**DRUG DELIVERY: A NANOMEDICINE APPROACH**

Sharon Sagnella¹² and Calum Drummond³*

¹Australian Centre for NanoMedicine, School of Chemical Engineering, University of New South Wales, Sydney, NSW 2052
²Children’s Cancer Institute Australia, Lowy Cancer Research Centre, University of New South Wales, Randwick, NSW 2031
³CSIRO Materials Science and Engineering, Clayton South, VIC 3169

*Corresponding author: calum.drummond@csiro.au

**Introduction**

Conventional chemotherapeutic agents often fail, not due to their inability to kill cancer cells, but because of their inability to distinguish cancer cells from normal cells resulting in suboptimal efficacy combined with severe toxic side effects. The development of second-generation ‘molecularly targeted’ chemotherapeutic agents has emerged as one strategy to circumvent this lack of specificity. However, similar to their first-generation counterparts, many of these second-generation drugs are hydrophobic, making formulation difficult, and upon systemic administration, suffer from nonspecific biodistribution, rapid clearance and rapid degradation, in part because of their small size. For these reasons, many second-generation chemotherapeutic agents have largely failed in their quest for enhanced efficacy combined with reduced systemic toxicity (1–3).

In the past few decades, nanomedicine, the exploitation of the unique properties of nanoscale and nanostructured materials in medical applications, has been explored extensively as a promising strategy in the advancement of anticancer therapies with the ability to overcome many of the limitations common to chemotherapeutic agents (2–4). Nanoparticles have the potential to improve the biodistribution of chemotherapy drugs by protecting them from degradation, delivering them directly to the tumour site and/or preventing them from affecting healthy tissues.

Nanomedicine has seen the incarnation of a handful of nanoparticle-chemotherapy drug formulations approved for clinical use, the most well-known being Abraxane® (albumin-taxol nanoparticles) and Doxil® (doxorubicin long circulating liposomes), with several additional formulations currently in clinical trials (2–6). The majority of these formulations have not actually resulted in increased efficacy, but have made definite improvements in reducing toxic side effects (3).

Nanoparticles ranging in size from 1–1000 nm have been designed as drug delivery vehicles from a wide variety of materials including lipid-based amphiphiles (liposomes, hexasomes, cubosomes) (7–9), metallics (iron oxide, gold) (1,10,11), carbon nanotubes (1), mesoporous silicates (12), or polymers (polymer-based micelles, drug carriers, dendrimers) (1,2,13), as depicted in Fig. 1. These systems are designed such that chemotherapeutics are either physically encapsulated within or chemically conjugated to the nanoparticle.

**Targeting Nanoparticles to Tumours**

In order to specifically target nanomedicines to tumours, different approaches have been envisaged, with passive...
and active targeting of cancer cells having been shown to be valid approaches in preclinical and clinical studies (1,3,4,14). Passive targeting exploits the pathophysiological properties of the tumour vasculature which is generally highly disorganised with enlarged gap junctions between endothelial cells and compromised lymphatic drainage allowing for the extravasation of nanocarriers with sizes up to several hundred nanometres. Objects of this size cannot pass through the tight junctions that exist within the endothelial cell lining of the vessels of healthy tissues (Fig. 2) (1–6,13–15). Passive targeting is largely dependent on the ability of a drug nanocarrier to exhibit an increased circulation lifetime resulting in enhanced accumulation at the target site. Circulation time is dictated by the nanoparticle physicochemical properties (size, charge, biodegradability, solubility, shape, rigidity), which can be easily manipulated in the majority of the delivery systems described (1,13). The most common modification used to evade macrophage capture and increase circulation time is accomplished by making the nanoparticle surface hydrophilic through the addition of a polyethylene glycol (PEG) coating on the surface (2,3,13,16). The majority of the nanoparticle-drug formulations used clinically and in development rely mainly on passive targeting.

