. 2007).

16.2 The Emerging Era of Polymeric Nanoparticles

Nanoscale materials are perfect candidates being used as platforms for drug delivery for targeted treatment of cancer. Recent developments in nanotechnology have led to the development of several nanostructures of different sizes, shapes, core physical, and surface properties under investigation for possible medical use, specifically for cancer therapy (Krishnan et al. 2010). Taken as a whole, nanotechnology has made substantial strides in cancer chemotherapy progress in the last ten years (Rivero-Buceta et al. 2019; Padhi et al. 2020). The use of nanoparticles allows reduced doses of the drugs administered (Salaam et al. 2018). Fascinatingly, nanotechnology is opening the solution to accomplish and improve the performance and sustainability limitations of traditional delivery systems. From the very beginning, nanoparticles have emerged as a possible candidate for the delivery of therapeutic agents to specified organs, tissues/cells, and for minimizing drug delivery problems (Parveen and Sahoo 2008; Verma et al. 2017). A further important categorization of nanoparticles is inorganic nanoparticles and polymeric nanoparticles centered on their compositional structure (Khalid and El-Sawy 2017). In this subsection, the emerging era of polymeric nanoparticles has been discussed in brief.

Interestingly, polymeric nanoparticles are colloidal particles (submicron) that are preferred for the distribution of medicinal items (actives) to the targeted site. It may be of different shapes including spherical, branched, and core-shell. Generally, polymeric nanoparticles are synthesized using formulation techniques such as

solvent evaporation, solvent diffusion, spontaneous emulsification, polymerization, and many more methods. In polymeric nanoparticles, the targeted anticancer drug is usually adsorbed/conjugated or encapsulated inside or on the surface of the nanoparticles for specific/targeted drug delivery (Kumari et al. 2016).

In particular, targeted polymeric nanoparticles efficiently deliver the anticancer agent to a specific location in a controlled manner. Therefore it offers the efficient therapeutic potential for cancer treatment with minimal toxicity to normal cells (Khalid and El-Sawy 2017). Overall, polymeric nanoplatform containing polymers is the most extensively used material in biomedical sciences, engineering as well as in our daily lives. Additionally, the implementation of such polymers is linked directly to their properties (viz. cross-linking nature, hydrophilicity, suitable functional group presence) especially in health engineering (Reddy and Rao 2016). Owing to this, it offers the ultimate benefits of stability, entrapment efficiency, biocompatibility, safety, drug loading, etc. Additionally, advances in polymer chemistry and colloid polymer chemistry have brought unbelievable perspectives into the domain of drug delivery (Fig. 16.2). Therefore, this could be used as a feasible medium for different cancer applications by customizing and influencing the attributes of nanomaterials (Vauthier and Bouchemal 2009). For example, nanospheres and nanocapsules have been typically used for anticancer drug delivery. In that, the nanosphere allows adsorption of the anticancer agent onto the particulate surface that is prone to degradation due to intensive processing (Peer et al. 2007).

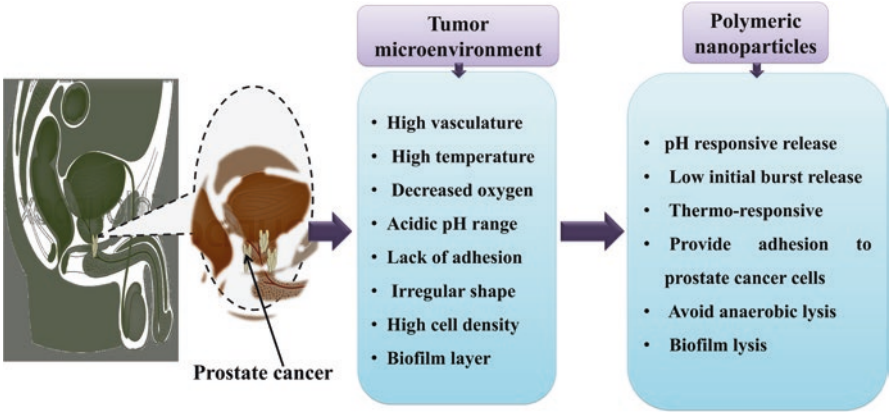

Fig. 16.2 Prostate cancer: hurdles for prostate cancer drug delivery and desirable properties of polymers for designing of polymeric nanoparticles for targeted delivery in prostate cancer. The tumor microenvironment in case of prostate cancer like high vasculature, high temperature, decrease in oxygen, change of pH surrounding the cancer cells to acidic pH, lack of adhesion, irregular shape, high cell density and biofilm layer limits for targeted delivery of anticancer drugs. Hence the polymeric nanoparticles are designed in such a way to deliver the chemotherapeutic drugs following the pH-responsive release with low initial burst release of the drugs. Other mechanisms may include temperature-sensitive drug delivery, increase in adhesion of drugs to prostate cells, avoiding the anaerobic lysis and biofilm lysis

Furthermore, the surface structures can also be configured to facilitate high loading and improve payload distribution that can be beneficial for the management of prostate cancer. Moreover, numerous advances in the use of polymeric nanoplatforms with several approaches (coating/layer-by-layer) allow the anticancer drug/antigen to be released over many days in a regulated approach (Agarwal et al. 2019). To summarize, owing to these remarkable properties of polymeric nanoparticles, it accounts for an alternative substitute for the effective treatment of prostate cancer.

16.3 Surface Engineered Polymeric Nanoparticles for Prostate Cancer

For effective drug delivery to the targeted site of prostate cancer, different types of polymeric nanoparticles can be effectively employed in prostate cancer therapy. As we know, the biological complexity in the body requires an appropriate root for nanocarriers. Therefore, polymeric nanoparticles are engineered to accumulate passively or actively in prostate cancer tumor sites, by regulating their hydrodynamic properties or by using target molecules to activate the surfaces of polymers. Concerning that, there are mainly two ways to surface engineer polymeric nanoparticles targeting prostate cancer, namely passive targeting, and ligand-centered targeting, to deliver anticancer molecules at the desired site (Fig. 16.3).

16.3.1 Passive Targeting

In passive targeting of anticancer molecules for efficient management of prostate cancer, the anatomical variations within normal and prostate cancer tumor tissue are utilized to deliver drugs for prostate cancer (therapeutic site) (Arya et al. 2019; Famuyiwa and Kumi-Diaka 2018). It may due to prostate cancer tumor vasculature, which varies significantly from normal prostate tissues. Generally, prostate cancer tumor contains heterogeneous cells of high vascular density and are generally larger sized. Additionally, the prostate cancer tumor is more permeable or leaky unlike the compact endothelium of typical blood vessels. Furthermore, widespread vascular mediator (viz. prostaglandins, bradykinin, vascular endothelial factor, nitric oxide, etc.) development facilitates the extravasations, despite the abovementioned pathophysiological conditions involving temperature, pH, and surface charge of prostate tumor. Passive targeting of polymeric nanoparticles for prostate cancer management has also been considered as an appropriate substitute for anticancer drug delivery (Arya et al. 2019; Hema et al. 2018). In this sub-section, we have emphasized architectured polymeric nanoparticles based on non-targeted drug delivery for prostate cancer management.

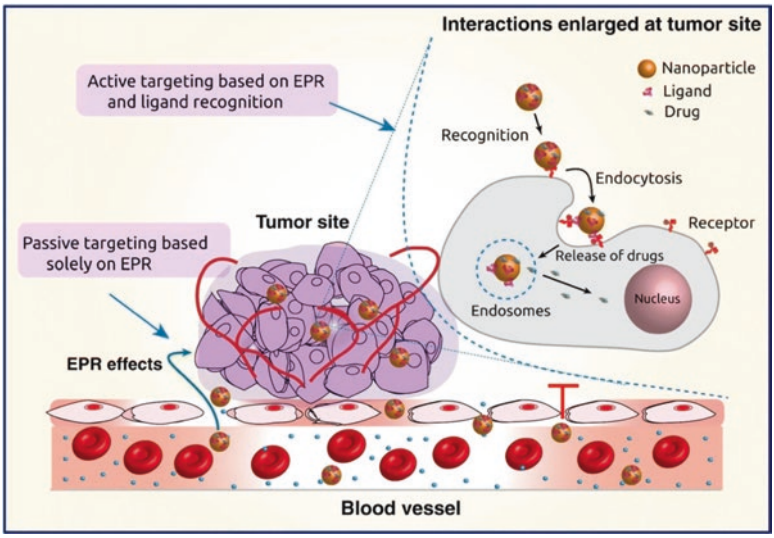

Fig. 16.3 Targeted (active targeting) and non-targeted (passive targeting) strategies for designing nanoplatform and its application for anticancer agent targeted delivery. Active targeting of polymeric nanoparticles is based on recognition of ligand conjugated drug by overexpressed receptors on the tumor cells. The drug gets internalized by receptor mediated endocytosis. Passive targeting of polymeric nanoparticles help to enhance the permeability and retention (EPR) effect. Accordingly, it enhanced the deposition and retention of the anticancer drug (high molecular weight) in prostate cancer solid tumors.'Reprinted with permission of "Current trends and challenges in cancer management and therapy using designer nanomaterials", Nano Convergence, Springer Nature from (Navya et al. 2019)

In passive targeting of polymeric nanoparticles for prostate cancer management, the enhanced permeability and retention effect enhanced the deposition and retention of the anticancer drug (high molecular weight) in prostate cancer solid tumors (Fig. 16.3). Generally, nanoparticular frameworks are still used to implement passive targeting when prostate cancer tumor volume raises the specific pathophysiological features of tumor vessels. Prostate cancer results in an increased enhanced permeability and retention effect, thanks to the leaky vasculature of the tumor and poor lymphatic flow (Arya et al. 2019).

As the dimensions of polymeric nanoparticles are usually smaller than those of the perforated blood vessels, they get accumulated in cancerous cells and stay stuck inside these tumors due to greater retention capacity as compared to normal tissues (Hema et al. 2018). Ample literature claimed that the microenvironment around the prostate cancer cells is different from normal prostate cells, which offers the passive targeting of anticancer molecules. Besides, the metabolic rate is also superior in prostate cancer cells. Furthermore, the acidic pH in the microenvironment is observed owing to the high use of glycolysis by cancer cells to retain the supply of oxygen and types of nutrients (Morshed et al. 2018). Besides, the assorted types of enzymes present in prostate cancer and the temperature of prostate tumors are

furnished with the passive targeting of anticancer molecules (Arya et al. 2019; Hema et al. 2018). Owing to this, the pH-responsive polymeric nanoparticles, temperature-sensitive polymeric nanoparticles, and enzyme responsive release of the anticancer agent from polymeric nanoparticles are gaining much attention from research scholars, which provides numerous merits over the conventional anticancer dosage forms.

The surface potential of tumor cells is highly negative than normal cells (Morshed et al. 2018). Therefore, the positive zeta potential of polymeric nanoparticles is majorly used for designing anticancer-targeted delivery, which offers cellular internalization as well as the subcellular localization of polymeric nanoparticles due to electrostatic binding forces. Finally, it is responsible for the overall cytotoxicity potential of selected anticancer molecules (Arya et al. 2019; Hema et al. 2018). Therefore, passive targeting is a superb way to engineer polymeric nanoplatform for well-organized prostate cancer treatment.

16.3.2 Ligand-Based Targeting

Abundant literature claimed that certain intracellular and extracellular receptors are over-expressed by the prostate tumor surface. Therefore, the targeting of these prostate cancer cell receptors may be desirable for the high deposition of the anticancer drug into prostate cancer tissue/cells. Targeting prostate cancer through architectured polymeric nanoparticles is an appealing prospect to deliver anticancer drugs owing to their aptitude to deliver active at specific locations/sites, consequently shielding normal cells or tissues from severe toxicity. Promising targets in prostate cancer have been folate receptors, prostate-specific membrane antigen, neuropilin-1 receptors, and transferrin receptors (Arya et al. 2019). In this type, ligands are integrated into the surface of the architectured polymeric nanoparticles system, this form of targeting connects with the right receptor at the desired prostate cancer tumor site. Fascinatingly, several kinds of ligands such as an antibody, peptides, aptamers, vitamins, carbohydrates, other small molecules, etc. (Fig. 16.4) have been selected in designing targeted architectures of polymeric nanoparticles for prostate cancer management (Agarwal et al. 2019; Hema et al. 2018). Ligand-based targeting can be implemented through the surface functionalization of the polymeric nanoparticles containing anticancer drugs, along with selected targeting moieties, which selectively recognize receptors/antigens of prostate cancer cells, increase their therapeutic effectiveness, and transcend multidrug resistance of cancer cells (Arya et al. 2019; Hema et al. 2018). Overall, ligand-based targeting offers notable merits including the release of anticancer molecules to the prostate cancer cells. It may form bonding with the cancer cell membrane and sustain/control the anticancer drug release or it can be internalized into prostate cancer cells via endocytosis mechanism.

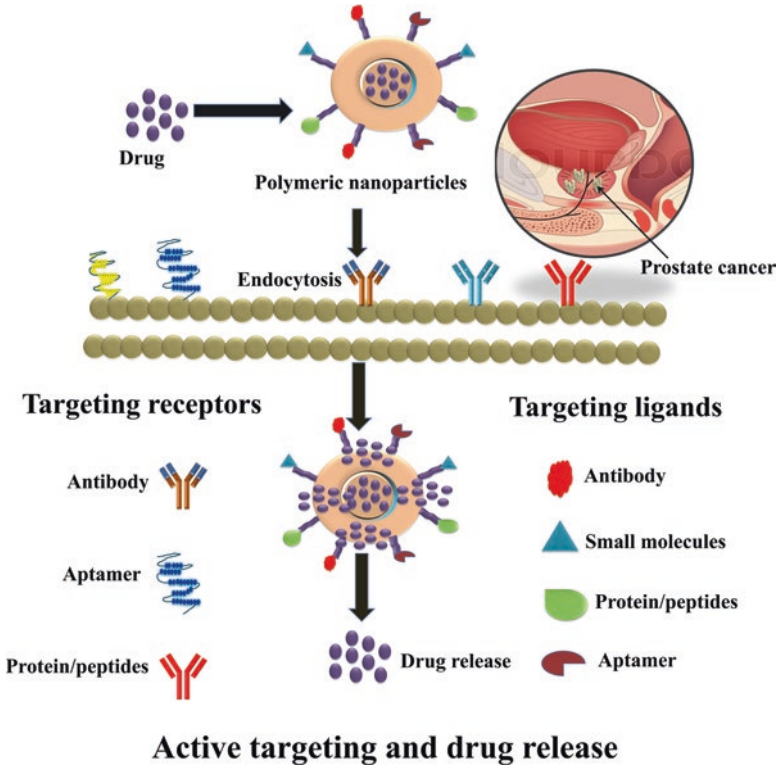

Fig. 16.4 Active targeting of architecture polymeric nanoparticles by overexpressed receptors and ligands for prostate cancer management. Ligands such as an antibody, small molecules, proteins/peptides, and aptamer are integrated on the surface of the polymeric nanoparticles, which offers the targeting with the right receptors such as aptamer, proteins/peptide, antibodies, etc. at the desired site of prostate cancer

16.4 Polymeric Nanoparticles for Targeting Prostate Cancer

There are currently several approaches available to produce targeted polymeric nanoparticles that incorporate a variety of theranostic molecules via the chemical process for prostate cancer management. Polymeric nanoparticles have shown tremendous potential in terms of the creation of polymeric nanoplatforms for prostate cancer molecular imaging, theranostics, etc. as stated in this section.

16.4.1 Solid Dispersion of Polymeric Nanoparticles

From their inception, solid polymeric nanoparticles are gaining huge attention from research fraternities, owing to their notable merits. Natural polyphenol viz. resveratrol is well known for its numerous biological/anticancer effects on different human

cell lines of cancer. However, low water solubility, limited bioavailability, and poor stability hinder its effectiveness in the battle against prostate cancer. A plentiful literature survey divulged the targeting ability (active/passive) of poly (lactic-*co*-glycolic acid) against assorted cancer types. Owing to this, Nassir et al. had developed resveratrol-loaded poly (lactic-*co*-glycolic acid) nanoparticles using polylactic-*co*-glycolic acid and resveratrol through nanoprecipitation technique. Herein, resveratrol loaded poly (lactic-*co*-glycolic acid) nanoparticles showed a significant decrease in cell viability against prostate cancer cells (LNCaP cells) with inhibitory concentration upto 50% and 90% (IC_{50} and IC_{90}) of 15.6 μM and 41.1 μM, which may be due to apoptosis, the hammering of the potential of mitochondrial membrane, reactive oxygen species production in LNCaP cells, deoxyribonucleic acid nicking, etc. Finally, this polymeric platform exhibited notable cytotoxicity in prostate cancer cells (LNCaP cells) as compared to plain resveratrol. Overall, Nassir et al., suggested that the resveratrol-loaded poly (lactic-*co*-glycolic acid) nanoparticles can be used in chemotherapy owing to their remarkable safety to normal prostate cells (Nassir et al. 2018).

As we know, in conjunction with immune stimulant adjuvant, precise immunotherapy seems to be an important technique to facilitate tumor regression and avoid recurrences in prostate cancer. A pioneering study reported the tumor-associated antigens (source-murine prostate cancer cell lines TRAMP-C2) encapsulated oral microparticulate vaccine using hydroxyl propylmethyl cellulose and ethyl cellulose through a spray dryer. This polymeric nanoplatform showed size-dependent cellular uptake that confirmed the principal role of particle size in antigen delivery. Additionally, the stability and protection of antigen from high acidic pH were achieved through a polymeric platform. The cyclophosphamide and granulocyte macrophage-colony stimulating factor combination with microparticulate vaccine offered a fivefold reduction in prostate cancer tumor volume in mice. Thus, oral microparticle vaccines can trigger a robust cell tumor response, with a substantial amount of resistance against tumor growth and progression in combination with clinically relevant agents (Parenky et al. 2019). Bharali et al. designed docetaxel-poly(ethylene glycol)-based poly (lactic-*co*-glycolic acid) nanoparticles using the solvent diffusion method and further engineered the surface of nanoparticles through conjugation of anti-CD24 for prostate cancer targeted delivery of docetaxel. In this, engineered nanoparticles demonstrated higher accumulation (~tenfold) in prostate cancer in contrary to non-conjugated nanoparticles, and accordingly reduced the overall prostate tumor mass and its viability. Herein, poly (ethylene glycol) offered longer time stability in blood circulation through minimizing opsonization. Additionally, high molecular weight poly (ethylene glycol) offers the maleimide functionalities for anti-CD24 conjugations. Enchantingly, nanoparticles played the role of nano-reservoir for docetaxel with sustained-release kinetics over an extended time. To conclude, the sustained release targeted docetaxel-poly(ethylene glycol)-based poly (lactic-*co*-glycolic acid) nanoparticles served as a docetaxel reservoir for a long duration, and thus, the nanoparticles may be able to remove the repeated doses of docetaxel in prostate cancer treatment (Bharali et al. 2017).

