Introduction

Effective cancer therapy always necessitates a sound understanding of cancer pathophysiology. Conventional chemotherapeutic agents are extremely limited in their treatment profiles because of their extremely poor solubilities, unfavorable pharmacokinetic profiles and non-specific distribution in the body which ultimately leads to severe toxicities [1]. The complex nature of cancer requires a multi-pronged approach in today’s day and age thus requiring a close interplay between biological scientists, clinicians and biomedical engineers to develop a delivery system that is robust enough to withstand the fair amount of challenges in a complex microenvironment.

Thus, the overall desirable goal is to prolong a patient's survival time, prevent relapse of a cancer episode and concomitantly reduce the toxicities due to chemotherapeutic agents. The compromised vasculature in a cancer environment has to be exploited in order to gain an upper hand to offset the chemotherapeutic drawbacks. The lack of specificity, usually attributed to chemotherapeutic agents can be largely overcome by this drug loaded nanocarriers [2]. Drug delivery accompanied by either passive or active targeting is fraught with many challenges and limitations [3]. The biggest challenge is to properly identify and then direct the chemotherapeutic agent to that particular target. This is usually accompanied by an extremely limited solubility for most of these chemotherapeutic agents, poor pharmacokinetic profile and a generally higher toxicity. These limitations can be overcome by loading these drugs into nanocarriers and allowing passive targeting to occur because of the compromised vasculature. Even if the nanosystem is designed for active targeting to occur, primarily passive targeting occurs first followed by active targeting [4]. Targeted anti-cancer agents, by themselves have found success in recent times with prominent examples such as Gleevec® (Imatinib mesylate), Herceptin® (Trastuzumab) and Iressa® (Gefitinib). Hence, this paves the way and the need for development of a targeted nanocarrier system loaded with these drugs for a better efficacious profile with minimal toxicity.

Adaptation of the Tumor Microenvironment

The deep knowledge and understanding of the tumor microenvironment enables researchers to design strategies based on several different conditions such as underlying pH, vascular irregularities, hypoxic environment, and metabolic state. These morphological changes can be exploited to design drug delivery systems that can be specifically targeted to these regions. Angiogenesis (defined as the formation of new blood vessels from existing ones) is a very important characteristic that allows the tumors to thrive providing them an enriching supply of oxygen and nutrients. It is regulated by a systematic control of activators and inhibitors [8]. Immature tumor vasculature undergoes extensive remodeling resulting in irregular shaped and dilated blood vessels [9]. In 1971, eminent scientist Judah Folkman suggested that tumor growth might be curtailed by prevention of recruitment of new blood vessels [10]. This very finding forms an important basis of active tumor targeting to endothelial cells by nanosystems [11]. During the initial stages of tumor growth, the cells primarily use diffusion to obtain nutrients limiting their size to approximately 2 mm³ [12]. Accordingly, the tumor cells must begin to recruit new blood vessels in a process called angiogenesis. The blood vessels then continue to proliferate rapidly producing a severely irregular and aberrant vasculature [13], thus resulting into regions with high blood or poor blood supply. Tumor vessels can become excessively leaky due to deficient basement membranes and incomplete endothelial linings caused by the extremely compromised ability of endothelial cells to completely envelop the proliferating cells forming the vessel walls.

There are some additional factors present intracellularly at elevated levels, which pose a significant contribution to neo-angiogenesis, thus
recruiting an extensive network of blood vessels that feed the tumor [14]. Some of these factors comprise of vascular endothelial growth factor [15], basic fibroblast growth factor [16], bradykinin [17], and nitric oxide [18]. More notably, vascular endothelial growth factor (VEGF) increases the permeability of blood vessels by causing a significant increase in the quantity of fenestrations or rather minute openings between cells [15].