As a means of increasing recognition of target cells by nanoparticles, active targeting has been implemented. Active targeting utilises specific ligands such as peptides or antibodies that bind to molecules specifically expressed or overexpressed on target cells. Thus, active targeting does not actually improve overall accumulation at the tumour site, but rather enhances cellular uptake of the particles following their passive extravasation due to the leaky vasculature (2,3,17). Transferrin and folate ligands are two examples of commonly used active targeting moieties in nanomedicine formulations targeting tumours (18,19). The only clinically approved actively targeted nanomedicines are antibody–drug conjugates used in the treatment of leukemias and lymphomas (3,20). Currently, no actively targeted nanoparticle formulations are approved for clinical use, with only a small number in clinical trials. Despite the ample evidence and extensive research effort supporting the benefits of both passively and actively targeted nanomedicines in the treatment of cancer, clinically, both strategies have met with only moderate success. This is likely due to the fact that the complexity of the tumour microenvironment (tumour heterogeneity, vascularity, location) is commonly overlooked and will have a major effect on nanoparticle extravasation, accumulation, and penetration into the tumour. The tumour microenvironment is highly heterogeneous in composition with as much as half of its volume occupied by non-cancerous cells and dense extracellular matrix (Fig. 3) (3,14). Furthermore, the hyperpermeable nature of the tumour vasculature, while being ideal for allowing nanoparticles to enter into tumour tissue, also allows fluid to leak from the vessel into the tumour microenvironment, thereby causing extraordinarily high interstitial pressure throughout the tumour interior (3,14,21). The interstitial pressure tends to increase with increasing tumour volume and remain lower in the outermost areas of the tumour. Finally, malignant cells within solid tumours tend to be tightly packed and are heterogeneous in nature (3,14). Thus, while the leaky nature of tumour vessels can promote nanoparticle deposition and accumulation, the microenvironment creates a number of barriers that prevent these delivery systems from effectively accessing tumour cells and thus reaching their full potential as the ‘silver bullets’ of anticancer therapies.

Beyond Tumour Targeting
As the understanding of how the tumour microenvironment prevents nanoparticles from accessing their targets improves, strategies can be developed to bypass these barriers. While the initial research effort into the utilisation of nanoparticles as drug delivery vehicles focused mainly on passive and active targeting of tumours, in more recent years, studies have slowly begun to design...
nanoparticles that can address and overcome the current limitations of nanoparticles as drug delivery vehicles.

One strategy that has been employed which can circumvent many of the barriers encountered by nanoparticles upon extravasation from the tumour vessels is to target nanoparticles to the tumour vasculature (3,21). Tumour blood vessels tend to express or overexpress certain cell surface and extracellular matrix proteins that are either not present or present only at low levels in normal vessels, making them ideal as potential targets. Since the luminal surface of tumour vessels is completely accessible to circulating compounds, nanoparticles targeting the tumour endothelium can bind to their target molecules without the need to penetrate into the tumour to deliver their contents. This feature eliminates the problems associated with passing through the multiple cell layers between the endothelium and the tumour cells, penetrating the high cell density common within tumours, obstruction from the extracellular matrix, and high interstitial pressure. Tumour vessel targeting has been accomplished through the addition of antibody fragments and peptides that bind to tumour vessel associated extracellular matrix proteins such as the EDB (extra domain-B) domain of fibronectin and the fibrin–fibronectin complex, as well as peptides designed to bind specifically to receptors and molecules that are highly expressed on tumour endothelial cells such as certain integrin receptors, aminopeptidase-N (CD13) and nucleolin (2,3,21).

While there are some obvious benefits to this strategy, it is not a suitable option for avascular or poorly perfused tumours. Furthermore, chemotherapeutics delivered within the local tumour vessel environment via nanoparticle targeting to tumour vessels can still suffer from poor intratumoural distribution. Studies have shown that chemotherapy drugs themselves generally only penetrate about three to five cell diameters in from the blood vessel, with little to no drug reaching distant tumour cells and this can result in the development of drug resistance (21,22).

The ability of nanoparticles to carry their contents deep within the tumour would be of huge benefit to enhancing the efficacy of chemotherapeutics. Certain active tumour vessel targeting peptides have been suggested to possess tumour-penetrating properties as well. Ruoslathi et al. demonstrated that the addition of a cyclic peptide called iRGD, which contains an arginine-glycine-aspartate (RGD) sequence, to the nanoparticle surface resulting in a system that first targets integrin receptors in the tumour vasculature via the RGD motif. Upon binding, the sequence undergoes proteolytic cleavage of the peptide, exposing a new binding motif specific for neutrophilin-1, allowing for deep penetration of the tumour tissue (21–23).

Additionally, studies have begun to focus on developing an understanding of how nanoparticle characteristics such as size, shape, and surface properties affect their ability to penetrate into tumours. It has been demonstrated in vivo in a small number of studies that only extremely small particles (those with diameters of 12 nm or less) can properly penetrate into tumours (3,11,24). Unfortunately, nanoparticles within this size range will be rapidly cleared through the kidneys and thus are unlikely to be able to effectively accumulate within the tumour, creating a ‘nanoparticle size paradox’ in which larger particles...
are necessary for increased circulation time while small particles are necessary for tumour penetration. Wong et al. have attempted to address this paradox through the development of 100 nm ‘multistage’ nanoparticles designed to be broken down by tumour-associated proteases into smaller 10 nm nanoparticles following extravasation, which can then effectively fully penetrate into the tumour (25). Similarly, Sagnella et al. have developed amphiphile pro-drugs that self-assemble into nanoparticles of ~200–300 nm, which are then degraded into smaller bioactive molecules by enzymes specific to the tumour site (9).

In addition to size, shape and surface charge can also affect tumour penetration. Studies have indicated that particle aspect ratio can play a role in tumour penetration, with higher aspect ratio particles (i.e., those that are more cylindrical rather than spherical) exhibiting improved penetration (26). Similarly, negative surface charge has been shown to be of benefit for enhancing penetration as well (3). However, the role of nanoparticle characteristics on tumour penetration is still poorly understood and more research is necessary to elucidate how these different properties can be altered to maximise tumour penetration.

Pre-treatment of tumours with drug, enzymes or inflammatory mediators or co-delivery of these molecules with nanoparticles has been implicated in increasing the ability of nanoparticles to deeply penetrate into tumours by breaking down the dense extracellular matrix barrier and/or increasing interstitial space. In a number of studies, the co-delivery of matrix-degrading enzymes such as collagenase, gelatinase and hyaluronidase has been shown to significantly enhance intratumoural transport of nanoparticles thereby greatly improving their efficacy (3,24,27). Similarly, pre-treatment of tumours with the hormone relaxin, which causes changes in collagen structure, resulted in a 2–3 fold increase in the delivery of large macromolecules (3,14). Tumour priming with low doses of the chemotherapy drugs paclitaxel and doxorubicin is yet another method which has been successfully used to expand interstitial space and enhance the penetration of larger nanoparticles ranging from 85–200 nm in size (28).

A final area that has been explored for enhancing nanoparticle delivery and efficacy in tumours is by designing systems that can be triggered to release their contents upon application of external stimuli such as heat, light, magnetic fields or ultrasound (29–31). Drug release can be restricted to a specific region by confining the external stimulus within that region. Furthermore, these external stimuli have been demonstrated to help improve the ability of larger nanoparticles to effectively distribute throughout the tumour (14,30). The most successful example of this type of system is Thermodox®, a temperature-sensitive PEGylated liposomal doxorubicin delivery system currently in Phase 3 clinical trials (3). While this strategy holds a lot of promise, stability of such systems and difficulties associated with effectively and specifically applying loco-regional stimuli have mostly prevented them from clinical success.

Conclusion

The use of nanomedicines in localised drug delivery has received a lot of attention over the past couple of decades and resulted in several clinically approved formulations. These systems have been shown to have a number of advantages over conventional chemotherapeutics; however, they have not yet reached their full potential as anticancer agents. This is likely due to the fact that until more recently, features of the tumour microenvironment that can create barriers to effective nanoparticle delivery have been largely overlooked. With improved understanding of how the tumour microenvironment affects nanoparticle delivery and distribution within tumours, strategies can be developed to better address and overcome the shortcomings of current delivery systems. Thus, future anticancer therapies using nanomedicine can be envisioned to specifically kill all cancer cells within the tumour while leaving normal tissue in the body virtually untouched.

References


References continued on page 20
References continued from page 8