Singh and co-authors claimed a higher level of folate receptor expression in prostate cancer tissues (obtained from the patient) as compared to the normal tissue of the human body. Keeping this in mind, the authors developed the folic acid conjugated resveratrol – docetaxel nanoparticles using fluorescein sodium salt and starch by planetary ball mill and further coated it with polycaprolactone and poly (ethylene glycol). It demonstrated that the nanoparticles resulted in 65.90% apoptosis, which was higher than the single anti-cancer molecule. Additionally, immunofluorescence studies confirmed the enhancement in intracellular concentration of resveratrol and docetaxel in the cytoplasm was due to receptor-mediated endocytic delivery of the entrapped drug. Moreover, polycaprolactone- poly (ethylene glycol) copolymer demonstrated greater hydrophilicity and degradability than polycaprolactone polymer, which was confirmed from cell culture studies. In addition, resveratrol/docetaxel coating with polycaprolactone- poly (ethylene glycol) boosted the bioavailability and sustained the release of resveratrol/docetaxel. For this reason, this research gave a promising insight into the biomedical use of resveratrol and docetaxel-based nanoparticles for prevention and treatment (Singh et al. 2018). A shortage of an efficient delivery vector appeared as a key obstacle in developing efficient ribonucleic acid interference therapy. In 2017, Evans et al. designed the folate targeted amphiphilic cyclodextrin with di-stearoyl phosphatidyl ethanolamine poly (ethylene glycol) 5000-folate for precise targeting delivery of ribonucleic acid interference to prostate cancer cells. The endosomal release of small interfering RNA was achieved by using pH-sensitive synthetic fusogenic peptide GALA that contains amino acid (30) endosomal escape peptides. Herein, the uptake of RNA interference-loaded cyclodextrin nanoparticles has been reduced by incubation of excess free folate. In conclusion, it was inferred that the folate targeted amphiphilic cyclodextrin vector would be a suitable candidate for effective prostate cancer treatment (Evans et al. 2017).

Fitzgerald et al. designed the small interfering ribonucleic acid and cyclodextrin complex. Further, PEGylated adamantane was conjugated with an anisamide-targeting ligand that helped to target the sigma receptor of prostate cancer cells. Finally, developed nanosized functionalized complex offered stability and protection to small interfering ribonucleic acid from serum-induced nuclease degradation. Additionally, i*n vitro* study confirmed that the targeted complex offered superior cellular uptake as well as knockdown of the PLK1 gene in prostate cancer cells. To our awareness, it would be the only evidence that cyclodextrin had been used to deliver small interfering ribonucleic acid to prostate cancer cells through their sigma-receptor in the form of integration complexes with adamantane derivatives. Therefore, in the future, it could be a suitable carrier for the targeted treatment of prostate cancer (Fitzgerald et al. 2016). In another pioneering work, Zhang and co-investigators designed small hairpin ribonucleic acid-loaded polymeric nanoparticles and used them as folate receptor-targeted nanoparticles by utilizing a folate-targeted H1 nanopolymer for hairpin ribonucleic acid delivery. Herein, androgen receptor-targeted hairpin ribonucleic acid significantly suppressed prostate cancer growth with a prolonged survival rate in hormone-independent prostate cancer tumor-bearing mice. This study confirmed that the nanoparticles inhibited deoxyribonucleic

acid damage repair signaling pathways. These nanoparticles increased the sensitivity of hormone-independent prostate cancer to radiotherapy. Therefore, developed nanoparticles can be preferred as a radiosensitizer in hormone-independent prostate cancer, which can maintain superior treatment aptitude. In the future, such therapy can be combined with dose-escalated radiotherapy for better treatment against treatment-resistant prostate cancer (Zhang et al. 2017b).

Dhas and colleagues developed stable, nanosized bicalutamide-loaded folic acid conjugated chitosan functionalized poly (lactic-*co*-glycolic acid) nanoparticles through the nanoprecipitation method for prostate cancer management. In brief, folic acid and chitosan conjugate were fabricated to coat nanoparticles, which sustained the release of the drug for 120 h. Besides, the cell proliferative assay was confirmed by the dose-dependent cell cytotoxicity. Herein, folic acid functionalization enhanced the folate receptor binding ability of developed nanoparticles. Furthermore, developed nanoparticles accomplished remarkable cell viability (56.4%) than non-functionalized nanoparticles (43.8%). Overall, the cell proliferative assay confirmed that nanoparticles enhanced the therapeutic efficacy and demonstrated higher cell cytotoxicity as compared to plain drug and non-functionalized nanoparticles (Dhas et al. 2015). Huerta and co-authors developed spherical, nanosized, and stable nimesulide-loaded nanoparticles prepared from poly (lactic-*co*-glycolic acid) and eventually coated them with chitosan through the emulsion-solvent evaporation method. In brief, stronger interaction with the cell membrane resulted due to the positively charged surface, which further helped to increase the *in vitro* nanoparticles uptake. Besides, prepared polymeric nanoparticles displayed remarkable PC-3 cell growth inhibition. Furthermore, nanoparticles showed interference in the permeability of nimesulide, and consequently resulted in low carrier cytotoxicity, whereas it preserved the overall pharmacological potential of the encapsulated active. Hence, nanoparticles would be a co-adjuvant therapy for prostate cancer treatment, that can offer the targeted delivery through intratumor injection (Huerta et al. 2015). Therefore, solid polymeric nanoparticles can be preferentially used for prostate cancer treatment through passive as well as active targeting due to their tunable and versatile properties and remarkable merits over the conventional carriers.

16.4.2 Conjugated Polymeric Nanoparticles

As per literature, docetaxel appears to be a reliable therapy option that improves metastatic castration-resistant prostate cancer longevity and the quality of life of patients. While clinical trials with docetaxel-based therapy have been successful, total estimated toxicity and drug resistance restrict their therapeutic applications over the long-term period. In 2014, Hoang et al. evaluated the efficacy and safety of docetaxel through bone metastatic models of castration-resistant prostate cancer and subcutaneous by reformulating the docetaxel and poly (ethylene glycol) conjugated carboxymethylcellulose polymeric platform or cellax. In brief, carboxymethylcellulose was modified via acetylation, which offered superior conjugation ability,

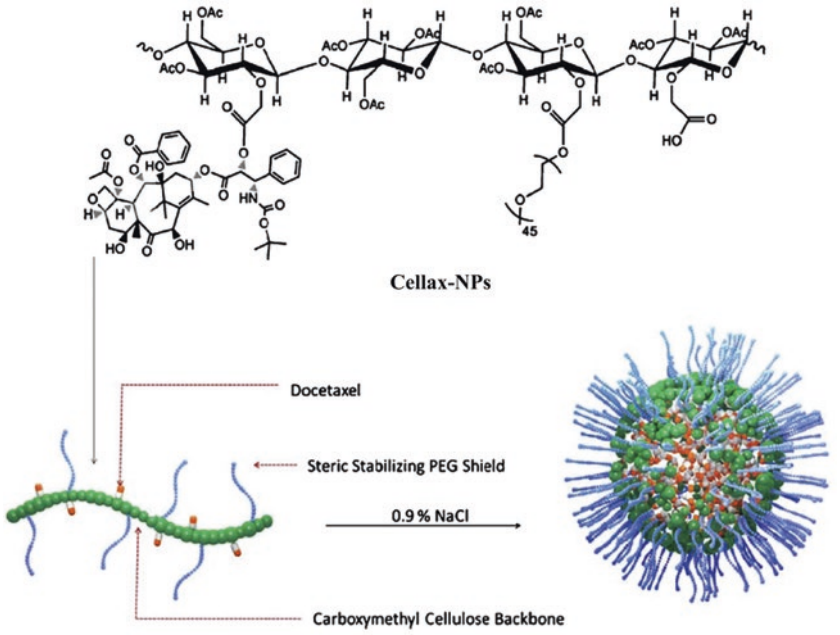

Fig. 16.5 Cellax polymer synthesis that contains poly (ethylene glycol) (PEG) as a steric stabilizing shield. Development of cellax nanoparticles (cellax NPs) followed by loading of docetaxel for prostate cancer management has been reported. At first, carboxymethylcellulose has been modified using the acetylation method that gives the superior conjugation ability. The acetylated carboxymethylcellulose, docetaxel, and poly (ethylene glycol) have been used to develop cellax. Finally, the cellax nanoparticles have been developed through a controlled nanoprecipitation method in presence of sodium chloride (NaCl). Reprinted with permission of "Docetaxel–carboxymethylcellulose nanoparticles display enhanced anti-tumor activity in murine models of castration-resistant prostate cancer", Elsevier, from (Hoang et al. 2014)

high yield, solvent solubility, and conjugation reproducibility. The acetylated carboxymethylcellulose, docetaxel, and poly (ethylene glycol) were used to develop cellax. The cellax nanoparticles were developed through a controlled nanoprecipitation method (Fig. 16.5).

Encapsulating docetaxel in nanoparticles extended their pharmacokinetic properties greatly and enhanced docetaxel bioavailability. Lastly, the enhanced permeability and retention effect of nanoparticles increased the tumor concentration of docetaxel, while limiting the overall systemic toxicity. Herein, the use of polymeric nanoparticles for docetaxel delivery offered the ability to deliver an eightfold high concentration of drug to mice with a reduction in previously reported side effects. It indicated the fact that cellax exhibited negligible side effects contrary to native molecules. In addition, cellax improved two to threefold survival rate followed by a notable increment in quality life of the mouse (model: prostate cancer metastases to bone). These findings offered a good promising viewpoint on the future translation of cellax to enhance metastatic castration-resistant prostate cancer treatment (Hoang et al. 2014).

Following docetaxel failure, cabazitaxel was recommended for metastatic castration-resistant prostate cancer, but still, the survival enhancement was only modest. Hoang et al. developed polymeric cabazitaxel conjugated nanoparticles for metastatic castration-resistant prostate cancer using cellx as a polymer that formed cabazitaxel conjugated cellax polymeric nanoparticles. Herein, owing to the high drug loading ability, biocompatibility, and safety of carboxymethylcellulose, carboxymethylcellulose-centered polymeric nanoplatform was synthesized. Interestingly, it reduced the overall toxicity due to the sustained release of cabazitaxel. Without initial burst release, polymeric nanoparticles sustained the cabazitaxel release in serum (10%/day). Consequently, it offered a 157-fold higher delivery of cabazitaxel to the docetaxel-resistant PC3 model of prostate cancer (PC3-RES), which was a 25-fold high dose than the pure cabazitaxel. Owing to that, it exhibited superior tumor inhibition in docetaxel-resistant castration-resistant prostate cancer mice models. Prepared nanoparticles demonstrated a tremendous potential to enhance metastatic castration-resistant prostate cancer therapy over therapeutically certified cabazitaxel (Hoang et al. 2017). Thus, conjugated polymeric nanoplatform with anticancer molecules offered minimal toxicity to normal cells, controlled/sustained release, good bioavailability, and accordingly superior prostate cancer tumor inhibition potential.

16.4.3 Polymer-Lipid Hybrid Systems

The newly reported form of malignancy in males is prostate cancer and for efficient prostate cancer treatment, combined chemotherapy has proven to be a successful method. About this, Chen et al. reported the co-delivery of curcumin: cabazitaxel system, aptamer conjugated curcumin, and cabazitaxel co-delivered lipid-polymer hybrid nanoparticles using aptamer conjugated poly (lactic-*co*-glycolic acid) – poly (ethylene glycol) and L-α-phosphatidylcholine from soybean (lipid system). The designed hybrid lipid-polymer nanoparticles provided the polymeric core and lipidic shell for anticancer molecules. Besides this, the aptamer conjugation in the hybrid system offered targetability towards the prostate cancer tumor cells. Owing to the smaller nanosized dimension (200 nm) of developed nanoparticles, it exhibited appreciable cytotoxicity and longer systemic circulation. In this hybrid system, poly (ethylene glycol) offered several merits including low immunogenicity, high flexibility, low toxicity, etc. The decoration of aptamer on the surface also assisted in sustaining the release of anticancer molecules. As result, these hybrid lipid nanoparticles sustained the release of curcumin and cabazitaxel. Herein, these drug-loaded nanoparticles accomplished good cell inhibition and superior prostate cancer tumor accumulation. Additionally, the curcumin: cabazitaxel (2:5) co-delivery system confirmed the potential of a hybrid lipid polymer nanoparticles system that offered synergistic effects for prostate cancer treatment. Hence, the concurrent administration of curcumin and cabazitaxel would open a new door for the efficient treatment of prostate cancer (Chen et al. 2020).

In another research work, Chen et al. synthesized cabazitaxel loaded bombesin-polyethylene glycol-1, 2-distearoyl-sn-glycero-3-phosphoethanolamine contained hybrid lipid-polymer nanoparticles. Cabazitaxel and bombesin-polyethylene glycol-1,2-distearoyl-sn-glycero-3-phosphoethanolamine have been used as polymer conjugated targeted ligand by employing the nanoprecipitation technique. These nanoparticles containing negative surface charge assisted in reducing the toxicity, which was important for prostate cancer therapy. The positive surface load enabled the hybrid lipid-polymer nanoparticles to efficiently get associated with the prostate cancer cell surface, promote cell penetration and facilitate the process of internalization. *In vitro* cytotoxicity of hybrid lipid-polymer nanoparticles demonstrated that the prepared nanoparticles were more efficient than the native form when evaluated in prostate cancer cell lines (LNCaP cells). Additionally, nanosized polymeric nanoparticles were conducive to passive tumor targeting due to enhanced permeability retention effects that resulted in successful tumor accumulation of nanosized polymeric nanoparticles. In general, the nanoparticles provided cancer cells with cabazitaxel sufficient to enhance the anti-tumor efficacy (Chen et al. 2016).

Recently, Yeh et al. also reported the synthesis of doxorubicin and vinorelbine-loaded liposomes. Doxorubicin and vinorelbine-loaded liposomes have been prepared through a thin lipid method and were further functionalized with SP204-polyethylene-1, 2-distearoyl-sn-glycero-3-phosphoethanolamine to form doxorubicin and vinorelbine loaded liposomes. Interestingly, surface-engineered nanoparticles demonstrated enhanced intracellular drug delivery with superior cytotoxicity due to the functionalization of the SP204 targeted peptide. Overall, targeted nanoparticles were observed to have remarkable antitumor activity as well as promoted the overall survival rate in the studied mice. Hence, targeted functionalized polymeric nanoplatform with the lipidic system had notable potential in engineering targeted drug delivery for the management of prostate cancer (Yeh et al. 2016). Concisely, a polymer-lipid hybrid system may be an appropriate candidate for intracellular anticancer drug delivery in prostate cancer management and other cancer treatment.

16.4.4 Polymeric Micelles

Polymeric micelles have been explored for the maintenance of therapeutic dosage levels at specific sites. Furthermore, polymeric micelles have covalent interlinking in the center and shell to enhance structural integrity and excellent stability. Feng et al. accomplished the synthesis of prostate-specific membrane antigen targetted nanosized and stable prostate cancer-binding peptide (modified glycol chitosan lipoic acid) based on docetaxel micelle using prostate cancer binding peptide modified glycol chitosan – lipoic acid conjugate and docetaxel using emulsion/solvent evaporation method (Fig. 16.6). Owing to cross-linking among composites, micelle offered a slower docetaxel release than the plain micelle. Additionally, the targeted micelle was able to achieve higher cellular uptake and cytotoxicity as compared to the non-targeted micelle. Furthermore, micelle showed stronger anticancer activity

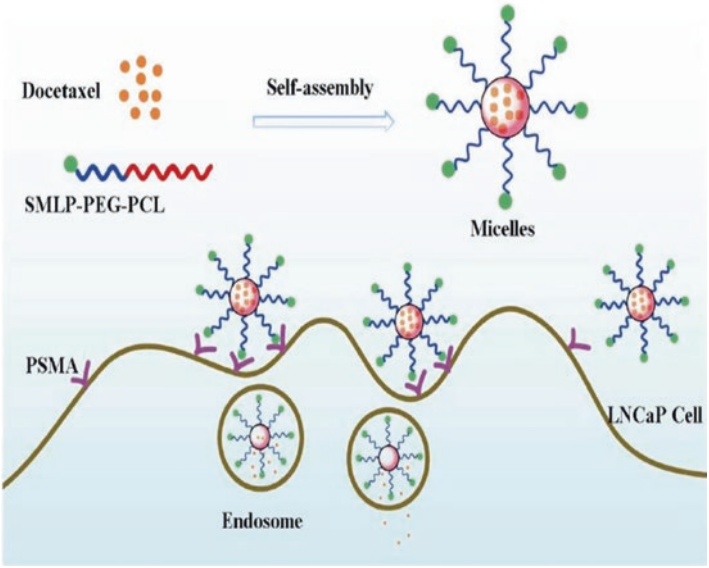

Fig. 16.6 Prostate-specific membrane antigen (PSMA) ligand conjugated docetaxel loaded small molecular ligand of PSMA-poly (ethylene glycol)-polycaprolactone (SMLP-PEG-PCL) micelle for prostate cancer cells (LNCaP cells) targeted therapy. Herein, the conjugation of the targeted ligand in docetaxel micelles shows the targeted delivery of docetaxel micelle into cancer cells as well as high cellular uptake. Reprinted with permission of "PSMA ligand conjugated poly(ethylene glycol)-polycaprolactone polymeric micelles targeted to prostate cancer cells", PLoS ONE from (Jin et al. 2014)

against LNCaP tumor xenograft models. Therefore, this combination study presented a novel perspective of effectively launching innovative targeted plus cross-linked polymeric micelles for delivery of a lipophilic anticancer agent for effective cancer treatment (Feng et al. 2019).

In another pioneering work, Jin et al. designed the prostate-specific membrane antigen ligand conjugated docetaxel-polycaprolactone-polymeric -micelle for prostate cancer targeted therapy. The formulated stable and nanosized polymeric micelle accomplished the sustained release of docetaxel with remarkable stability over 7 days. As the ester group of polycaprolactone was liable to an acidic environment, the docetaxel release was found to be slow in pH 7.4 than the pH 5.5. Furthermore, the conjugation of the targeted ligand in micelles offered a lower IC_{50} value as compared to the non-targeted micelles. Besides, it also achieved a fivefold higher cellular uptake. The observed results indicated the fact that the prepared nanosized micelle can be used for the targeted delivery of anticancer agents in prostate cancer treatment (Jin et al. 2014).

Repeated therapies involving chemical agents usually fail to achieve the required therapeutic efficacy because of cancer stem cells and micro RNA-regulated chemoresistance. The expression of micro RNAs can alter because of defective signaling pathways, including hedgehog signals. Previously published literature claimed that

the concurrent administration of cyclopamine and paclitaxel successively inhibited the paclitaxel resistance cells. In 2017, a published pioneering study assembled the poly(ethylene glycol)-*block*-poly(2-methyl-2-carboxylpropylene carbonate-paclitaxel – graft dodecanol) and poly(ethylene glycol)-*block*-poly(2-methyl-2-carboxylpropylene carbonate- cyclopamine – graft dodecanol) polymer-drug conjugates using polyethylene glycol-*block*-poly(2-methyl-2-carboxylpropylene carbonate-graft dodecanol) into polymeric- cyclopamine @ polymeric- paclitaxel assembled micelles through film hydration method. Polymeric – cyclopamine @ polymeric – paclitaxel micelles achieved good stability, which offered lower burst release of cyclopamine and paclitaxel at pH 5.3 and 7.4. Furthermore, polymeric-cyclopamine @ polymeric- paclitaxel micelles containing ester bonding and amide bonding showed excellent stability at neutral pH which resulted in lower drug release (but not significant). Furthermore, the modification in pH from 7.4 to 5.3 resulted in the cleavage of both the abovementioned bodings in the prepared micelle. Interestingly, the polymeric- cyclopamine @ polymeric- paclitaxel micelles suppressed the prostate cancer tumor colony generation. Moreover, it also inhibited the hedgehog signaling plus upregulated prostate cancer tumor suppressor micro RNAs. The polymeric- cyclopamine @ polymeric- paclitaxel micelles therapy demonstrated significant prostate cancer growth inhibition in the orthotopic prostate tumors in selected mice. In the future, this combination i.e. polymeric – cyclopamine @ polymeric – paclitaxel micelles could be used for combating chemoresistance in prostate cancer therapy (Yang et al. 2017).

Liu and Huang synthesized brush type biodegradable and G3-C12 modified poly (oligo (ethylene glycol) mono-methyl ether methacrylate-co-G3-C12)-g-poly(e-caprolactone) using R-opening polymerization, reversible addition-fragmentation transfer polymerization, and polymer post-functionalization. Further, poly (oligo (ethylene glycol) mono-methyl ether methacrylate-co-G3-C12)-g-poly(e-caprolactone) was used for the delivery of bufalin that helped in castration-resistant prostate cancer-centered targeted treatment. The biocompatible and biodegradable micellar nanoparticles offered controlled release (about 62% after 24 h) of bufalin at pH 7.4 due to the degradation of esterase in the micelle. Further, it improved the overall anticancer efficacy in both studies. Herein, the G3-C12 incorporation in nanoparticles enhanced the inhibition of tumor growth ($P < 0.05$) in contrary to non-targeted nanoparticles. Owing to more cellular apoptosis and improved anticancer efficacy, these prepared micellar nanoparticles could be used as a potential substitute for castration-resistant prostate cancer treatment (Liu and Huang 2016). Therefore, in the future, polymeric micelle could be a choice for prostate cancer therapy due to the maintenance of therapeutic dosage levels at specific sites.

16.4.5 Polyplexes

In an attempt to comprehend the involvement of micro ribonucleic acid *in vivo* and to support tailored gene therapy for micro ribonucleic acid-associated prostate cancer, new developments in convenient micro ribonucleic acid delivery techniques

using prostate cancer-driven nanoparticles have presented crucial details. In this esteem, Zhang and co-authors designed the polyarginine peptide (R11) – branched disulphide polyethyleneimine (SSPEI)-micro RNA-145 nanocarrier for the targeted delivery of micro RNA-145. In brief, R11 conjugated SSPEI (R11- SSPEI)-micro RNA-145 demonstrated bioactivity at a wide concentration range. Furthermore, it demonstrated the dose-dependent reduction in the reported signal that confirmed the functional action of micro RNA-145. The systemic administration of prepared polyplexes accomplished the dramatic inhibition of prostate cancer tumor growth followed by prolonged survival duration (Zhang et al. 2015). In the upcoming days, the established targeted polyplexes for systemic administration could be explored as potential nanocarriers for prostate cancer therapeutic utilization.

16.4.6 Miscellaneous Polymeric Nanoparticles

The life-threatening systemic cytotoxicity of approved anticancer agents in normal cells/tissues is among the significant issues for impactful cancer chemotherapy. Plentiful literature divulged the polymeric nanocapsules that offered astonishing merits including the protection of enzymatic degradation of active molecules, the stability of drug molecules, excellent loading of the hydrophobic drug (in the oily core of nanocapsules), etc. Additionally, it modified the pharmacokinetics of anticancer agents and lowered normal cell toxicity. In 2020, the Shitole group developed quercetin and docetaxel-based polymeric nanocapsules using luteinizing-hormone-releasing hormone as an active target), poly (ethylene glycol) (as a spacer), and poly (lactic-*co*-glycolic acid) through interfacial deposition method with an optimized molar ratio of docetaxel: quercetin (1:3). Herein, polymeric nanocapsules offered the sustained release of active and poly (ethylene glycol) provided stability to nanocapsules in serum. Prostate cancer cell lines (PC-3 and LNCaP) confirmed the targetability of the luteinizing hormone-releasing hormone. Besides, the combination of docetaxel: quercetin incorporated polymeric nanocapsules showed superior cell inhibition activity than the single drug-loaded nanocapsules.

Further, polymeric nanocapsules exhibited higher cell toxicity than non-targeted polymeric nanocapsules. Therefore, optimized polymeric nanocapsules may be a preferential choice for the successful treatment of prostate cancer (Shitole et al. 2020). Concisely, the employment of polymeric nanocapsules in the domain of prostate cancer treatment has shown to exhibit a transformative effect. Additionally, it also improved the stability of anticancer molecules viz. peptides, hydrophobic agents, etc. The high surface area of polymeric nanoparticles permits the superior loading of anticancer agents and also allows for targeted delivery with minimal leakage of drugs in the biological environment. Architectured polymeric nanoplatforms targeted treatments of prostate cancer have been summarized in Table 16.1.

Table 16.1 Architectured polymeric nanoplatforms targeted treatment of prostate cancer

Sr. no.	Anti cancer agent	Polymer name	Synthesis method	Particle size (nm)	Zeta potential (mV)	Polydispersity index	Prostate cancer cell lines	Features	References
Solid polymeric nanoparticles									
1.	Resveratrol	Poly(lactic-*co*-glycolic acid)	Nanoprecipitation method	202.8	–	0.17	LNCaP	It exhibited greater cytotoxicity to LNCaP cells and no adverse cytotoxic effects.	Nassir et al. (2018)
2.	Tumor-associated antigens	Hydroxypropyl methylcellulose	Spray dryer	4920	7.92	–	–	It reduced the tumor volume and offers a reduction in T-regulatory cells.	Parenky et al. (2019)
3.	Docetaxel	Poly (lactic-*co*-glycolic acid), poly (ethylene glycol)	Solvent diffusion method	200	–	–	PC3, LNCaP, PC3M, LNCaP-AI	It increased the accumulation of docetaxel and reduced prostate tumor mass and viability	Bharali et al. (2017)
4.	Resveratrol, docetaxel	Polycaprolacto, poly (ethylene glycol)	Planetary ball milled	36.6	−40.5	–	PC3 and PC3-R	It demonstrated a significant enhancement in cell apoptosis and confirmed the elevated expression of the folate receptor.	Singh et al. (2018)
5.	Ribonucleic acid interference	Cyclodextrin	–	200	Neutral	–	LNCaP, PC3	It confirmed that the folate-targeted cyclodextrin is a suitable carrier for small interfering ribonucleic acid complexes.	Evans et al. (2017)
6.	small interfering RNA	Cyclodextrin	–	288.90	+10.28	0.395	DU145	It targeted nanoparticles offers the small interfering ribonucleic acid high levels and cellular uptake	Fitzgerald et al. (2016)

7.	Small hairpin RNA	β-Cyclodextrin	Electrostatic interaction	181.10	32.80	–	PC3, 22Rv1	It significantly suppressed prostate cancer tumor growth and demonstrates the inhibition of prostate cancer cell growth, increased cell apoptosis, cell cycle arrest, etc.	Zhang et al. (2017b)
8.	Bicalutamide	Chitosan	Nanoprecipitation method	206.90	+21.7	–	DU 145 (human)	It offers tissue/cell-targeting or site-specific delivery.	Dhas et al. (2015)
9.	Nimesulide	Chitosan	Emulsion solvent evaporation method	393	+10	–	PC-3	It achieved the highest cytotoxic effect with drug-loaded nanoparticles on PC-3 cells	Huerta et al. (2015)
Conjugated polymeric nanoparticles									
10.	Docetaxel	Cellax-nanoparticles	Controlled nanoprecipitation process	122	−0.8	0.14	Human PC3	It enhanced the docetaxel efficacy and safety and bone metastatic mice models of castration-resistant prostate cancer.	Hoang et al. (2014)
11.	Cabazitaxel	Cellax nanoparticles	Nanoprecipitation process	96	–	0.115	PC3-RES	It showed superior tumor inhibition in the docetaxel-resistant castration – resistant prostate cancer model and improved long-term survival rate (70%)	Hoang et al. (2017)

(continued)

Table 16.1 (continued)

Sr. no.	Anti cancer agent	Polymer name	Synthesis method	Particle size (nm)	Zeta potential (mV)	Polydispersity index	Prostate cancer cell lines	Features	References
Polymer-lipid hybrid system									
12.	Cabazitaxel, curcumin	Polymer-lipid hybrid	Nanoprecipitation method	121.3	−23.5	0.15–0.19	LNCaP	It showed good cell inhibition ability and high tumor accumulation, which provides remarkable tumor inhibition efficiency.	Chen et al. (2020)
13.	Cabazitaxel	Polymer-lipid hybrid	Nanoprecipitation Method	184.9	26.5	–	LNCaP	It exhibited remarkable anti-tumor activity.	Chen et al. (2016)
14.	Doxorubixin, vinorelbine	Polymer-lipid hybrid	Thin lipid method	–	–	–	PC3	It improved the overall doxorubicin and vinorelbine therapeutic efficacy via increasing tumor apoptosis, reducing tumor angiogenesis.	Yeh et al. (2016)
Polymeric micelles									
15.	Docetaxel	Modified chitosan	Emulsion/solvent evaporation method	397	–	0.213	LNCaP	It exhibited higher cellular uptake, cytotoxicity, and stronger anticancer activity against LNCaP.	Feng et al. (2019)
16.	Docetaxel	Polycaprolactone	–	50.50	–	0.044	LNCaP	It achieved a much lower IC_{50} and exhibited a fivefold higher fluorescence intensity.	Jin et al. (2014)

17.	Paclitaxel, cyclopamine	Micelles	Film hydration method	–	–	–	DU14, PC3	It inhibited hedgehog signaling and suppressed the prostate cancer tumor colony formation.	Yang et al. (2017)
18.	Bufalin	Modified poly caprolactone	Common solvent approach	66.10	−13.45	0.15	DU145	It exhibited good biocompatibility, biodegradability, and improved anticancer efficacy, and exhibited more cellular apoptosis.	Liu and Huang (2016)
Polyplexes									
19.	miR-145	Branched polyethyleneimine	–	240	−28.7	0.26	PC3, LNCAP	It provides the dramatic inhibition of prostate tumor growth followed by prolonged survival duration.	Zhang et al. (2015)
Miscellaneous									
20.	Docetaxel, quercetin	Poly (lactic-*co*-glycolic acid), Poly (ethylene glycol)	Interfacial deposition method	120–150	−20 to −40	0.2	PC-3, LNCaP	It enhanced cell inhibition activity and showed higher cell cytotoxicity, caspase-3 activity.	Shitole et al. (2020)

16.5 Stimuli-Responsive Polymeric Nanoparticles

The exploitation of biodegradable polymer-centered architectured nanocomposites (i.e. polymeric nanoparticles) as emerging drug delivery vehicles has received considerable interest in recent years. Regrettably, albeit with architectured polymeric nanocarriers at the prostate cancer tumor site, bioavailability, and release of drugs seemed difficult to control. Fascinatingly, these polymeric nanoparticles are believed to be potentially efficient therapeutic carriers to respond to inimitable external stimuli (Alsehli 2020). Therefore, to deal with this predicament, effective methodologies have been preferred to synthesize a stimulus (viz. light, temperature, pH, redox potential, etc.) responsive polymer nanocarrier, which demonstrates the response to prostate tumor-containing stimulus reactions (Fig. 16.7).

Briefly, the literature reported the fact that the altered pH value observed under pathologic conditions (acidic pH of prostate tumor microenvironment) has been widely preferred to encourage anticancer molecules to be released into a target

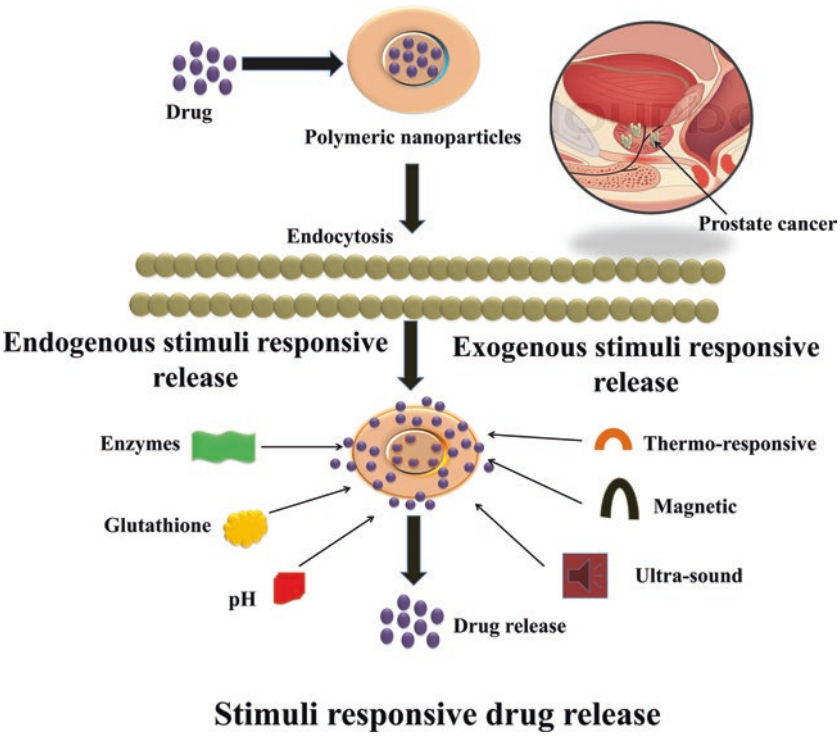

Fig. 16.7 Endogenous and exogenous stimuli-responsive polymeric nanoparticles for prostate cancer management. Effective methodologies have been preferred to synthesize endogenous stimuli enzymes, glutathione, pH, etc. for release of drug to the targeted site. In another case, exogenous stimuli mainly light, temperature, and ultrasound have been reported as responsible for drug release to the targeted site of prostate cancer

biological or intracellular organ of prostate tumor (Mura et al. 2013). Similarly, in the case of temperature, due to their phase-transition compliance concerning temperature variations, temperature-responsive polymers have been extensively examined for smart drug delivery in cancer management. In this perspective, the temperature functions as an external/internal stimulus (Mohammed et al. 2018). A responsive nanoarchitecture polymeric nanocarrier offers the switchable release of anticancer molecules. In this, the actual difference in oxidizing extracellular and reducing intracellular space potential furnishes the triggered (controlled/sustained) release of anticancer molecules (Zhang et al. 2017a).

As we know, prostate cancer is among the most aggressive diseases and is distinguished by the over-expression of various enzyme forms, which are either connected to or secreted by the membrane (Barve et al. 2014). The utilization of enzymes (natural biological) as triggers to activate anticancer molecule release by weakening certain bonds to disassociate the architecture of polymeric nanoparticles structures thereby offering new approaches to prostate cancer management (Barve et al. 2014; Isaacson et al. 2017).

16.5.1 pH-Responsive Polymeric Nanoparticles

Literature referred to the fact that ginsenoside compound K (protopanaxadiol metabolite) has noteworthy anticancer activity. Not withstanding these characteristics, ginsenoside compound K's weak low solubility and low permeability dramatically reduces its efficiency and thereby restricts its therapeutic use. In, 2020 Zhang et al. developed the ginsenoside compound K – *O*-carboxymethyl chitosan nanoparticles using ginsenoside compound K and *O*-carboxymethyl chitosan through the ionic cross-linking method. Further, these prepared spherical polymeric nanoparticles demonstrated the remarkable particle size and polydispersity index, that helped for permeability enhancement. The negative zeta potential of nanoparticles indicated the superior stability of nanoparticles in dispersion. Besides, nanoparticles exhibited the pH-dependent (pH: 5.8) sustained drug release (58.56%, up to 96 h), which may be due to the protonation of *O*-carboxymethyl chitosan containing amino groups present in lower acidic pH. Furthermore, nanoparticles enhanced the cytotoxicity and cellular uptake of ginsenoside compound K in selected prostate cancer cell lines (PC3 cells). Additionally, nanoparticles boosted caspase-3 and caspase-9 activities. Therefore, a polymeric nanoplatform involving nanoparticles would be an exceptional nanocarrier for effective prostate cancer management (Zhang et al. 2020).

In 2017, Yan et al. reported the synergistic effect of epidermal growth factor receptor peptide also known as GE11 targeted and pH-responsive co-delivery of docetaxel and curcumin for prostate cancer management. In this context, GE11 conjugated poly lactic-*co*-glycolic acid –poly (ethylene glycol) conjugate was synthesized via cross-linking of GE11, polylactic-*co*-glycolic acid – poly (ethylene glycol) – maleimide. Curcumin and docetaxel-loaded G11 nanoparticles were

prepared using poly (lactic-*co*-glycolic acid) – poly (ethylene glycol) – epidermal growth factor receptor peptide, GE11 conjugated poly (lactic-*co*-glycolic acid) – poly (ethylene glycol), and docetaxel via solvent displacement method. Herein, nanoparticles demonstrated 85.2% of the cumulative release of curcumin, which followed a sustained release at pH 5. Whereas, docetaxel release was found to be mild pH-dependent. Interestingly, the engineered nanoparticles containing cis-aconitic anhydride bonds helped to enable the release of curcumin/docetaxel from prepared nanoparticles via bond cleavage at pH < 6.5. Furthermore, it improved the cancer cell inhibition rate and tumor tissue growth. Hence, docetaxel and curcumin prodrug can open a novel perspective for prostate cancer treatment through concurrent GE 11 targeting along with enhanced permeability and retention effect of polymeric nanoparticles (Yan et al. 2017).

Reports cited the fact that usage of curcumin is limited for treating cancer because of its quick oxidative degradation, causing poor stability and bioavailability, in physiological conditions. In 2015, Thangavel and co-authors had developed curcumin encapsulated nanoparticles, which additionally provided the radical scavenger effect. Interestingly, pH-sensitive curcumin encapsulated pH-sensitive redox nanoparticle were designed using self-assembling methoxy-poly (ethylene glycol)-b-polylactide (i.e. amphiphilic block co-polymers) employing poly (ethylene glycol) – b – poly [4-(2,2,6,6-tetramethylpiperidine-1-45oxyl) aminomethylstyrene]) conjugated with reactive oxygen scavenging nitroxide radicals. They claimed that the entrapment of curcumin and nitroxide radicals suppressed the curcumin degradation in physiological conditions. Herein, drug release from nanoparticles at pH 7.4 was found to be slower and 60% of the curcumin remained encapsulated in micelle even at 72 h. Furthermore, nanoparticles induced strong apoptosis than native curcumin. Besides, prepared nanoparticles increased the bioavailability and significant reactive oxygen species scavenging activity that resulted in the suppression of prostate cancer tumor growth. Therefore, prepared nanoparticles can be a suitable alternative to overcome the aforementioned limitations to heighten their overall therapeutic potential (Thangavel et al. 2015).

Pearce and co-authors engineered the glutamate urea hyper branched polymer conjugated-doxorubicin-polymeric microparticles using glutamate urea as a prostate-specific membrane antigen targeting ligands and poly (ethylene glycol) hyper branched polymer. The loading of doxorubicin onto hyper branched polymer through hydrazone formation offered the controlled release of doxorubicin. These nanoparticles exhibited the pH-dependent (simulated endosomal conditions) *in vitro* release (90%) over 36 h. In total, this developed hyperbranched polymer confirmed the feasibility of controlled plus stimuli-responsive doxorubicin delivery for prostate cancer treatment. Herein, the hydrolysis of the hydrazone bond offered doxorubicin release in the endosome, which imparted cytotoxicity and triggered apoptosis.

Interestingly, after conjugation of ligand on the surface of the hyperbranched polymer, the developed polymeric nanoparticles demonstrated the concentration and time-dependent cellular uptake. Therefore, the hyperbranched polymeric

approach is an exceptional theranostic for prostate cancer treatment and there is a requisite to explore it in clinically relevant models (Pearce et al. 2017). Despite the merits of established methods and technologies, resistance in cancer therapy is still challenging for scientific fraternities. In recent decades, zein (plant protein) is majorly preferred for designing tunable drug delivery as a carrier form.

In 2017, Thapa et al. designed a dual drug-loaded polymeric nanoplatform for the treatment of metastatic prostate cancer. Herein, stable, nanosized vorinostat and bortezomib zein polymeric nanoparticles have been prepared by phase separation method that demonstrated controlled pH-dependent release in respective *in vitro* dissolution media. Besides this, polymeric nanoparticles showed higher cellular uptake, remarkable cytotoxicity with apoptosis. The drug-loaded nanoparticles exhibited towering anti-migration effect and pro-apoptotic protein induction, which confirmed the overall effectiveness of prepared polymeric nanoparticles in prostate cancer treatment. Owing to minimal toxicity and enhanced *in vivo* antitumor effects, it opened up a new vista for quality and effective prostate cancer treatment (Thapa et al. 2017). Therefore, pH-responsive polymeric carriers may be a suitable alternative that could assist in the release of anticancer agents at prostate cancer tumor/targeted site.

16.5.2 Enzyme-Responsive Polymeric Nanoparticles

The engineering of poorly soluble small anti-cancer molecules in polymeric prodrug form has grown into extremely promising themes in androgen-independent prostate cancer management. Yuan et al. synthesized the esterase-sensitive galectin 3 binding peptide-mediated esterase-sensitive tumor-targeting polymeric prodrug of camptothecin using camptothecin, oligo (ethylene glycol) monomethyl ether methacrylate for the treatment of prostate cancer. Briefly, in oligo (ethylene glycol) monomethyl ether methacrylate -co- camptothecin -co- galectin 3 binding peptide, camptothecin was linked using a β-thioester bond (i.e. esterase responsive bond). The obtained polymeric nanoparticle showed ~77% camptothecin release (24 h) in the presence of esterase and about 20% cumulative camptothecin (within 24 h) in absence of esterase. Esterase offered the hydrolysis of the β-thioester bond and released the camptothecin from the polymeric nanoparticles. Besides, attachment between polymers to galectin 3 binding peptides performed a pivotal role in boosting cytotoxicity by actually targeting the galectin-3 receptor. The multifunctional polymeric nanoparticles of camptothecin reported better aqueous solubility, remarkable stability, greater intracellular penetration, and increased cytotoxicity in DU145 cells *in vitro* as compared to native camptothecin. Due to the enhanced permeability and retention effect and galectin 3 binding peptide-mediated tumor targeting, it showed superior anticancer efficacy and diminished *in vivo* toxicity. The study

illustrated that in the management of androgen-independent prostate cancer, polymeric nanoparticles of camptothecin may be an impressive polymeric prodrug (Yuan et al. 2020).

Literature reported that cabazitaxel is a novel inhibitor for the treatment of metastatic castration-resistant prostate cancer. It is a 2nd generation, highly promising taxane compound. Unfortunately, it had solubility and targetability issues which restricted its therapeutic uses. Barve et al. developed the enzyme responsive cabazitaxel polymeric-micelle using 2-[3-(1,3-dicarboxypropyl)ureido]pentanedioic acid (i.e. targeting ligand) and cabazitaxel for successful prostate cancer treatment. In the micelle formulation, enzyme responsive 2-[3-(1,3-dicarboxypropyl)ureido]pentanedioic acid was cleavable due to the presence of the matrix metalloproteinase-2 enzyme. Generally, matrix metalloproteinase-2 is overexpressed in the prostate cancer tumor microenvironment which may favor the design of enzyme responsive drug delivery systems. Fascinatingly, micelle demonstrated very low critical micelle concentration, high entrapment, and drug loading ability. Furthermore, it exhibited enzyme-dependent release of cabazitaxel from polymeric micelle due to the cleavage of the 2-[3-(1,3-dicarboxypropyl)ureido]pentanedioic acid bond. Remarkably, polymeric micelle furnished remarkable cellular uptake of cabazitaxel in prostate cancer than the free cabazitaxel molecules. Hence, polymeric micelle showed exceptional tumor growth inhibition ability in mice than the free as well as a plain micelle. Therefore, cabazitaxel polymeric micelle may be a potential substitute for advanced prostate cancer therapy (Barve et al. 2020). Concisely, an enzyme responsive architectured polymeric nanoplatform would be an outstanding class of drug delivery carrier for stimuli-responsive delivery of anticancer molecules.

16.5.3 Ultrasound-Triggered Polymeric Nanoparticles

Literature reports claimed the fact that ultrasound has demonstrated notable impacts in enhancing the delivery of cancer therapy in conjunction with microbubbles. In 2020, Fagerland and colleagues reported the preparation of cabazitaxel-loaded nanoparticles using PEGylated poly[2-ethyl-butyl cyanoacrylate known as cabazitaxel –poly (ethylene glycol)- poly[2-ethyl-butyl cyanoacrylate nanoparticles using the miniemulsion polymerization method. Herein, prepared nanoparticles were used with microbubbles and ultrasound to reduce the tumor size and prostate volume in the preferred transgenic adenocarcinoma of the mouse prostate model. The intravenous administration of prepared nanoparticles resulted in the accumulation of cabazitaxel in the spleen and liver as compared to pure cabazitaxel. Unfortunately, the application of ultrasound and microbubbles combination with nanoparticles did not exhibit any significant difference in overall therapeutic performance including histology grading or proliferation (Fagerland et al. 2020). Therefore, in the future, the optimization of ultrasound quality attributes may assist to improve the entire presentation of anticancer ultrasound-triggered therapy.

16.5.4 Dual-Responsive Polymeric Platforms

The rise in cancer stem cells is directly associated with chemoresistance, which stymies prosperous chemotherapy. Synergistic treatments (a combination of anticancer agents) for both bulk tumor cells and cancer stem cells have proven effective to reverse chemoresistance and treat prostate cancer. In recent attempts, Lin and co-researchers developed the dual responsive (pH and enzyme sensitive) polymeric nanocarrier for docetaxel and rubone (miR-34 activator gene) to the cancer stem cells targeting and taxane resistant prostate cancer treatment. They prepared docetaxel-loaded rubone prodrug micelle by encapsulating docetaxel into poly (diisopropylaminoethanol) (i.e. pH-responsive) and rubone prodrug conjugate with polycarbonate (i.e. glutathione responsive). Herein, the prepared micelle demonstrated remarkable stability and offered to target ability by the enhanced permeability and retention effect. Furthermore, micelle underwent disassembly/expansion due to the poly (diisopropylaminoethanol) protonation. Moreover, glutathione-induced disulfide bond cleavage in the acidic microenvironment of endocytic vesicles. Owing to this, it resulted in the rapid release of rubone and docetaxel into the cytoplasm of prostate cells. Finally, rubone up-regulated the miR-34a gene and accordingly increased the sensitivity of tumor cells towards the docetaxel. The distribution of several other lipophilic anticancer drugs for prostate cancer management can be accomplished by using this dual-sensitive approach (Lin et al. 2019). Overall, owing to its ability to boost the bioavailability of drugs in the prostate cancer tumor area, the resulting stimulation-responsive architectured polymer-centered nanocarriers have been extremely effective in prostate cancer management. Advances in endogenous and exogenous stimuli-responsive architectured polymeric nanoplatforms targeted treatments of prostate cancer have been summarized in Table 16.2.

16.6 Advances in Polymeric Nanoparticles for Prostate Cancer

16.6.1 Polymer-Based Superparamagnetic Nanoparticles

Recently, nanoscopic therapeutic systems integrating therapeutic agents, molecular targeting, and imaging functionality have taken on a growing dynamism and demonstrated considerable therapeutic prospects. Multifunctional polymeric nanoparticles have been prepared by Fang et al., which offered remarkable merits including controlled release of docetaxel, efficient magnetic resonance imaging contrast properties, and prostate cancer target abilities, etc. Briefly, docetaxel and superparamagnetic iron oxide were encapsulated into the shell of targeted nanoparticles, which were composed of poly (lactic-*co*-glycolic acid), poly (ethylene glycol), and Wy5a-aptamer. Herein, polymeric nanoplatforms helped in the controlled release of docetaxel from polymeric nanoparticles. Additionally, it boosted the contrast-enhanced aptitude. The incorporation of Wy5a-aptamer has demonstrated the

Table 16.2 Stimuli-responsive architectured polymeric nanoplatforms targeted treatment of prostate cancer

Sr. no.	Anticancer agent	Polymer name	Synthesis method	Particle size (nm)	Zeta potential (mV)	Polydispersity index	Cell lines	Features	References
pH-responsive polymeric nanoparticles									
1.	Ginsenoside compound K	*O*-carboxymethyl chitosan	Ionic cross-linking method	173	−29.6	0.29	PC-3	It enhanced the cytotoxicity and cellular uptake, aqueous solubility, and stability of Ginsenoside Compound K.	Zhang et al. (2020)
2.	Docetaxel, curcumin	Polylactic-*co*-glycolic acid – polyethylene glycol	Solvent displacement method	166.7	−37.5	0.213	LNCaP cells	Polymeric nanoparticles sustained the release of actives and improved the inhibition rate of the prostate cancer cell.	Yan et al. (2017)
3.	Curcumin	Methoxy-poly(ethylene glycol)-b-polylactide	–	35	–	0.085	PC-3	It increased the stability of curcumin and enhanced cancer cell death by apoptosis.	Thangavel et al. (2015)
4.	Doxorubicin	poly (ethylene glycol) hyperbranched polymer	–	–	–	–	LNCaP cells	It demonstrates the pH-dependent controlled release profile and significantly reduces the subcutaneous prostate tumor volume.	Pearce et al. (2017)
5.	Vorinostat, bortezomib	Zein	Phase separation method	160	–	0.20	PC3, DU145, LNCaP	It offers a pH-dependent controlled release and exhibited minimal toxicity.	Thapa et al. (2017)
Enzyme-responsive polymeric nanoplatforms									
6.	Camptothecin	Oligo (ethylene glycol) monomethyl ether methacrylate	–	70.3	−17.7	0.26	DU145	It demonstrates superior anticancer performance and less in vivo toxicity, better water solubility, good stability, higher intracellular uptake	Yuan et al. (2020)

7.	Cabazitaxel	2-[3-(1,3-(dicarboxypropyl) ureido]pentanedioic acid	Solvent evaporation method	196	0.56	0.13	C4-2 cells.	It exhibited higher cellular uptake and better inhibition of tumor growth.	Barve et al. (2020)
Ultrasound-triggered polymeric nanoplatforms									
8.	Cabazitaxel	PEGylated poly[2-ethyl-butyl cyanoacrylate	Miniemulsion polymerization	–	–	–	–	It improved cabazitaxel delivery.	Fagerland et al. (2020)
Dual responsive polymeric platforms									
9.	Docetaxel, rubone	Poly(diisopropylaminoethanol)	Nanoprecipitation method	45.07	2.5	–	DU145-TXR, PC3-TXR	It improves the sensitizing prostate cancer tumor cells towards docetaxel and showed significant inhibition of taxane-resistant tumor progression.	Lin et al. (2019)

targeted delivery of actives to prostate cancer cells. Furthermore, *in vivo* experiments confirmed the noticeable antitumor magnetic resonance imaging ability with significantly lowering systemic toxicity. Hence, in the future, this multifunctional polymeric Wy5a-aptamer functionalized docetaxel-loaded superparamagnetic iron oxide-based polymeric nanoparticles can be preferred as an efficacious therapy for castration-resistant prostate cancer management (Fang et al. 2020).

To address systemic chemotherapy barriers, various vehicles have been created to encapsulate and distribute anticancer molecules, viz. dendrimers, polymeric nanoparticles, micelle, etc. One of the key drawbacks of such vehicles is that drug delivery and progression of treatment cannot be tracked in real-time. Awareness of the biodistribution of an anticancer molecule is essential for prostate cancer targeted application. Concisely, for the design of comprehensive substitute cancer therapies, i.e. drug carriers, which can also act as tracers and contrast reagents, are essential. Theranostic magnetic nanoparticles that simulcast both imaging agents and therapeutic agents are of significant meaning for the treatment of prostate cancer. In 2013, Wadajkar and co-authors developed the thermoresponsive polyarginine peptide modified poly[*N*-isopropylacrylamide-acrylamide-allylamine] magnetic nanoparticles using poly[*N*-isopropylacrylamide-acrylamide-allylamine] coated superparamagnetic nanoparticles followed by conjugation of prostate cancer-specific peptide for active targeting of prostate cancer tumors. Poly [*N*-isopropylacrylamide-acrylamide-allylamine] has a 40 °C critical solution temperature that provides thermo-responsive behaviors in the formulated nanoparticles. Additionally, it offers the amine functionalities for bioconjugation in nanoparticles. Further, prepared nanoparticles exhibited excellent superparamagnetic behavior before and after the conjugation of peptide (i.e. R11). It also demonstrated the remarkable biocompatibility with normal prostate cells up to 500 mg/mL concentration of nanoparticles. The cellular uptake by PC3 and LNCaP cells was found to be dose-dependent and showed more distribution in prostate cancer tumors. Herein, bioconjugation of magnetic nanoparticles declined (30%) the magnetic resonance in tumors as compared to plain poly [*N*-isopropylacrylamide-acrylamide-allylamine] magnetic nanoparticles (0%). In conclusion, the application of prepared nanoparticles offered a potential platform for targeting and monitoring prostate cancer, i.e. therapeutic and diagnostic applications (Wadajkar et al. 2013).

Eupatorin (flavonoid) showed anticancer activity, but had poor aqueous solubility, and accordingly low bioavailability. The rapid degradation of eupatorin limited its efficacy. In this pioneered work, the poly (ethylene glycol) methyl ether-*block*- poly lactic-*co*-glycolic acid @ iron oxide nanoparticles were used as a possible nanocarrier for delivery of eupatorin to DU-145 and LNCaP human prostate cancer cells. In brief, nanoparticles have been prepared by the nanoprecipitation method. The obtained nanoparticles demonstrated initial burst release in 24 h (30%) and further sustained the eupatorin release up to 200 h. Herein, coated superparamagnetic iron nanoparticles with poly (ethylene glycol) methyl ether-*block*- poly (lactic-*co*-glycolic acid) provided superior biocompatibility and increased the uptake by prostate cancer cells. The increased apoptosis and necrosis rate decline claimed that the eupatorin encapsulation in poly (ethylene glycol) methyl ether-*block*- poly lactic-*co*-glycolic acid-coated iron oxide nanoparticles accomplished the enrichment in anticancer activity in DU-145

and LNCaP human prostate cancer cell lines than the pure form of eupatorin. Hence, eupatorin encapsulated nanoparticles can be preferred as a suitable surrogate for pharmacological applications (Tousi et al. 2020).

In another study, Nagesh and colleagues have illustrated prostate-specific membrane antigen targeted cyclodextrin, pluronic F127 coated docetaxel-loaded superparamagnetic polymer-coated nanoparticles for prostate cancer treatment. In brief, cyclodextrin coating in prepared nanoparticles offered the hydrophobic cavity for the physical entrapment of hydrophobic anticancer molecules (docetaxel), whereas the coated pluronic polymeric chains offered the stability of the nanoparticles in suspension. These nanoparticles demonstrated efficient internalization of prostate cancer cells. Additionally, prostate cancer cells demonstrated the down-regulation of anti-apoptotic proteins, induction of the expression of apoptosis-associated proteins, inhibition of chemo-resistance associated protein, etc., which confirmed the anticancer potential of docetaxel loaded superparamagnetic polymeric nanoparticles. Therefore, the preparation could be appropriate for prostate cancer-targeted treatment (Nagesh et al. 2016).

The combined effect of chemotherapy and photothermal treatment provided a more accurate supply of active in the nanosystem. The literature claimed that the photothermal agents are majorly preferred for cancer thermal therapy, in which near-infrared light converts into cytotoxic heat. Owing to the astonishing properties of selenium nanoparticles including biocompatibility, anticancer efficacy, minimal toxicity, etc, it is gaining much attention from the researchers as a possible mediator and drug carrier for anticancer therapy. In 2020, Liu and co-authors reported doxorubicin incorporated poly (ethylene glycol) functionalized copper and selenium nanoparticles for prostate cancer treatment. Briefly, the hydrophilic nature of doxorubicin conjugated nanoparticles has been increased by polyethylene glycol-2000 linking on the surface of the nanoparticles that offered the admirable solubility for nanoparticles. They mentioned that near-infrared achievable selenium nanoparticles provided the heat energy which resulted in the release of encapsulated doxorubicin from polymeric nanoparticles. Further, functionalized nanoparticles offered high cellular uptake ability, superior cytotoxicity against DU145 and LNCaP cells. The prepared nanoparticles of red blood cells biocompatibility showed the dose relied on the hemolytic effect that confirmed the reduction in toxicity of the polyethylene-based nanoparticles and without poly (ethylene glycol) nanoparticles (water as positive control). Based on findings, it was stated that the functionalized nanoparticles exhibited insignificant hemolysis, which accordingly confirmed the biocompatibility of prepared nanoparticles (Liu et al. 2020).

16.6.2 Polymeric Nanoparticles Bearing Radionuclide, Magnetic Resonance Imaging Agents and Metal Nanoparticles

An abundance of research has shown that there is no successful method of treating androgen-resistant prostate cancer lethality. In 2019, Wang et al. designed the multifunctional paclitaxel-loaded pluronic- polyethyleneimine gold nanoparticles for

androgen-resistant prostate cancer treatment by incorporating organic and inorganic material with gold nanoparticles using the film emulsification method. It exploited the usage of gold nanoparticles that offered photodynamic effects, photothermal effects, chemotherapy, etc. effects. In brief, pluronic- polyethyleneimine nanoparticles helped to develop micelles, which encapsulated paclitaxel. Paclitaxel helped to arrest the prostate cancer tumor cell cycle, whereas nanoparticles demonstrated the controlled release of paclitaxel, TRPV6 cation channel blocking, amplifying the cell cycle arrest. Increasing the temperature and producing reactive oxygen species offered improved cellular toxicity as well as apoptosis. It also enhanced the tumor targeting and achieved low toxicity in liver function in androgen resistant prostate cancer treatment followed by minimal side effects to other normal body organs. Final nanoparticles offered a detailed, productive, and broad strategy for deadly androgen-resistant prostate cancer as a particular synergistic framework combining several therapeutic protocols with negative toxicity (Wang et al. 2019). "All in one" technologies should be designed for targeted, bio-consistent, and synergistic merits to battle the heterogeneity of cancer.

In 2019, Poudel et al. designed the docetaxel-coated copper sulfide nanoparticles followed by wrapping of lanreotide linked poly (ethylene glycol)-di-stearoyl phosphatidylethanolamine platform, which offered targeted delivery of docetaxel with prolonged circulation time by avoiding unwanted docetaxel release. After near-infrared irradiation, a photothermal carrier assisted in the release of docetaxel from nanoparticles to the targeted site in a regulated manner. Furthermore, compared to the non-targeted polymeric platform and free/plain anticancer drugs, the induction of apoptotic markers increased tumor aggregation and maximized growth inhibition of the tumor. Therefore, owing to the excellent results of this study, it may offer futuristic applications for promising prostate cancer treatment (Poudel et al. 2019).

Bulmahn et al. developed the multifunctional hierarchical nano-formulation using chitosan-coated poly (lactic-*co*-glycolic acid) loaded with docetaxel and interleukin-8 and small interfering RNA was bound with upconversion nanoparticles (upconversion nanoparticles) through electrostatic force for castration-resistant prostate cancer treatment. Fascinatingly, this theranostic of prepared nanoparticles offered concurrent gene therapy and chemotherapy along with image-guided combination therapy using bimodal optical and magnetic resonance imaging agents in combination. Furthermore, this novel multifunctional hierarchical nanoformulation accomplished a dramatic reduction ($p < 0.001$) in IC_{50} in preferred PC-3 cells than the free form of docetaxel. Owing to the enhanced anticancer potential, it could be preferred as an image-guided combination therapy for castration-resistant prostate cancer management (Bulmahn et al. 2020). Menon et al. designed the nanosized, stable 8-dibenzothiophen- 4-yl-2-morpholin-4-yl-chromen-4-one (radiosensitizer) loaded peptide conjugated poly (lactic-*co*-glycolic acid) i.e. NU7441-R11-PLGA nanoparticles. It provided the prostate cancer-targeted sustained delivery (up to 3 weeks). Interestingly, these prepared polymeric nanoparticles exhibited biphasic release of radiosensitizer. The cellular uptake of PC 3 cells relied on the dose and magnetic field. *In vitro* study claimed that the 8-dibenzothiophen- 4-yl-2-morpholin-4-yl-chromen-4-one exhibited an effective radiation sensitizer in

prostate cancer cell lines. The combination of poly (lactic-*co*-glycolic acid) with peptides, which contains 8-dibenzothiophen- 4-yl-2-morpholin-4-yl-chromen-4-one and an appropriate imaging agent will thus help to monitor, target, and image nanoparticles and effectively radiosensitive prostate cancer cells (Menon et al. 2015).

16.6.3 Miscellaneous Advanced Polymeric Platform

Conventional approaches to cancer care are suffering from several drawbacks, such as non-selective differentiation toward healthy cells and cancer cells. Regarding this, in 2020, Changizi et al. designed folic acid conjugated polymeric gold nanoparticles for the active targeting of prostate cancer cells. These prepared nanoparticles offered real-time fluorescence as compared to non-targeted polymeric gold nanoparticles ($p < 0.001$) and along with that it induced radiosensitivity in LNCaP prostate cancer cells. The polymeric gold nanoparticles uptake was significantly ($p < 0.01$) raised in prostate cancer cells as compared to normal cells owing to folic acid conjugation on the surface of nanoparticles. Furthermore, prostate cancer cells showed a higher cytotoxic effect because of the presence of folate receptors on the surface of prostate cancer cells. Finally, these nanoparticles and ionizing radiation showed a significant synergistic dose-dependent effect ($p < 0.01$) on the induction of apoptosis and necrosis of cells. Concisely, obtained nanoparticles lead to several merits (higher and specific cellular uptake/accumulation) that reserved a room as a radiosensitizer for futuristic anticancer applications (Changizi et al. 2020).

In another study, Guo et al. prepared the gold nanoparticles-poly (ethylene glycol) transferrin targeting ligand (negative charge) and gold nanoparticles polyethyleneimine- folic acid (positive charge) for small interfering ribonucleic acid delivery. Small interfering RNA was attached to the surface of modified folic acid nanoparticles via electrostatic forces. Herein, the surface-modified gold nanoparticles resulted in lower cytotoxicity in prostate cancer cells. Furthermore, gold nanoparticles poly (ethylene glycol) with the transferrin targeting ligands demonstrated receptor-mediated nanoparticles uptake in PC3 cells that confirmed the transferring targeting ligands receptors in prostate cancer cells. On the other hand, folate-receptor targeting ligands with gold nanoparticles-polyethyleneimine offered small interference ribonucleic acid delivery in LNCaP cells. Overall, both these nanoparticles can be a suitable substitute for small interference ribonucleic acid delivery in prostate cancer therapy (Guo et al. 2016). Therefore, polymeric nanoparticles are successful candidates for combined cancer therapy as vehicles for pharmaceutical products or molecules. They deliver remarkable advantages in terms of stability, biocompatibility, biodegradability, adaptability, contrary to inorganic nanoparticulate systems. Overall, polymeric nanoparticles containing revolutionary polymers that respond to specific microenvironmental tumor conditions including reduced pH, high levels of reactive oxygen species, overexpressed enzymes, etc. may be used to activate regulated delivery of anticancer drugs. Advances in multifunctional architectured polymeric nanoplatforms targeted treatments of prostate cancer have been summarized in Table 16.3.

Table 16.3 Multifunctional architectured polymeric nanoplatforms targeted treatment of prostate cancer

Sr. no.	Anticancer agent	Polymer	Synthesis method	Particle size (nm)	Zeta potential (mV)	Polydispersity index	Features	References
Polymer-based superparamagnetic nanoparticles								
1.	Docetaxel	Poly (lactic-*co*-glycolic acid)	Precipitation method	154.3	−38.4	–	It improved the contrast-enhanced magnetic resonance imaging capability and controlled the drug release	Fang et al. (2020)
2.	–	Poly[*N*-isopropylacrylamide-acrylamide-allylamine	–	147	25.7	0.26	It demonstrates the significantly high peptide accumulation in prostate cancer tumors and can be used as a targeting carrier and imaging agent for prostate cancer treatment.	Wadajkar et al. (2013)
3.	Eupatorin	Poly(lactic-*co*-glycolic acid)	Nanoprecipitation method	58.5	−34.16	0.167	It increased apoptosis and suppresses proliferation.	Tousi et al. (2020)
4.	Docetaxel	Poly(ethylene glycol)	Chemical precipitation method	139.5	−10.9	<0.2	It enhanced the cellular uptake of docetaxel and exhibited superior anti-cancer activity.	Nagesh et al. (2016)
Polymeric nanoparticles bearing radionuclide/magnetic resonance imaging agent/metal nanoparticles								
5.	Doxorubicin	Poly (ethylene glycol)	–	100	–	0.12	It enhanced cellular uptake, *in vitro* cytotoxicity, etc., and improves the aqueous solubility of doxorubicin	Liu et al. (2020)
6.	Paclitaxel	Pluronic-polyethyleneimine	Film emulsification method	147	14.5	0.18	It offers several merits including blocking the TRPV6 cation channel, controlling drug release, and enhancing cell cycle arrest, and improving cellular toxicity along with apoptosis, and enhancing tumor targeting.	Wang et al. (2019)

7.	Docetaxel	Poly (ethylene glycol)-di-stearoyl phosphatidyl ethanolamine	–	186.1	−16.4	0.18	It enhanced the accumulation of docetaxel in the prostate cancer tumor site and showed maximum tumor growth inhibition.	Poudel et al. (2019)
8.	Docetaxel	Chitosan	Solvent evaporation method	235	+17	–	It offers the simultaneous delivery of chemotherapy with gene therapy and enabled image-guided combination therapy.	Bulmahn et al. (2020)
9.	8-dibenzo thiophen- 4-yl-2-morpholin-4-yl-chromen-4-one.	Poly (lactic-*co*-glycolic acid)	Single emulsion technique	274	−24.52	0.12–0.16	It provides the prostate cancer-targeted sustained delivery of 8-dibenzo thiophen- 4 – yl – 2 -morpholin-4-yl-chromen-4-one.	Menon et al. (2015)
Miscellaneous advanced polymeric platform								
10.	Gold nanoparticles	Spiropyran, vinyl imidazole	–	55.9	−28.85	–	It exhibited higher cellular uptake and demonstrates a higher cytotoxic effect.	Changizi et al. (2020)
11.	Gold nanoparticles	Poly (ethylene glycol), polyethyleneimine	–	~33, ~105	~−45, ~+53	–	It increased the endogenous gene silencing.	Guo et al. (2016)

16.7 Challenges

The recent dramatic increase in the number of polymeric nanostructures approved by the food and drug administration is used in the clinical setup of cancer. Additionally, there are several attempts made to enhance the therapeutic characteristics of anticancer drugs which are already formulated. Interestingly, the polymeric nanoparticles have displayed appreciable bioavailability and regulated biodistribution by utilizing stimuli responding pathways for the release of anticancer drugs in the body. We witness that, including all characteristics as a "magic bullet" for managing prostate cancer and other types of cancers, comprehensive efforts in preclinical and clinical technology are being carried out to create combined therapy. Also, food and drug administration has authorized nearly distinct nanodrugs (primarily polymeric nanoparticles) for prostate cancer and other cancer treatments. The synthesis and use of polymeric nanoparticles have boosted and revolutionized a lot of interest as a personalized medicine option for prostate cancer treatment. The manufacturing of reliable polymeric nanoparticles in the prostate cancer treatment domain is ensured by closer collaboration across academia and industry with the usage of innovative technologies.

The strategic focus is also on the efficient bonding of anticancer drugs to improve drug retention. The theranostic polymeric nanoparticles can be efficiently used for assembling separate nanoparticles for cancer diagnosis, imaging, and simultaneously targeted anticancer drug delivery. In the future, special attention may be provided to the design of theranostic polymeric nanoparticles that can track the prostate cancer stage. These versatile polymeric nanoparticles may be helpful for the early detection and successful treatment of prostate cancer and can be used during the process of prostate cancer stage monitoring. The theranostic polymeric nanoparticles for clinical purposes should have a greater capacity for tissue penetration and greater biosafety parameters with lower cell toxicity. In addition to this, these polymeric nanoparticles can uphold the poorly soluble anticancer molecules that can offer the reasonable retention of anticancer drugs in the prostate cancer tumor cell or tissue microenvironment. Recently, stimuli-responsive polymeric nanoparticles have demonstrated a substantial potential in the delivery of the oral anticancer agent. Therefore, such stimuli-responsive polymeric nanoparticles can be used to design innovative polymeric nanoparticles, which can assist to recognize genetic mutation, epigenetic changes, and deoxyribonucleic acid mismatch. Additionally, it can be designed to deliver anticancer molecules in the prostate cancer tumor and release the drug at various stages of prostate cancer.

Despite the plentiful advantages of polymeric nanoparticles, the biocompatibility and biodegradation of polymeric nanoparticles is still a big concern for study and development. The design and development of polymeric nanoparticles have also resulted in solving the problem of biotoxicity i.e. nanotoxicity and biodegradability. In the event of nanotoxicity, the polymeric nanoparticles' shape, particle size, and uniformity of nanoparticles should be better managed.

In the case of biodegradability, nanoparticles should be fully broken down and removed from the patient's body following the release of the payload. Besides, to resolve such a major issue, several biodegradable polymers (viz. chitosan, poly (lactic-*co*-glycolic acid), polylactide, etc.) can be preferred as a potential substitute for targeted prostate cancer therapy. Besides this, appropriate polymerization can be preferred to synthesize biocompatible polymeric nanoparticles. Interestingly, polymeric nanoparticles displayed controlled release of the anticancer agent; therefore, it can be preferred as a better alternative for the intravenous route. In a future point of view, theranostic polymeric nanoparticles can be potentially approved as personalized medicines in prostate cancer, in which the nature of these nanoparticles may be tailored to improve diagnostic or therapeutic outcomes by factors viz. patient age, prostate cancer stage, etc. Also, polymeric nanoparticles can be synthesized and filled with immunologic cell mortality initiating agents to promote prostate cancer tumor cell deaths. Additionally, the area of interest will be polymeric nanoparticles including complex features and multifunctional attributes (i.e. theranostic nanosystems), but with a simple design.

16.8 Conclusion

Concisely, prostate cancer is a multifaceted male reproductive system-related frequent non-skin cancer type that necessitates being dealt with successfully on many fronts. The biological complexity and diversity of prostate cancer are still limiting steps for scientific advancement. Fascinatingly, the efficacy of anticancer agent-loaded polymeric nanoparticles has greatly improved in chemotherapy as demonstrated by abundant preclinical/clinical findings. Accordingly, various tactics for attempting to change the physiochemical attributes of polymeric nanoparticles are possible, which are accurately loaded with various approved chemotherapeutics and further targeted to prostate cancer cells/tissue. Out of reported techniques, stimuli-responsive drug delivery demonstrates excellent therapeutic action. In that, two different anticancer molecules can be delivered to the prostate cancer cells/tissues using several signaling mechanisms in a prostate cancer tumor, which furnishes the synergistic output (known as synergistic therapy) for the prostate cancer treatment. Other pioneering studies reported that the anticancer-loaded/functionalized polymeric nanoparticles showed noteworthy thermionic emission. Therefore, it destroys the prostate cancer tumor cells by boosting the local temperature of the prostate cancer tumor microenvironment, which improves the release of anticancer drugs. Overall, architectured polymeric nanoparticles are providing revolutionary platforms for efficient prostate cancer treatment at different stages followed by remarkable advantages as compared to other nanocarriers. In the future, to confirm the prediction and reliable pharmacokinetics of anticancer drugs for humans, the *in vitro* and/or *in vivo* models for the assessment needs to be improved.

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Chapter 17
Cellular Internalization and Toxicity of Polymeric Nanoparticles

Santwana Padhi and Anindita Behera

17.1 Introduction

Cancer is a highly complex disease caused by defect or instability of the genes causing alteration in cellular pathology such as abnormal growth and replication of the cells due to genetic mutation, chromosome translocation and gene malfunction and disabled apoptosis (Mansoori et al. 2007; Padhi et al. 2015). It is a major public health problem worldwide being the leading cause of death in developed countries and second leading cause of death in developing countries. An estimated 12.66 million people were diagnosed with cancer across the world in 2008, and 7.56 million people died from the disease (Ferlay et al. 2010). By 2030, the global burden is expected to grow to 21.4 million new cancer cases and 13.2 million cancer deaths (Bray et al. 2012). Of all cancers, lung cancer is the leading cause of mortality in males with an estimated 1.61 million cases diagnosed in 2008 across the world whereas ovarian and breast cancer accounts for most number of deaths among gynecological malignancies in females (Siegel et al. 2012).

In spite of the fast progression of the disease and the increased global burden, the conventional treatment modality for cancer is restricted to surgical resection, radiation, chemotherapy which has limited efficacy because of lack of specificity, inadequate drug concentration at tumor sites, poor drug delivery, drug resistance and significant damage to noncancerous tissues (Padhi et al. 2018). These limitations results in poor diagnosis and ineffective treatment which therefore necessitates the

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development of novel and innovative drug deliveries with accurate diagnosis, higher therapeutic indices and better safety profile. Over the past few decades, there have been significant advances in the field of nanotechnology based therapeutics especially its integration with oncology. This field of nanotechnology or more precisely nanomedicines has gained wider acceptance and has emerged as a solution to the drawbacks of conventional therapy bringing a revolution in the both cancer diagnostics and therapeutics (Padhi et al. 2020; Verma et al. 2017).

Nanomedicines can be polymer based, metal based or lipid based systems of which polymer based systems are more favorable owing to its high stability and better targeting ability (Cuenca et al. 2006; Behera et al. 2020a, b). Nanoparticles are defined as colloidal particles ranging from 10 nm to 1000 nm in size. These nanoconstructs offer various advantages over the traditional deliveries such as stability, high specificity, controlled release, high payload, improved solubility and bioavailability (Heath and Davis 2008). Its capability to bypass multidrug resistance and ability to accommodate and transport molecules of varied physical and chemical nature makes it a promising drug delivery system for an anticancer model nanoparticle based therapeutics such as Nab-paclitaxel or Abraxane®, a solvent-free formulation of paclitaxel loaded albumin nanoparticles (Abraxis Biosciences Ltd), have been approved by United States Food and Drug Administration for treatment of metastatic breast cancer (Montero et al. 2011) while many others s are undergoing various phases of clinical trials (Young et al. 2011; Wei et al. 2007). Apart from therapeutic use, nanoparticles can also serve as valuable diagnostic tool for early detection of cancer or biomarkers so that the treatment can be initiated at early stage of disease progression. Nanotechnology based diagnostics include- quantum dots (Gao et al. 2004)**,** bionanobarcodes (Nam et al. 2003), nanocantilevers (Klein et al. 2005) and nanowires (Zheng et al. 2005), nanotubes (Xiao et al. 2009). Moreover, intelligent multifunctional nanosystems referred to as theranostics can be optimally designed to offer the advantage of combinatorial use of therapy and diagnosis thereby allowing simultaneous detection, imaging, and transport and release of the therapeutic agent (Kelkar and Reineke 2011; Kim 2007).

The pathophysiological condition and the anatomical changes that occur in the tumor tissue and its microenvironment allow several advantages for the delivery of nanoparticles which can be engineered to achieve site specific delivery via passive or active targeting (Padhi and Behera 2020). The physiology of the normal and tumor tissue differ in various aspects. Passive targeting takes the advantage of these changes to deliver the drug at the tumor sites (Khuroo et al. 2014). Normal vasculature is highly ordered, 8–10 μm in diameter and uniformly structured. In contrast, tumor blood vessels are 20–100 μm in diameter, leaky in nature, with abnormal, distended capillaries and sluggish and irregular flow (Brown and Giaccia 1998). They also possess wide fenestrations of 200 nm to 1.2 μm allowing the nanoparticles to permeate easily into the extravascular spaces resulting in their high accumulation inside the tumors. This phenomenon of action nanoparticle is more commonly referred to as the enhanced permeation and retention effect (Maeda et al. 2000). Another form of passive targeting involves the conjugation of drug to tumor specific

molecule which when comes in contact with the tumor microenvironment converts into an active substance (Sinha et al. 2006).

The tumor is also known to over-express certain receptors or antigens, and this fact can be utilized to functionalize the nanoparticles to recognize the receptors on the tumor surface (active targeting) leading to its preferential accumulation within the tumor via receptor mediated endocytosis (Behera and Padhi 2020). Despite of the novel applications of nanomedicines in biomedical domain, there are still some gaps to be filled, such as the fate of nanoparticles and their toxicity assessment on human health. Some of the key factors to contemplate for a successful treatment framework are the behavior of the entities after human body exposure and its accumulation up to a certain extent at the intended sites. Furthermore, because of the differences in size, the safety assessment applicable to nanomedicine will be vastly different from that of bulk drugs, and they may react differently relying on their properties.

Due to a lack of data, integrative studies are being prompted to better understand nanomedicine internalization and toxicity furthering to implement specific evaluation protocols or nanomaterial modifications for safe nanomedicine architecture. The current review provides an understanding of the physicochemical attributes affecting the cellular internalization and the critical need for toxicological evaluation of nanomedicines. In a nutshell, the aim of this review is to provide a comprehensive compilation of the factors that affect the cellular internalization along with the nanomedicine safety and toxicity considerations.

17.2 Factors Affecting Cellular Internalization of Polymeric Nanoparticles in Tumors

Phagocytosis and pinocytosis are the two principal endocytic pathways by which cellular internalization occurs (Foroozandeh and Aziz 2018). Large particles are often internalized by phagocytosis pathways (Behzadi et al. 2017), while particles in the nano range are taken by the tumor cells by pinocytosis, which occurs through either adsorptive pinocytosis or receptor-mediated endocytosis (Panariti et al. 2012). Caveolin-independent or clathrin-independent (~90 nm), caveolae-mediated (60 nm) and clathrin-mediated endocytosis (120 nm) are the major pinocytic uptake pathways, as well as clathrin-independent or caveolin-independent endocytosis (~90 nm). The different endocytic pathways by which a particle enters a cell are presented in Table 17.1.

The interaction of nanocarriers in the biological milieu is determined by their size, shape, and surface reactivity with the cells or tissues with which they interact. Furthermore, the interaction is influenced by the cell type engaging with nanocarriers and the cellular processing networks (Nel et al. 2009). The interaction between nanoparticles and cells takes place at the plasma membrane, where, depending on the physicochemical attributes of the nanocarriers such as size, composition,

Table 17.1 Endocytic pathways for particle entry into a cell

Endocytic pathway	Characteristics	Reference
Phagocytosis	Cells involved are macrophages, monocytes, neutrophils, and dendritic cells Receptors involved are Fc receptors, complement receptors, mannose/fructose receptors and scavenger receptors Uptake of nanoparticles is dictated by its physicochemical attributes	Hillaireau and Couvreur (2009) and Swanson (2008)
Clathrin mediated endocytosis (CME)	Cells procure nutrients and plasma membrane components like cholesterol, while the transferrin carrier provides iron The process undertakes receptor-specific uptake or by non-specific adsorptive uptake CME occurs in the region of plasma membrane that rich in clathrin. It accounts for the budding vesicle's voluntary co-assembly into a structured facade that produces and stabilizes the membrane curvature.	Conner and Schmid (2003) and Schmid et al. (2006)
Caveolae dependent endocytosis	Caveolae are usually flask-shaped membrane structures present in epithelial as well as non-epithelial cells Involved in processes such as cell signaling, transcytosis, and regulation of lipids, fatty acids, membrane proteins, and membrane tension.	Pelkmans and Helenius (2002)
Macropinocytosis	It does not use lipid rafts or pit-forming proteins in any way. Particles and dissolved molecules in the extracellular fluid are taken up by the endocytic vesicle Acts as an entry portal for microbial pathogens, including many bacteria and viruses	Lim and Gleeson (2011)

surface charge, reactive functional groups on the surface significantly change the plasma membrane's integrity (Rothen-Rutishauser et al. 2019).

Particle size, shape, surface charge, surface hydrophobicity/hydrophilicity, and surface functionalization are the chief physicochemical attributes of nanoparticles that impacts cellular uptake endocytotic trajectory as well as cytotoxicity (Patnaik et al. 2021). The major factors that impact the cellular internalization of polymeric nanoparticles in tumors are discussed below:

17.2.1 Particle Size

The particle size of nanoparticles plays an important role in assessing efficiency of cellular uptake (Zhu et al. 2013) as well as its toxic implications in living cells (Nel et al. 2009). Furthermore, the size of the nanoparticles was discovered to play a significant role in deciding the uptake pathway. Pinocytosis or macropinocytosis are

the endocytic pathways that allow smaller sized nanoparticles ranging in size from a few to several hundred nanometers to penetrate cells. Nanoparticles with a size range of approximately 250 nm to 3 m have been showcased to have appropriate in vitro phagocytosis, whereas nanoparticles with a size range of 120–150 nm are uptaken through clathrin- or caveolin-mediated endocytosis, with a maximum size limit of 200 nm recorded for nanoparticles using this stated pathway (Rivolta et al. 2012). The size of caveolae is essential in the caveolae-mediated pathway which hinders the uptake of larger nanoparticles. The central element in nanoparticles drug delivery is to avoid nanoparticles being engulfed by the reticuloendothelial system and to extend their blood circulation time, thereby increasing bioavailability. In such a scenario, raising the size of nanoparticles leads to a higher clearance rate (Gendelman et al. 2015). To construct efficient and safe nanoparticles for clinical applications, it is imperative to understand the involvement of nanoparticles size in cellular uptake processes.

Jiang et al. explored the size-dependent interaction and internalization of transtuzumab-conjugated nanoparticles with a size of approximately 2–100 nm in metastatic breast cancer (ErbB2+) cells and reported that nanoparticles of 40–50 nm had the highest cellular uptake, which may be attributed to the optimum antibody intensity on the particle surface, which may have stimulated maximum cross-linking in the over-expressed membrane receptors (Jiang et al. 2008). Smaller sized polymeric nanoparticles with sizes less than 25 nm have demonstrated to employ a new pathway to penetrate the perinuclear region of cells through non-degradative vesicles beyond the endo/lysosomal pathway, as reported by Lai and coworkers (Lai et al. 2007). This pathway was not facilitated by clathrin or caveolae dependant endocytic pathway. The cellular uptake of herceptin functionalized gold nanoparticles by breast cancer cells (SK-BR-3) was found to be size dependent. Nanoparticles in the size ranges of 25–50 nm showed the maximal rate of cellular internalization (Hoshyar et al. 2016).

17.2.2 Particle Shape

The shape of the particles is also an important factor that influences blood circulation time and tumor accumulation. The internalization of spherical gold nanoparticles and polymeric nanoparticles was greater than that of non-spherical ones (Zhang et al. 2008). It was reported elsewhere that spherical gold nanoparticles were taken up more by cells as compared to rod-shaped gold nanoparticles (Chithrani et al. 2006). Nonetheless, particle form influences not only tumor cell internalization but also its association with reticulo endothelial system, as well as pharmacokinetic and tumor retention as well. Cellular uptake of layered double hydroxide nanoparticles with fluorescein isothiocyanate in different morphologies such as hexagonal sheets and rods were investigated by Xu and coworkers (Xu et al. 2008). Clathrin-mediated endocytosis was noted to be the pathway for cellular internalization. Layered

double hydroxide – fluorescein isothiocyanate nanospheres were maintained in the cytoplasm, while microtubules pushed layered double hydroxide – fluorescein isothiocyanate nanorods towards the nucleus.

17.2.3 Surface Charge

Surface charge is another important factor that affects nanoparticles uptake in cells. Nano surface alteration has been used to manipulate the surface charge of nanoparticles to be either cationic or anionic (Zhu et al. 2012). It is a known fact that in the cells positively charged nanoparticles have a higher internalization rate in the cells than neutral and negatively charged nanoparticles (Rivolta et al. 2012). Positively charged nanoparticles, on the other hand, has the propensity to disrupt the cell membrane integrity and thereby enhance toxicity (Lovrić et al. 2005). Positively charged ones, in particular tend to trigger cell death. The pharmacokinetic profile of the charged nanoparticle must be taken into account, as charged nanoparticles typically associate more strongly with serum proteins in the biological environment, resulting in faster blood clearance than neutral particles. It is possible to construct nanoparticles that shed their PEG shielding following tumor extravasation, exposing cationic particles that can engage with target tumor cells (Ernsting et al. 2013).

Molecular dynamics simulations were used by Li and Gu to study the association of charged and neutral nanoparticles with cell membrane. When compared to neutral nanoparticles, charged nanoparticles had greater binding to the cell membrane. Furthermore, by the charge density of nanoparticles, the membrane can completely envelope them (Li and Gu 2010). Another study looked into the associations of cationic and anionic gold nanoparticles with cellular membrane using molecular dynamics simulation. The results show that as the charge density of gold nanoparticles increases, so does the damage to the cell membrane caused by their penetration (Lin et al. 2010). These results indicate that adjusting the surface charge densities of gold nanoparticles to maximize absorption while mitigating cytotoxicity may be a way to regulate the associations between cells and the nano system, which are essential traits for any nanoparticles being proposed for biological applications.

A study was conducted in chitosan nanoparticles of sizes around 215 nm. In all the studied cell lines, the cellular uptake rate was also positively associated with the surface charge. Following internalization, the positively charged nanoparticles escaped from the lysosome and manifested perinuclear localization, while negatively and neutrally charged nanoparticles tend to co-localize with the lysosome. These findings were crucial in establishing the body of knowledge needed to fabricate chitosan-based nanoparticles that are both effective and precise in their application (Yue et al. 2011).

Cationic gold nanoparticles demonstrated a fivefold higher uptake rate than their anionic counterparts when evaluated in breast cancer cells (SK-BR-3) (Cho et al. 2009). The researchers also discovered that half of cationic gold nanoparticles

penetrate into cells by creating disruptions in the cell membrane by non-endocytosis pathways whereas anionic and neutral nanoparticles only enter cells through endocytosis mechanisms.

A study report by Jiang et al. inferred that chitosan nanoparticles with a greater surface charge, either positive or negative were taken up by macrophages more readily than neutral particles, emphasizing the relevance of electrostatic interaction in the process of phagocytosis (Jiang et al. 2017). Positively charged chitosan nanoparticles were internalized by cells to a higher degree than negatively charged nanoparticles when evaluated in non-phagocytic cells such as human liver cell line L02 and human hepatoma cell line SMMC-7721. The plausible reason for the observed effect may be due to the attractive/repulsive forces between the negatively charged CM and the cationic/anionic nanoparticles (Arvizo et al. 2010).

17.2.4 Conjugating Ligands

Surface modulation with targeting ligands on the surface of nanoparticles is a crucial step in drug delivery applications of nanoparticles to reduce toxicity, enhance stability, monitor and modulate cellular internalization of nanoparticles, which thereby impacts their biological fate (Chompoosor et al. 2010). Polyethylene glycol, carboxylic acid group, neutral functional groups such as hydroxyl groups, and the positive amine group are the most common surface functional groups encountered on nanoparticles ends up in increasing the increased amount of amine group results in a higher positive surface charge, which ends up in increasing nanoparticles uptake into cells (Lorenz et al. 2006). Carboxylic acid functional groups, on the other hand, raise the negative charge of nanoparticles, which improves their penetration.

While many nanoparticulate systems and animal models have demonstrated to be effective, this concept may display some associated drawbacks. To begin with, cellular internalization progresses only after nanoparticle extravasation and cellular penetration starts only in the vicinity of micro vessels, culminating in reduced tumor penetration and clustered drug uptake (Li and Huang 2008). The biological efficacy of small interfering RNA payloads transported through non-targeted and transferrin conjugated nanoparticles in tumors was compared by Bartlett et al. (Bartlett et al. 2007). They reported no differences in biodistribution between the targeted and non-targeted nanocomplexes, but the targeted complex had higher gene silencing capacity. The researchers inferred that the ligand‘s primary role was to maximize intracellular absorption rather than directing the complexes to tumor tissue. Other groups have published similar findings as well (Kirpotin et al. 2006; Li et al. 2008).

Jiang and colleagues looked into the variations in cellular absorption of pristine polystyrene nanoparticles and amino-functionalized counterparts (Jiang et al. 2010)**.** The findings showed that amino-functionalized polystyrene nanoparticles had a higher internalization rate than pristine polystyrene nanoparticles, and that the former were absorbed primarily through clathrin-mediated endocytosis while the latter

were uptaken through clathrin-independent endocytosis. The importance of surface chemical alteration in cellular interactions with nanoparticles is highlighted by this striking difference.

17.3 Toxicity of Nanoparticles

Toxicity is of prime concern for all anticancer agents. The cumulative toxicity of a cytotoxic agent may decrease its therapeutic efficacy. Haematological and non-haematological toxicities are the important toxicity patterns observed for all cytotoxic drugs. Conventional chemotherapy involves the use of free drug solutions administered through intravenous bolus or infusion (Ewesuedo and Ratain 2003). Upon administration they bind to body tissues and plasma proteins resulting in a diminished therapeutic potential and an increased toxicity. This ultimately leads to lack of specificity ultimately causing harm to non-cancerous healthy tissues also (Ratain and Mick 1996; Tipton 2003). These factors pose a great challenge to effective anticancer treatment. The unspecific action of cytotoxic agents leads to tissue and organ toxicity leading to a diminished therapeutic potential (Powis 1991).

A decrease in the size of nanoparticles results in an increased particle surface area which enhances the interaction of different chemical moieties onto the nanoparticle surface resulting in toxicity (Suh et al. 2009). Tissue distribution of the nanoparticles depends on their size. It has been previously reported that 26% of 100 nm, and 10% of 500 nm particles were distributed in the mucosal and lymphatic tissues of the intestines (Suh et al. 2009). The specific surface properties of nanomaterials also have an effect in predicting the toxicity profile. They have the ability to penetrate major organs such as lungs, small intestine to produce organ-specific toxicity.

As particle surface makes an intimate contact with the biological membrane to produce a therapeutic effect, hence it plays a pivotal role in determining the toxicity profile of the system. The presence of oxygen or ozone and transition metals on the particle surface leads to the production of reactive oxygen species ultimately leading to inflammation at certain sites (Risom et al. 2005; Donaldson and Stone 2003). But suitable surface coatings can mask the reactive groups anchored to the surface leading to diminished toxicity.

Nanoparticles have an inherent property to aggregate when stored for some period of time and this unique property may help in determining their toxicity profile. Aggregation leads to increased particle size resulting in more macrophage clearance compared to unaggregated ones. Particle aggregation results from the use of high concentration of nanoparticles leading to reduced toxicities when compared with the use of lower concentration of nanoparticles (Gurr et al. 2005; Takenaka et al. 2001). Most of the aggregates are observed to be larger than 100 nm size. Hence the use of higher concentration of nanoparticles may lead to produce less undesirable toxic effects as compared to lower concentration of same nanoparticles.

Nanoparticles of 200 nm in size enter the cell passively but larger particles are taken up by macrophages by specific receptor mediated, actin-dependent mechanism. The absenceof receptors on the surface of erythrocytes imply that they will not be able to take up particles. But it was observed that the 1 μm particles were not taken up at all but smaller sized particles entered the cells passively which was confirmed by electron microscopy with 0.02 μm titanium dioxide particles (Peters et al. 2006) Hence the uptake of nanoparticles by red blood cells is size dependent but surface charge plays a minor role (Rothen-Rutishauser et al. 2006). But surface charge plays an important role in the uptake of nanoparticles by platelets. It has been reported that positively charged particles induces platelet aggregation as well as thrombi formation which is not observed with negatively charged particles (Nemmar et al. 2002).

Proteins present in the blood binds to nanoparticle surface to form complexes which increases the nanomaterial mobility in entering the deep layers of tissues which may result in altering their structure and functionality leading to toxicity (Nel et al. 2006). Proteins with high concentrations and high association rate constants will initially occupy the nanoparticle surface, but may also dissociate quickly to be replaced by proteins of lower concentration, slower exchange, and higher affinity. Apolipoproteins present in blood bind to nanoparticle surface to form complexes which are taken up by the specific receptors expressed on the cell surface. It has been reported that complexes of apolipoprotein E nanoparticles have the capability to produce neurotoxicities (Cedervall et al. 2007; Kim et al. 2007).

Functional changes of proteins of such complexes may be another mechanism by which particularly small nanoparticle, with their large surface area as a binding interface, may induce protein mal-functioning, which may lead to the pathogenesis and adverse health effects (Borm and Kreyling 2004).

The distribution of nanoparticles depends on their route of administration and provides a fair idea regarding its toxicity aspect. Nanoparticles given orally are found to be distributed in lungs, brain, liver, kidney and are reported to pass through the gastro intestinal (Hagens et al. 2007). Nanoparticles have the ability to cross the transplacental membrane which may affect the embryos development ultimately leading to death when give intraperitoneally (Vega-Villa et al. 2008). Intravenously administered nanoparticles are distributed in the liver, spleen, bone marrow, lungs and thereafter rapidly cleared from the systemic circulation by the action of splenic macrophages (Moghimi et al. 2005).

On inhalation smaller sized nanoparticles are distributed in the distal air pathways whereas larger ones are retained in the upper airways. They have the ability to reach the ganglia after translocation (Medina et al. 2007). These nanosized materials often cause cardiovascular and respiratory disorders resulting in death of a significant segment of human population.

It has been cited in the literatures that the polymers and the excipients used in the formulation of polymeric nanoparticles needs to be intelligently chosen to minimize the associated toxic effects. Polymers and surfactants are routinely employed in the formulation processes. The nature and concentration of the surfactant used estimates the particle size and drug release pattern. Excipients that are biocompatible,

biodegradable, and non-toxic can make up an optimal drug delivery system; hence it becomes imperative to evaluate the toxicity of excipients used in formulation of nanocarriers. Garth et al. investigated the oral toxicity of span 20 in rats for a time period of 6 months (Fitzhugh et al. 1960)**.** There was a gradual enlargement of the common bile duct as the dosage was increased. There was no evidence of carcinogenicity in male and female mice fed with span 60 (4%). With span 60 at 4% of the given diet, however, renal enlargement and a higher prevalence of nephrosis was noted. When human subjects were administered span 60 at a concentration of 6 g/day for 28 days, no harmful side effects were reported (Waldstein et al. 1954)**.** When span 60 was given at dose levels of 0.5, 2, or 4% of the diet for 80 weeks to the rodents, no signs of carcinogenicity were also detected (Hendy et al. 1978). Poloxamers have been linked to anti-carcinogenic effects in numerous studies. When evaluated in multidrug-resistant cancer cells, they are suggested to enhance the sensitivity of chemotherapeutic drugs (Singh-Joy and McLain 2008).

In addition, biodegradable polymers have become increasingly popular as drug delivery vehicles in recent decades which are known to be readily metabolized in the body. The widespread usage of polyglycolide in pharmaceutical applications is attributable to the degradation product glycolic acid which is a natural metabolite produced by hydrolysis. Glycolic acid is released in large quantities once the polymer begins to degrade. Glycolic acid is either excreted unchanged by the kidneys or removed as carbon dioxide and water via the tricarboxylic acid cycle (Agrawal and Ray 2001)**.** Despite the fact that glycolic acid is resorbable at higher concentrations, they may also raise the acid's localized concentration, which could be harmful to the adjoining tissues (Kornhauser et al. 2009)**.** The degradation route of poly (lactic-co-glycolic acid) is rapid than that of polylactic acid. Lactic acid and glycolic acid are known to be the biodegradation products of poly (lactic-co-glycolic acid) (Gunatillake et al. 2006). The compatibility of platelets in human blood samples with poly (lactic-co-glycolic acid) nanoparticles was investigated by Xue and colleagues and they reported that the poly (lactic-co-glycolic acid) nanoparticles were platelet compatible at concentrations less than 10 g/ml because no platelet activation or aggregation was observed (Li et al. 2009). As evidenced by histopathological assays, Semete and colleagues demonstrated that poly (lactide-co-glycolic acid) nanoparticles were safe in cell culture and when administered orally at concentrations up to 60 mg when evaluated in bagg albino (balb/c) mice (Semete et al. 2010). The aggregation behavior of sodium laurate-based poly (lactic-co-glycolic acid) nanoparticles in simulated blood fluid was investigated by Kim et al. When compared to PEGylated poly (lactic-co-glycolic acid) nanoparticles, the nanoparticles demonstrated to have a larger size in simulated blood fluid, which could provoke a serious problem when administered intravenously because it has a propensity to block blood vessels. Furthermore, poly (lactic-co-glycolic acid) nanoparticles impacted 80% of red blood cells, while PEGylated poly (lactic-co-glycolic acid) nanoparticles inflicted less damage based on particle concentration used (Kim et al. 2005).

17.4 Conclusion

The reticuloendothelial system engagement with nanoparticles, extravasation and penetration of nanoparticles through highly permeable tumor tissues and the optimal drug release pattern around or within the target tumor cells are enlisted as vital all biological barriers to successful drug delivery applications. The particle size of approved cancer nanomedicines falls in the range of 80–130 nm. However, there is growing interest in fabricating smaller sized particles (lesser than 50 nm) for better tumor permeability as well as penetration. Nanomedicine research endeavours has concentrated on refining the physicochemical characteristics of nanoparticles such as particle size and shape, surface charge, ligand density, release profiles to achieve optimized pharmacokinetics and delivery.

Currently, research is based on the permeability side of the enhanced permeability and retention effect approximation, with less attention on the retention component, which is influenced by the tumor‘s compromised lymphatic drainage. Much of quantitative functional imaging techniques need to be designed to investigate lymphatic functionality in tumors and how this component affects the interstitial fluid pressure, nanoparticle penetration, extravasation and retention. With advances in technical understanding, logical nanomedicine architecture can be recognized to ensure the desired therapeutic impact in image-stratified patients. In addition, it is essential to determine the safety profile of different excipients used in the development of nanocarriers, as well as the final formulations. This is a critical criterion for establishing the safety of a formulation intended for use in the treatment of a specific disease. More strict and developed regulatory aspects on the safety of nano-formulation excipients are mandated. More research is needed, however, to determine the overall safety of nanoparticle-based delivery systems. Particulars of *in vivo* safety characterization of nanocarrier excipients, as well as the evolved nano-formulation, will enable up possibilities for developing a safe and effective nanomedicine. The safety of the formulation, which is dictated by the toxicological effects of its components, will determine the future of an effective nanoparticulate-based delivery vehicle.

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Chapter 18
Prospects and Challenges in the Treatment of Solid Tumors

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Abbreviations

ALA	Amino levulinic acid
DNA	Deoxyribose nucleic acid
MAL	Methyl amino levulinic acid
WHO	World health organization

18.1 Introduction

Cancer is one of the major immunological disorders in the world. It is defined as an uncontrolled growth of cells. Cancer is defined as a group of more than 100 different diseases characterized by the uncontrolled growth of abnormal cells in the body" (Britannica). According to the medical dictionary, "Cancer is not just one disease, but a large group of almost 100 diseases. Its two main characteristics are the uncontrolled growth of cells in the human body and the ability of these cells to migrate from the original site and spread to distant sites". Cancer is the second leading cause

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of death worldwide and is expected to cause 13.2 million death incidents by 2030 (Padhi et al. 2018). Globally, about 1 in 6 deaths occurs due to cancer. Approximately 70% of cancer deaths occur in low- and middle-income countries (Padhi and Behera 2020). Around one-third of deaths from cancer are due to the five leading behavioral and dietary risks, namely high body mass index, low fruit and vegetable intake, lack of physical activity, tobacco use, and alcohol use. A tumor may be defined as an abnormal mass of tissue that usually does not contain cysts or liquid areas and it often leads to malignancy (Khuroo et al. 2014). Solid tumors may be benign (not cancer), or malignant (cancer). Different types of solid tumors are named for the type of cells that form them. Examples of solid tumors are sarcomas, carcinomas, and lymphomas. Leukemias (cancers of the blood) generally do not form solid tumors (Connolly et al. 2003).

The physiology of tumors is uniquely different from that of normal tissues, and the associated complexity of tumor complexity leads to the failure of applied therapeutic regimens (Padhi et al. 2015). The hostile pathophysiological microenvironment is largely determined by an abnormal tumor microcirculation. Blood flow can vary considerably despite similar histological classification and primary site. Blood flow is not regulated according to metabolic demands as is the case in normal tissues. Increased vascular permeability has been demonstrated, with extravasation of blood plasma expanding the interstitial fluid space, and due to lack of functional lymphatics, the hydrostatic pressure in the tumor interstitium drastically increases (Vaupel P. 2009). Interstitial hypertension forms a 'physiological' barrier to the delivery of therapeutic macromolecules in cancer cells. The interstitial space of the tumor is three to five times larger than in most normal tissues and contains a relatively large quantity of mobile fluid, that is, fluid that freely moves. There is a pH gradient across the cell membrane. Interestingly, this gradient is the reverse of that found in normal tissues. The formation of tumor and structure of tumor is represented in Figs. 18.1 and 18.2 (Baba and Câtoi 2007; Vaupel et al. 1988).

Different kinds of solid tumors are named according to the type of cells they are composed of:

- Sarcomas: Cancers arising from connective or supporting tissues such as bone or muscle.
- Carcinomas: Cancers arising from glandular cells and epithelial cells of the body that line body tissues.
- Lymphomas: Cancers of lymphoid organs such as lymph nodes, spleen and thymus, which produce and store infection-fighting cells. These cells also occur in almost all tissues of the body, and lymphomas, therefore, may develop in a wide variety of organs.

Various types of solid tumors are depicted in the following figure (Fig. 18.3) (Gavhane et al. 2011).

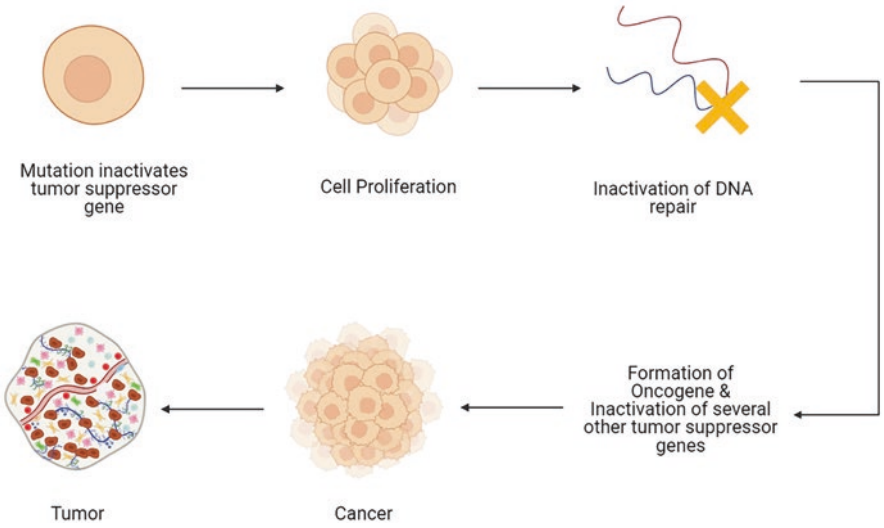

Fig. 18.1 Formation of tumor involving the stages of cell proliferation and formation of oncogene. Mutation in cells inactivates the tumor suppressor gene and results in cell proliferation by inactivating the repair of DNA. This results in formation of oncogenes and inactivation of several tumor suppressor genes resulting in growth of solid tumor

18.2 Current Treatment of Solid Tumors

Treatment of solid tumors is often a challenge due to various factors like age and sex of the patient, position of the tumor as well as stage of cancer. Often the treatment modality comprises a combination of chemotherapy, radiation therapy, and surgery. Surgical removal is preferred when the clinical condition of the patients is stable enough to withstand the consequences of the same (Zhang et al. 2016). It is generally followed by chemotherapy and radiation therapy as per the patient's clinical condition. Radiation therapy damages the DNA of cancerous tissue leading to cellular death. To spare normal tissues (such as skin or organs which radiation must pass through to treat the tumor), shaped radiation beams are aimed from several angles of exposure to intersect at the tumor, providing a much larger absorbed dose at the targeted site than in the surrounding healthy tissues (Vaupel et al. 2001). Besides the tumor, the radiation fields may also include the draining lymph nodes if they are clinically or radiologically involved with the tumor, or if there is thought to be a risk of subclinical malignant spread. It is necessary to include a margin of normal tissue around the tumor to allow for uncertainties in daily setup and internal tumor motion. These uncertainties can be caused by internal movement (for example, respiration and bladder filling) and movement of external skin marks relative to the tumor position (Jhanwar et al. 2005; Puls et al. 2011).

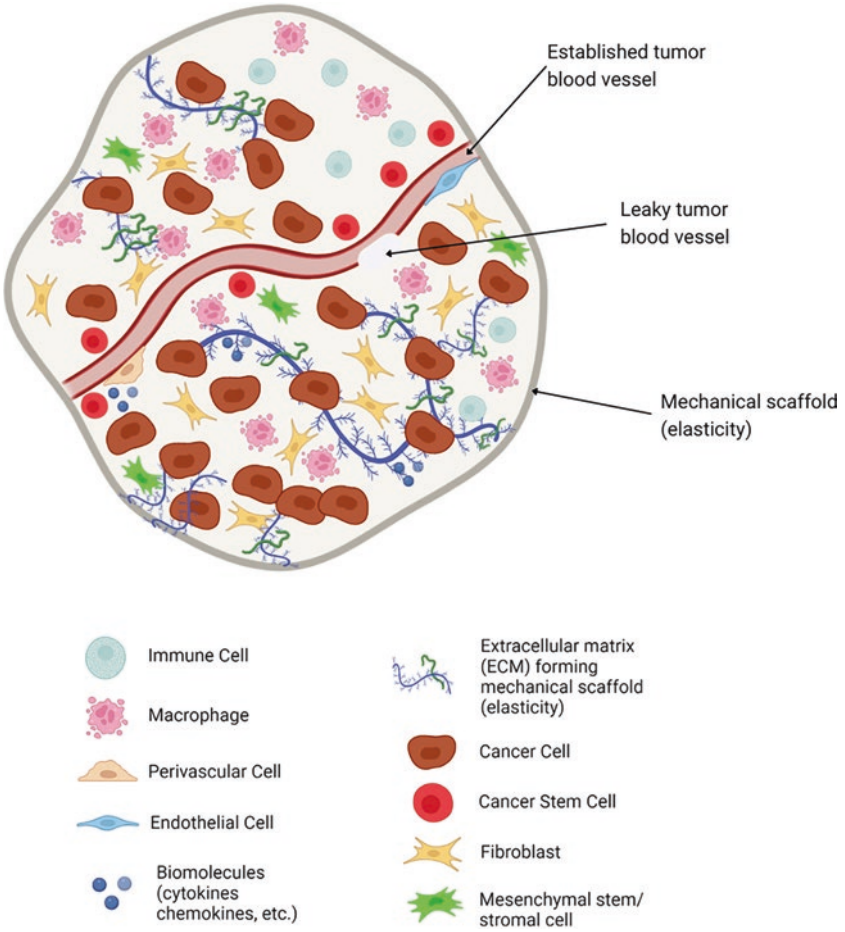

Fig. 18.2 Tumor depicting the cancer stem cells and mesenchymal stem cell affecting biomolecules. The figure depicts the tumor cells containing different types of cells like immune cell, macrophages, perivascular cells, endothelial cells, cancer stem cells, cancer cells, fibroblasts and mesenchymal stem, stromal cells and different biomolecules like cytokines and chemokines

18.2.1 Chemotherapy

- Chemotherapy involves the use of one or more anti-cancer drugs as part of a standardized chemotherapy regimen. Chemotherapeutics may be given with curative intent. It may aim to improve the quality of life of patients or to reduce symptoms (Tripathi 2013; Foye et al. 2002; Hassan et al. 2021). Various drug candidates are utilized in the treatment of solid tumors which are depicted in Table 18.1.

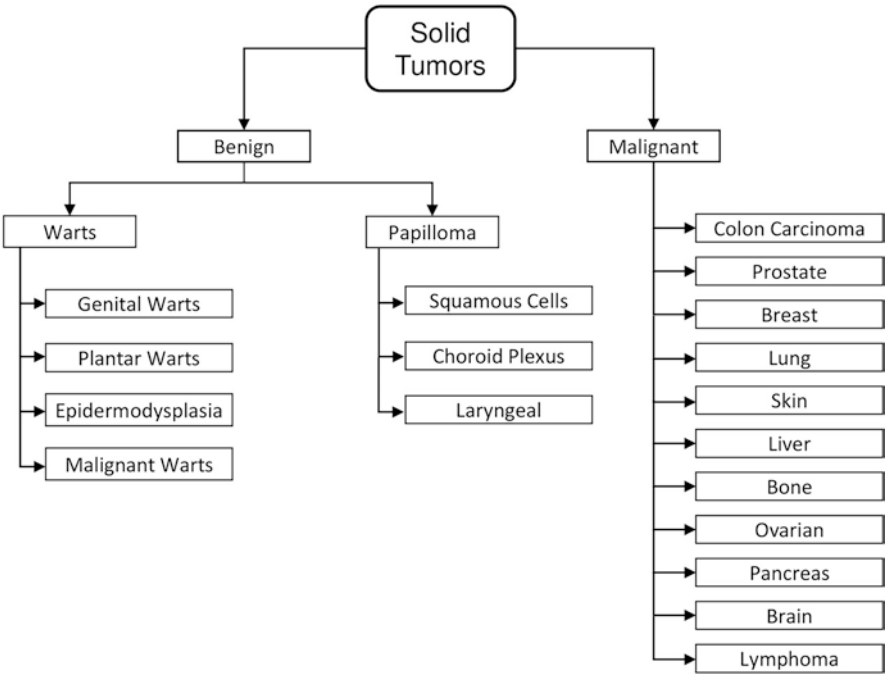

Fig. 18.3 Classification of different types of solid tumours. Solid tumors may be benign (not cancer), or malignant (cancer). Different types of solid tumors are named for the type of cells

18.2.2 Radiotherapy

Radiotherapy which is generally abbreviated as RT, XRT, or RTx, is prominently used in the treatment of cancer, in combination with chemotherapy. In radiation therapy, ionizing radiations are used to kill malignant cells which are delivered by a linear accelerator. Radiation damages the DNA of cancerous cells, resulting in the termination or suppression of tumor growth. Radio sensitivity is measured as a response of cancer cells to the amount of radiation which plays a key factor to determine the dose of radiation therapy (Citrin 2017).

Radiation dose is calculated in cGy, which is defined as a unit of absorbed radiation dose that is equal to one hundredth (10^{-2}) of a gray, or 1 rad. It is used primarily in epithelial cell cancer (carcinoma), leukemia, and lymphoma. Most carcinomas are moderately radiosensitive & require a higher dose to treat the disease, while treatment of leukemia and lymphoma requires a relatively low amount dose (Loi et al. 2017). Some cancers are extremely radio-resistant which often requires a considerably higher dose to produce a radical cure. Renal cell cancer is one such type of cancer that requires a higher radiation therapy dose. Melanoma cancer is treated with immunotherapy along with radiation therapy to ease the treatment (Ma et al. 2003; Weber and Wieder 2006; Triantopoulou et al. 2013).

Table 18.1 Classification of anticancer drugs for solid tumors

Sr. No.	Category	Examples
A. Cytotoxic agents		
1. Alkylating agents		
1.1	Nitrogen mustards	Mechlorethamine, cyclophosphamide, isophosphamide, chlorambucil, melphalan
1.2	Ethylenimine	Thiotepa
1.3	Alkyl sulfonate	Busulfan
1.4	Nitrosoureas	Carmustine, lomustine
1.5	Triazine	Dicarbine, temozolomide
1.6	Methylhydrazine	Procarbazine
2. Platinum co-ordination complexes		
	Platinum co-ordination complexes	Cisplatin, carboplatin, oxaliplatin
3. Antimetabolites		
3.1	Folate antagonist	Methotrexate, pemetrexed
3.2	Purine antagonist	6-Mercaptopurine, 6-thioguanine
3.3	Pyrimidine antagonist	5-fluorouracil, capecitabine, cytarabine
4.	Microtubule-damaging agents	Vincristine, vinblastine, vinorelbine, paclitaxel, docetaxel, estramustine
5.	Topoisomerase-I inhibitor	Topotecan, irinotecan
6.	Topoisomerase II inhibitor	Etoposide
7.	Antibiotics	Actinomycin D, doxorubicin, daunorubicin, epirubicin, mitoxantrone, bleomycin, mitomycin C
8.	Miscellaneous	Hydroxyurea, L-asparaginase, tretinoin, arsenic trioxide
B. Targeted drugs		
1.	Tyrosine-protein kinase inhibitor	Imatinib, nilotinib
2.	Epidermal growth factor reeptor inhibitor	Gefitinib, erlotinib, cetuximab
3.	Angiogenesis inhibitor	Bevacizumab, sunitinib
4.	Protease inhibitor	Bortezomib
5.	Unarmed monoclonal antibodies	Rituximab, transtuzumab
C. Hormonal drugs		
1.	Glucocorticoids	Prednisolone
2.	Estrogens	Fosfertrol, ethinylestradiol
3.	Selective estrogen receptor modulators	Tamoxifen, toremifene
4.	Selective estrogen receptor down regulators	Fulvestrant
5.	Aromatase inhibitor	Letrozole, anastrozole, exemestanea
6.	Antiandrogen	Flutamide, bicalutamide
7.	5-α reductase inhibitor	Finasteride, dutasteride
8.	Gonadotropin releasing hormone analogs	Nafarelin, leuprorelin, triptorelin
9.	Progestins	Hydroxyprogesterone

18.2.2.1 Types of radiotherapy

A. **Teletherapy or external beam radiation**

It delivers **2**D beams using kilovoltage therapy X-ray units; medical linear accelerator that generates high energy X-rays which are identical to other sources like a medical linear accelerator that generates high energy. It consists of a single beam of radiation delivered to the patient from various dimensions, either from the front side or back side or sometimes from both sides (Zoyla et al. 2006; Pollack and Zagars 1997; Mijnheer et al. 2013).

B. **Contact X-ray brachytherapy**

It is commonly known as electronic brachytherapy or the papillon technique. X-rays are applied close to the tumor to especially treat rectal solid tumors. The X-ray tube is inserted through the anal region to the rectum and stabilized against the cancerous tissue. Then high doses of X- rays are emitted directly into tumor tissue (Myint and Gerard 2018; Sun et al. 2019; Rao et al. 2018).

C. **Sealed source radiation therapy (brachytherapy)**

In this technique, the radiation sources are precisely placed directly at the site of the cancerous tumor. Radiation only affects a precise localized area, which reduces exposure to healthy tissue. It provides advantages over external beam radiation therapy. This technique needs lesser time frame as compared to other therapies (Dale et al. 1998; Mayles et al. 2007; Astrahan et al. 1990).

D. **Systemic radioisotope therapy (unsealed source radiotherapy)**

It is a form of targeted therapy. This technique is generally used in the treatment of goiter (overgrowth of the thyroid gland). In this technique, the isotope is attached to another molecule, antibody(s), or protein, which penetrates to the desired tissue via infusion. Other applications are the infusion of meta-iodo benzyl guanidine to treat neuroblastoma (Bakht et al. 2011; McEwan, 1997; Breen et al. 1994).

18.2.3 Surgery

In the primary stage of solid tumors, surgeons prefer curative surgery when the tumor is localized to a specific area of the body. The surgery proceeds through minor incisions through skin, muscles & sometimes bones. After surgery it becomes painful for patients. There are the following types of surgery performed in the treatment of solid tumors (Moreno et al. 2013; Gervasoni et al. 2007; Di Martino et al. 2009).

Table 18.2 Application & outcome of photodynamic therapy

Sr. No.	Type	Indication	Outcome
1.	Squamous cell carcinoma	MAL-PDT	Better than cryosurgery
		ALA-PDT	Better than 5-fluorouracil
2.	Basal cell carcinoma	PDT	Better than cryotherapy & surgery
3.	Non-small-cell lung cancer	PDT	Improved surgical outcome in combination & lengthen survival, relieved symptoms

ALA aminolevulinic acid, *MAL* methyl aminolevulinic acid, *PDT* photodynamic therapy

A. **Cryosurgery**

The area which needs to be operated is surrounded by an extremely cold environment by means of nitrogen or argon gas. This destroys abnormal tissue. It is utilized especially in early-stage skin cancers, in some precancerous growths on the skin, and cervical cancer (Gage 1998; Blackwood and Cooper 1972; Korpan 2012).

B. **Lasers**

In this technique, powerful beams of light are used as a surgical source, focusing on very tiny areas, so they can be used for pinpoint surgery. Lasers shrink/destroy tumors or growth. These types of surgeries are performed on the body surface or internal lining of internal organs (Huang et al. 2008).

C. **Photodynamic therapy**

Some chemotherapeutic agents react to a certain type of light. This method is used often to treat or relieve skin cancer, mycosis & non-small cell lung cancer. Some combinations are explained in the following table (Table 18.2) (Yanovsky et al. 2019).

18.3 Advantages and Disadvantages of Treatments

The current treatment of solid tumors involves the combination of surgery, chemotherapy, and radiation therapy, which is also termed adjuvant therapy.

18.3.1 Advantages

Post-operative adjuvant therapy is now a standard consideration in many solid resettlement tumors, significantly reducing the risk of recurrence. The magnitude of efficacy across different tumor types was studied by Wara (1981). Immunity plays

an important role in the treatment of solid tumors. Immunomodulation has been pursued in most melanoma tumors (Stevens and Rodriguez 2015).

Massari et al. studied complex algorithms for the determination of the most appropriate adjuvant therapy in breast cancer patients (Massari et al. 2017). The balance between therapeutic outcome, acute toxicity and long-term side effects is an important consideration for adjuvant therapy and should be determined on an individual patient basis. The age of the patient and the tolerance to treatment are the factors that affect the study. The tolerability of more intensive chemotherapy regimens may decrease with increasing intensity of therapy (Riethmüller et al. 1994).

18.3.2 Disadvantages

There are major advances in the current treatment of solid tumors, but the treatment does not hold the nerve of the disease because of the following factors:

(a) Less patient compliance.
(b) Targeting single molecular abnormalities or cancer pathways achieved acceptable clinical responses that have modestly affected survival in some cancers.
(c) Targeting a single drug does not lead to a cancer cure
(d) Toxicity and the associated complications of therapy pose a limiting factor for implementation (Mceachron et al. 2020; Patnaik et al. 2021).

Due to the above-mentioned reasons, the current treatment modality does not apply to all the population of cancer, which forces the researcher to explore novel therapy for treating cancer.

18.4 Challenges

Cancer is the second leading cause of death worldwide. Global demographic characteristics predict an increased cancer incidence in the next decades, with 420 million new cancer cases annually expected by 2025. Breast, colorectal, prostate, and lung cancers are the most frequently diagnosed cancers worldwide. (Fukumura et al. 2018).

18.4.1 Immune Evasion Mechanisms in Tumors

Regular anticancer immunity requires the identification and elimination of early malignant cells that express tumor-associated antigens. It presents a complex with human leukocyte antigens on the surface of the tumor cell. Regular anticancer immunity needs identification and disruption of early malignant cells and

interactions with dendritic cells, antibodies, cytokines, macrophages, plasma cells, and helper T cells (Rodríguez et al. 2003). Although the immune mechanism provides prevention of malignancy with the help of immunosurveillance, which contributes a great deal to the elimination of malignant tissue, it can also lead to immunoediting & moulding of tumor immunogenicity. Malignant cells exploit diverse molecular pathways to obstruct immune-mediated destruction by impairing cellular components of body defense mechanisms related to tumor identification & dismissal (Real et al. 2001). Human leukocyte antigen is obligatory for defense system identification and successive disruption of malignant tissue by a body defense mechanism, as tumor antigen could surface up in a human leukocyte antigen restricted mode to be identified by T-cell receptors. Disabled human leukocyte antigens – I expression inhibits the stimulation of the cytotoxic defense mechanism (Elpek et al. 2007). Human leukocyte antigens - II expression influences the antigen-presenting ability of antigen-bearing cells. Anomalous expression of human leukocyte antigens – G by malignant tissue favours immune breakout by virtually blocking the activities of all immune cells. Exploring a novel treatment for tumors depending on T cell activation should be taken into account (Kim et al. 2018; Sha et al. 2020).

18.4.2 Clinical Implementation of Next-Generation Sequencing Technologies

In the last decade, diagnosis of tumor type by analyzing the genetic expression has been standard practice, like somatic mutations, epigenetic modification, and altered gene expression are key characteristics of the tumor. While genetic assessment of tumor has been restricted to specific biomarkers in use for the treatment, development in the sequencing of nucleic acid and execution of various genome analysis tools empowered for detection of genomic alteration are identification of genomic variation associated with tumor are in the pipeline (Calabria et al. 2016; Paolillo et al. 2016). NGS (next-generation sequencing) provided indications regarding genomic markers and therapeutic targets of novel clinical application on the failure of standard treatment. Sanger sequencing is an accurate and sensitive approach that allows recognition of potential abnormal variants, limited to a single amplimer being explored. Similarly, qualitative & quantitative alterations in gene expression profiling constitute major challenges in tumor studies. Micro-arrays & reverse transcription – polymeric chain reactions are coherent perspectives yet limited to genes present on array (Perera et al. 2018; Niravath et al. 2017).

Genomic abnormalities are uncommon, non-uniform in nature, and distributed across various types of tumors. Prediction of mutations in all tumor types requires a comprehensive, multiplexed, and highly sensitive sequencing technique. Management of a massive amount of genomic database in primary datasets requires complex bioinformatics to analyze the data properly and requires a larger time

frame for therapeutic decision-making. Difficulty in clinical interpretation of a high quantity of non-informative data is a secondary issue. (Lo et al. 1997; Bianchi 2015).

18.4.3 Conducting Biomarker-Driven Clinical Trials

Early phase clinical trials account for the critical connection between the preclinical phase and phase III randomized clinical trials. High attenuation rates and elevated costs, when associated with exceptional possibilities are driven by tumor genomics and assurance of personalized medicines give novel approaches for conduction and phrasing of phase I & II clinical trials. The crucial task arises for the successful and methodical transformation of drug candidates in the drug development pipeline (Tan et al. 2009; Garon et al. 2015). The inclusion of analytically and scientifically validated biomarkers in judiciously developing, hypothesis-testing and conducting clinical trials offers an encouraging way to achieve the desired objective. The establishment of a pharmacological audit trail furnishes a mode for access and management of risk in drug development and increases rationality in decision making. Appropriate preclinical designs are essential for pharmacokinetic and therapeutic efficacy modelling as well as validation of biomarkers. Scientific and analytical validation must ensure the usefulness of the biomarker for the desired activity, according to the stage of development and the impact on the trial (Garrido-Laguna et al. 2011; Fiste et al. 2020). Specifically, they are exploratory or used to make decisions within the trial. To be maximally useful at an early stage, these must be in place before the commencement of phase I trials. Validation and qualification of biomarkers then continue through clinical development (Bailey et al. 2014).

The precision of cancer medicine is highly irregulated due to the abnormal mutation frequency of tumors during phases of clinical trials. Genotype-enriched clinical trial designs classify tumor types according to histology, basket trials, independent of histology, include patients with differential tumor idiosyncrasy to identify matched therapy within the framework of Phase I and II clinical trials. These trials incorporate random strategies to include manual control in cases where patients are being treated with non-targeted therapy, assessing the predictability of biomarkers (Hu and Dignam 2019).

18.4.4 Tumor Heterogeneity and Resistance

Drug resistance is the major challenge in targeted therapy. Tumor cells acquire various mechanisms to resist the targeting agent. Sequential monotherapies offer promise in temporarily addressing problems of acquired resistance, but are limited by the ability of tumor cells to adapt and evolve new resistance mechanisms to persist in the drug environment (Lim and Ma 2019). According to recent studies on drug resistance and progression models, under-targeted therapy is a result of a minute subpopulation of cells that can endure drugs and eventually develop further mutations allowing them to re-grow and become the dominant population in treatment

resistant tumors. This tissue appears to have developed via a sub-clonal mode that results in mutations, which differs from the standard mutation (Parikh et al. 2019). Currently available methods allow for a more comprehensive and holistic analysis of tumor heterogeneity in the issues that are associated with spatial and temporal heterogeneity. Emerging technologies such as liquid biopsy and single-cell methods allow for studying targetable drivers of minimal residual disease and contribute to pre-emptive combinatorial targeting of both drivers of the tumor and its minimal residual disease cells (Meacham and Morrison 2013).

The development of the cancer process involves somatic mutations. Cells that acquire certain mutations gain a survival advantage and dominate localized tumor areas by displacing those lacking these genomic alterations (Dagogo-Jack and Shaw 2018). This process is enhanced by consecutive clonal expansions. According to this model, all cells within a tumor would be biologically similar, and thus equally susceptible to acquiring mutations and spawning new sub-clones. Tumor initiation and progression are based on a relatively minor population of cancer stem cells, which are ancestors of a much larger population of differentiated ones with limited proliferative capacity (Lawson et al. 2018). Targeting single molecular abnormalities or cancer pathways has achieved good clinical responses that have modestly affected survival in some cancers. However, targeting a single hallmark or pathway with a single drug ("magic bullet") will not likely lead to cancer cure (Burrell 2014).

18.5 Perspective

18.5.1 Better Classification of Tumors

After the discovery of the microscope, the cancer was classified as a cellular disorder, after which it got diversified according to cell type. There are many classifications of cancer, but more commonly the classification is based on morphological features, site of the tumor, and cell type (Yuan et al. 1996). However, different patients having the same morphological type may have a diverse clinical cause. Hence the need to recognize the factors may improve the prognostic accuracy needs to be envisaged. Soon after the molecular mechanisms responsible for the uncontrolled cell proliferation came into the picture, the capacity to evade apoptosis, the tendency to invade adjacent tissues, trigger angiogenesis and disseminate, it became pertinent to classify tumors according to these features and this classification may help in allowing a better prognostic rating of the individual patient's tumors (Maeda et al. 2000; Matsumura and Maeda 1986). As the search for new biomarkers increases, analysis of the same will be essential for all clinical trials to better select patient populations for optimal therapy. Currently, tumor heterogeneity continues to be the major challenge that inevitably leads to relapse. To address the heterogeneity of the tumor population, we need to develop complex therapies that would eliminate the bulk of the tumor as well as the critical sub-populations (Greish et al. 2003; Lo et al. 1997; Bianchi 2015).

18.5.2 Simplification and Acceleration of the Drug Development System

More than 500 antineoplastic agents are in the clinical phase of development. The persistent progress of our understanding of uncontrolled cell proliferation and the other key features of the transformed phenotype may cause this number to grow further in the upcoming future. The approved biologics are already expensive, too high for their indiscriminate use in all patients with the same histopathologic tumor type, due to the involvement of high development cost. All these figures point out the need of accelerating the entire process of drug development, so that we will be able to recognize promising agents earlier, exclude inactive agents, and pursue clinical development shooting for registration of a small, selected number of compounds with a high chance of success (Tsao et al. 2007; Li et al. 2011; Gill and Sargent 2006; Oneda and Zaniboni 2019).

18.5.3 Design of Trials

Phase III trials are often randomized when the results of preclinical, phase I, and II results are promising. Value of the target delta of the trial surfaces as a determining factor in the success of the clinical trial. If the delta value is very low (approximately 10%), the chances of success are going to be high, but the number of patients needed to demonstrate this small difference should be very high as well, making the cost and the time high and long. (Donald 2015) Furthermore, even if the trial is successful, the relevance of a 10% improvement may be marginal. Two key questions drive the choice of the target delta in comparative clinical trials, stating 'what is the plausible delta' and 'would that delta be worthwhile for patients (Sahebjam et al. 2013). So far, priority has been given to the first question rather than to the second; this is due to two reasons:

1. The recognition that 'superstars' in the treatment of common solid tumors are the exception and that the progress in oncology is incremental.
2. The higher chances of having a successful trial if we keep the delta low enough to be very plausible (Hirakawa et al. 2018; Renfro and sargent 2017).

The scientific community and other forces of the society have ambivalent positions on these issues; on one hand, there is pressure for early approval of the new agents even in the face of preliminary data or with a non-impressive additional benefit, and on the other hand, the same scientific community state that they cannot afford the use of these agents for the relatively little gain in overall survival offered in the palliative setting. The solution seems straightforward, to raise the currently accepted bar for the target delta in planning comparative trials in the palliative setting of most common solid tumors. In this aspect, the market will not have to face the situation of a new host of expensive compounds with marginal efficacy approved based on large-scale randomized trials showing statistically significant improved efficacy

over standard treatment. Shifting the priority onto the second question, 'how worthwhile this difference is going to be', and thus using a higher delta to make the new treatment worthwhile has a major limitation which states that highly active compounds will be approved, but the cumulative effect of incremental improvements of other agents producing less spectacular results by themselves may be ignored (Burd et al. 2019).

18.5.4 Role of Nano-Formulation

There are various techniques applied in the treatment of solid tumors, of which adjuvant therapy is preferred over other therapies. Along with time due to resistance and the observed patient-to-patient genetic variation, adjuvant therapy also fails at times. Nano-enabled system for delivery of single or multiple antitumor agents can be an affordable solution in the treatment of solid tumors (Banik et al. 2016; Verma et al. 2017). Nanoparticulate systems are advantageous because of improved target-based drug delivery, controlled and sustained release of the encapsulated drug, alteration of physicochemical properties like solubility and bioavailability, altered blood plasma activity, and ability to co-deliver two or more therapeutic agents. The therapeutic efficacy of these nano-formulations is often superior due to their active and passive targeting capabilities (Behera and Padhi 2020).

The periphery of nanomedicine research is broadened; translation of nanosystems from the laboratory level to the bedside is now possible. In many countries, nano-formulations are approved by the medicinal advisory committee. Many nano-formulations exhibited appreciable clinical effects and now replaced many formulations completely (Chen et al. 2013). Out of numerous types of developed nano formulations, polymeric nanoparticles are the most suitable candidates for the treatment of solid tumors because they are easily degradable in biological systems, most of the polymers are derived from natural sources, the structural features of polymers are feasible to modify as per formulation requirement (Padhi et al. 2020). Commonly used polymers are chitosan, polylactic glycolic acid and polylactic acid, lecithin, gelatin, dextran, poly(amidoamine) etc. (Elsabahy and Wooley 2012; Bae et al. 2011). Furthermore, the remarkable number of nano formulations in preclinical investigations persuades the expectation of the forthcoming approval and clinical accessibility of more inventive nano formulations for the treatment of solid tumors (Bregoli et al. 2016; Amer 2014).

18.6 Conclusion

Cancer is a leading cause of death worldwide. Solid tumors are currently treated by adjuvant therapy, which has many loopholes and results in failure of treatment. Patient compliance and adherence to treatment modalities appear to be barriers to

cancer therapy. In the era of advanced drug delivery, nanoparticles are the most popular drug delivery vehicles, as these improve the bioavailability of the entrapped drugs, offer controlled drug release, and end up in patient compliance. The future foresees its multifunctional applications that enclose the required motives of early detection, tumor regression with reduced collateral damage, and structured monitoring of chemotherapy responses.

Hence, nanoparticles in the field of tumor treatment serve as an improvement of the therapeutic window of encapsulated drugs and provide an apt solution for future delivery problems for novel classes of drug molecules, ranging from biotechnology-derived products, natural products, and synthetic new chemical entities. Worldwide efforts are in progress for the clinical management of tumors. The signs of progress are in all aspects of treatment ranging from prevention, care to cure. In some types of solid tumors, the prognosis has improved significantly.

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