**Passive Targeting**

It relies heavily on the disease so that there can be a preferential accumulation of the drug loaded nano delivery system at the site of interest to further avoid any non-specific distribution. The Enhanced Permeability and Retention Effect (EPR) was first observed by Maeda and colleagues for the first time, in murine solid tumors discovered that when polymer-drug conjugates were administered intravenously, 10-100 fold higher concentrations could be achieved in the tumor due to the well-noted EPR effect as compared to free drug administration [19]. The permeability of the compromised vasculature and retention can lead to the accumulation of even macromolecules thus increasing their tumor concentration by 70-fold [20]. The foremost advantage in treating cancer with advanced, non-solution based therapies is this very inherent leaky vasculature present in the pathologically compromised cancerous tissues. This leaky and defective vascular architecture created due to the rapid vascularization which is a vital cog to enrich cancerous tissues. This leaky and defective vascular architecture allows the drug to leak into the tumor, size and the surface area of these particles. Conventionally, a particle must be at least 10 nm in diameter to avoid clearance by first pass renal filtration [21]. Passive targeting is largely possible through diffusion-mediated transport, which makes size a critically important factor. Larger molecules such as Dextran can accumulate in the tumor interstitium but will be internalized by the vascular surface. The distribution of smaller particles is usually more homogenous. Generally, the upper maximum limit for nanoparticles to undergo diffusion conveniently is around 400 nm [22]. The optimal size range of 40-200 nm will ensure longer circulation time, increased accumulation within the tumor mass and lower renal clearance [23]. Like particle size, particle shape also governs largely the route through which nanoparticles can be taken up within the tumor. Some of the most recent publications portray the effect that particle shape can have during cellular internalization [24]. For example, the effect of shape and geometry of contact of spherical and non-spherical polystyrene microparticles during phagocytosis by alveolar macrophages was discovered [25]. With these elliptical disk-shaped microparticles, it was noted that when the macrophage initially made a contact with these particles along the major axis, the particles were swiftly internalized in less than 6 minutes. However, when the primary contact was along the minor axis, the particles were not internalized for a very long time ranging up to 10 hours. It is only because of their symmetry that these spherical particles were swiftly internalized. This effect of shape was independent of particle size in this case. The only difference observed as regards to the size of the particles was the extent of uptake, which was only seen in particles in which the volume of the particle was significantly greater than the volume of the cell [25]. Conventionally, it has been noted that a nanoparticle must be at least 10 nm in diameter to avoid clearance by first pass renal filtration [21]. Many factors determine the appropriate size of the nanoparticle to be taken into the cell. As passive targeting is entirely reliant on diffusion-mediated transport into the tumor, size and shape is of foremost importance. Dreher and colleagues have shown that particles in the order of hundreds of nanometers in diameter show a greater propensity to accumulate in the tumor tissue, passively. Using dextran as a model macromolecule they showed that as the molecular weight is increased from 3.3 kDa to 2 MDa, the permeability and extent of penetration of a drug moiety is severely reduced [26]. Larger molecules were able to accumulate but were primarily distributed very superficially, as close as possible, to the vascular surface within the tumor [27]. However, smaller molecules could significantly penetrate more deeply into the tumor interstitium and achieve a more uniform distribution [28]. Novel methods of particle fabrication that allows for a greater control over their shape and size hold a very important role in designing these nanoparticles [29]. Some of the recently published work highlights the importance of particle size and shape on the eventual sub-cellular fate of these nanoparticles. In one of the studies, it was stated that spherical particles were taken up around 5 times more than rod-shaped particles, thus stressing upon the influence of shape of the nanoparticles on the uptake mechanism [30]. Along with particle size, particle shape and the curvature is a major factor in governing the extent of uptake. One of the studies demonstrated the shape-dependent internalization of nanoparticles into HeLa cells. They stated that the cylindrical particles had a greater uptake into the cells [31]. Even the aspect ratio of the nanoparticles plays a very important role in determining their uptake. They stated that particles with 150 nm diameter and 450 nm heights were taken into the cells approximately four times faster than symmetrical particles with an aspect ratio of 200. The general gist of the discussion states that rigid, spherical particles, which are particularly 100-200 nm in size, have the greatest propensity for prolonged circulation because they are large enough to avoid any liver uptake, but at the same time, are optimal in size to avoid filtration in the spleen. The design of non-spherical and/or flexible particles can, however, significantly extend the particle’s circulation time *in vivo* thus governing the biodistribution profile of these particles. For prolonged circulation of particles, uptake by liver and spleen must be avoided. Suitably tailoring the particles to sizes less than 300 nm or by keeping at least one dimension of the particle greater than 100 nm is a preferred way to prevent accumulation in the liver, while still conferring properties to the particle that allow it to navigate the sinusoids of the spleen. Particle size is greatly influences the mechanism of cellular uptake [32,33], namely the cellular internalization process is mediated by either phagocytosis, macropinocytosis, caveolar-mediated endocytosis or clathrin-mediated endocytosis [33], which is a major determinant dictate of the required conditions that a tailored nanoparticle experiences on internalization. Comprehensive information of the mode of entry into the cells is invaluable as this information can be used to fabricate an optimally engineered nanoparticle, which can be further targeted to specific intracellular microenvironments.

Surface characteristics also play a very important role in determining the extent of internalization of these nanoparticles into cells. Relatively, the surface can be modified by the polymer composition, thus governing an extra amount of hydrophobicity or hydrophilicity to these particles. Surface modification of these polymers by addition of Polyethylene Glycol (PEG) has been known to protect the nano-systems from opsonization and subsequent clearance by the Reticuloendothelial (RES) system) [22]. Furthermore, increasing the molecular weight of PEG chains will also increase the circulation time of these nanoparticles. Particularly for negatively charged nanoparticles, this PEG shield will confer more protection and thus prevent immediate clearance of these particles. Passive targeting, thus can be regulated by modifying the size, shape or in some cases, the surface dimensions of these nanoparticles. However, one major drawback of passive targeting is that it may not be able to distinguish the healthy tissue from the...
diseased one just like a chemotherapeutic regimen.

**Active Targeting**

The most important challenge in active targeting is defining the most suitable targeting agent or agents to selectively and successfully transport nanoparticle systems to cancerous tissue thus avoiding any kind of toxicity in the process. These strategies also then rely on the targeting agents’ or ligands’ capability to bind to the tumor cell surface with an extremely strong affinity to trigger receptor endocytosis. With such kind of interactions, the therapeutic agents will then be delivered into the tumor-specific regions.

Active targeting employs some kind of strong interaction such as ligand-receptor or other molecular recognition to confer more specificity to the delivery system. Eventually, it reduces the unwanted non-specific interactions and localization of the drug in peripheral tissues. Active targeting takes advantage of over-expression of certain receptors such as folate on the tumor cell surface. Nanosystems such as polymeric micelles [34,35] can be tweaked with their surface chemistries to confer more specificity. Conventionally, targeted nanocarriers have an edge over their non-targeted counterparts by being more efficacious at the site of delivery and also reducing any potential undesirable toxicities. Folate targeting is a classic example in terms of targeted drug delivery, as it has been extensively tried and tested over the past years. Folate receptor is over-expressed in a variety of cancer types such as ovarian carcinomas, osteosarcomas and non-Hodgkin’s lymphomas [36]. So, particles conjugated to folate receptor have greater chances of being internalized to a substantial extent, wherein the folate receptors are highly over-expressed. It has been reported that folate was conjugated to doxorubicin conjugated poly (D,L-lactic-co-glycolic acid (PLGA) – Polyethylene glycol (PEG) particles. These particles displayed enhanced cellular uptake and circulation times as compared to free Doxorubicin in folate-receptor positive cell lines. The enhanced cytotoxicity displays preferential targeting due to substantial internalization mediated by folate receptor active targeting [37].

Another such example of active targeting which can be done to identify the ideal ligands that serve the purpose of targeting is the development of a comprehensive strategy to screen antibodies from various phage libraries [38]. This method was primarily used to survey 2 antibodies (F5 and C1) to the human breast tumor cell line SK-BR3, which in turn binds to ErbB2, a growth factor that is overexpressed in human breast cancer and also in several other adenocarcinomas [39]. A research study used Doxil which is a commercial liposomal doxorubicin formulation. This liposomal system is then coupled to Polyethylene Glycol (PEG) conjugated to antibody F5. *In vivo* studies demonstrated that in mice treated with F5-coupled Doxil, tumor volume regression was very rapid and significant as compared to mice treated with free Doxil [39]. Similarly, liver targeting can be done through the asialoglycoprotein receptor.

Kim et al. [40] described that the nanoparticles which use the galactose moiety from lactobionic acid, biotin and diamineterminated poly (ethylene glycol) can demonstrate *in vitro* release of A11-transretinoic acid at a fairly constant rate over 1 month.

Aptamers can be also used for targeting using nanoparticles. Aptamers are short oligonucleotides of DNA or RNA that can engage in ligand binding. Through various high through-put processes such as SELEX (Systematic Evolution of Ligands by Exponential Amplification), these ligands can be screened and evaluated against potential targets. For example, prostate cancer cells highly over-express prostate-specific membrane antigen (PSMA) which can be targeted by the highly specific PSMA aptamer. Nanoparticles comprising of PLGA and PEG, encapsulating cisplatin and conjugated to the PSMA aptamer, can be used to target the cancer cells expressing PSMA more specifically as compared to free cisplatin. These kind of aptamer – targeted nanoparticles have greater biological significance in terms of targeted drug delivery [41].

Similarly, peptides have also shown great potential in terms of targeting for delivering various chemotherapeutic hydrophobic agents. These peptides are very cost-effective and easier to synthesize. Arginine-glycine-aspartic (RGD) sequence has a high affinity for αvβ3 integrins, which are highly expressed on tumor cells. Surface functionalization of poly(c-caprolactone)-poly(ethylene glycol) (PCL-PEG) polymeric micelles with this RGD sequence has been used to deliver Doxorubicin in Kaposi’s sarcoma cells. A 30-fold increase was noted in cellular uptake of the surface functionalized PCL-PEG micelles, as compared to non-functionalized micelles. Another such protein known as low-density lipoprotein receptor-related protein (LRP) is highly over-expressed by the blood-brain barrier and glioblastoma multi-forme, a tumor of the pituitary gland. Angiopep-2 is a complementary ligand which can be used for targeting purposes. Increased intracellular uptake was reported for Angiopep-2 conjugated PCL-PEG nanoparticles in U87 MG glioma cells, as compared to non-conjugated nanoparticles. Even when these particles were injected intravenously into mice bearing U87 MG glioma tumor, these targeted nanoparticles exhibited extensive localization in the tumors after crossing the blood-brain barrier, as compared to the non-targeted nanoparticles which showed some preferential localization due to the Enhanced Permeability and Retention effect (EPR) [42]. The conventional techniques used to target delivery of drugs to cancerous tissues may be used in a similar fashion to target imaging agents to the specific organelles. Whenever targeted agents can be deemed to be used in a clinical setting, the first and foremost assessment of the utility of a specific formulation in a particular patient may be undertaken with imaging agents to confirm specifically that the delivery system goes primarily to the cancerous tissues before any distribution to the peripheral organs at the commencement of any drug regimen. Studies using vasoactive intestinal peptide (VIP), which has ten-fold excessive receptors in breast cancer cells than normal breast cells, as a targeting agent for sterically stabilized liposomes have portrayed the fact that both passive and active targeting to breast cancer cells will occur *in vivo* in rats [43].

**Conclusion**

Both active and passive targeting has its own short-comings. There are significant hurdles to passive targeting that result in very low drug concentrations within the tumor leading to diminished efficacy. Similar to chemotherapeutic drugs, passive targeting may fail to clearly delineate between healthy and diseased tissue, thus posing potential toxicity problems. In case of active targeting, increasing the payload within the tumor cells is by no means any guarantee of delivery of the actual therapeutic agent to the target site, as its release may be hindered by the components within the cell. Endosomal escape and the subsequent delivery of the payload is always a challenge for targeted receptor-mediated cellular delivery. Tweaking the surface chemistry of these conjugated nanoparticles may compromise the stealth capacity of these polymers as PEGylation may not be possible to a sufficient extent. Encountering the tumor cells over-expressing receptors or proteins or such other regions of interest, without any barrier, is a big challenge for these targeted nanocarrier systems. If the stealth capacity of these carriers is compromised, then eventually these carriers will be rapidly cleared by the liver, spleen and other RES organs thus showing...
very little accumulation of these drug loaded targeted nanocarriers in tumor cells. Extensive research needs to be undertaken to develop these targeted nanocarriers to favorably alter biodistribution and increase the overall efficacy at the region of interest. Improving the specificity of the carrier, optimizing the loading and optimally tailoring the release of these nanocarriers are of paramount importance to significantly enhance the quality of cancer therapy.

**Conflict of Interest**

The author declares that there is no conflict of interest regarding the publication of this article.

**References**


