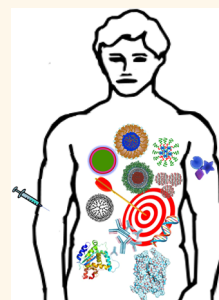


Facing the Truth about Nanotechnology in Drug Delivery

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ABSTRACT Nanotechnology in drug delivery has been manifested into nanoparticles that can have unique properties both *in vitro* and *in vivo*, especially in targeted drug delivery to tumors. Numerous nanoparticle formulations have been designed and tested to great effect in small animal models, but the translation of the small animal results to clinical success has been limited. Successful translation requires revisiting the meaning of nanotechnology in drug delivery, understanding the limitations of nanoparticles, identifying the misconceptions pervasive in the field, and facing inconvenient truths. Nanoparticle approaches can have real impact in improving drug delivery by focusing on the problems at hand, such as enhancing their drug loading capacity, affinity to target cells, and spatiotemporal control of drug release.



It is debatable when nanotechnology, as we now know it, began. Perhaps, we can trace the beginnings to the invention of the scanning tunneling microscope^{1,2} in 1980, as it and the subsequently developed atomic force microscope³ enabled manipulation of individual atoms and molecules. The nanotechnology fever we are experiencing now began when the United States launched the National Nanotechnology Initiative,⁴ the world's first program of its kind, in 2000. Since then, we have been bombarded by the dazzling images and cartoons of nanotechnology, such as nanorobots killing cancer cells resembling the plot of *Fantastic Voyage*. Tens of thousands of articles have been published on nanotechnology, and the press feed the public a steady diet of potential advances due to nanotechnology.

In this Perspective, the focus will be on drug-delivery aspects of nanotechnology, specifically, targeted drug delivery to tumors using nanoparticles. Nanoparticles designed for drug delivery have been called by many different names, including nanovehicles, nanocarriers, nanoconstructs, nanospheres, *etc.* Here, "nanoparticle" is used to represent all of these different formulations, including liposomes, polymer micelles, emulsion, and solid particles made of chitosan or poly(lactic-co-glycolic acid) (PLGA). Almost all papers on such nanoparticles end up with the same conclusion: nanotechnology has great potential for drug delivery. It is true.

The question, then, is to ask what can be done to turn this potential into tangible outcomes, *i.e.*, formulations that can benefit patients. It would be counterproductive only to talk about the potential for another decade. To achieve tangible outcomes, they first need to be defined. This, in turn, requires understanding the goals, which may depend on individuals.

Why Do Scientists Do What They Do? Scientists and engineers do their work because they love what they do. If the goal of research on nanotechnology is just to make something nano, new, and more complicated, then the progress made in the past decade has clearly achieved the goal, at least in part. The ultimate goal of any research in drug delivery, however, must be to develop drug-delivery systems, nanoparticulate systems in this case, to prevent, control, and treat debilitating diseases. Most scientists working in the pharmaceutical and biotechnology sectors, as well as in academia, want to develop nanoparticle formulations that can deliver drugs more effectively to the target site for enhanced efficacy and reduced side effects.

There are many diseases that need to be addressed. Diabetes patients still have to poke their fingers to measure blood glucose levels and to inject necessary quantities of insulin multiple times a day. Can this be made easier through nanotechnology? Heart disease is the leading cause of death in the United States. Can nanotechnology lower the mortality rate? Alzheimer's disease devastates not only the patients themselves,

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but also the patients' family members and friends. Can this disease be identified early and be treated effectively *via* nanotechnology? Many prescription opioid drugs are widely abused. Can nanotechnology be used to develop abuse-deterrent formulations? Cancer claims millions of lives each year. Can this be prevented by nanotechnology? Unfortunately, nanotechnology, with all of its hype and unwarranted high expectations, has not yet produced anything significant to deal with these issues. It is common to see studies on nanotechnology that just make things more complicated while achieving less than what traditional non-nanotechnology can do. Each investigator needs to have a clear goal in what they are doing, rather than simply making things more nano.

What Is Nanotechnology in Drug Delivery? Of the many subareas in drug delivery, most nanotechnology research has been focused on targeted drug delivery to tumors. This specific area will be used to define a goal and to assess the progress of nanotechnology in the past decade.

Quite frequently, Doxil and Abraxane have been used as examples of nanotechnology-based drug-delivery systems, mainly because they are in the nanometer size range. The development of Doxil, a PEGylated liposome formulation, began in the early 1980s and was approved by the US Food and Drug Administration (FDA) in 1995.⁵ The main reason for approval was the equivalent efficacy and reduced cardiotoxicity or improved safety profiles as compared with free doxorubicin.⁵ The promise of nanotechnology in drug delivery is to deliver a drug selectively to the target site for enhanced efficacy with reduced side effects. In that sense, a portion of the nanotechnology promises were achieved. Liposomes have been known for 60 years,⁶ and PEGylation has been known for 40 years.⁷ Abraxane is a simple formulation based on oil/water (o/w) emulsion.⁸ Paclitaxel-dissolved methylene chloride is

emulsified in albumin-dissolved aqueous solution to form an o/w emulsion, and subsequently homogenized to form nanodroplets. Albumin-coated paclitaxel nanoparticles are obtained by evaporating the solvent under reduced pressure. Albumin-coated nanocrystals can also be formed by simply adding albumin (as a surface modifier) to coarse drug crystals during milling⁹ or to the formed nanocrystals. The size of Doxil and Abraxane is certainly at the nanoscale, but neither of these formulations was inspired by modern nanotechnology. They were prepared by methods that were already widely practiced before the concept of modern nanotechnology evolved. Does any drug-delivery formulation become a nanotechnology system just because the size is at the nanoscale, regardless of how it is made? If that is the case, the current nanotechnology in drug-delivery systems is just a name change without any technological advances.

Understanding the Limitations of Nanotechnology. New generations of scientists need to focus on solving the problems that are indeed worthwhile. Tom Friedman, in his book *That Used To Be Us*,¹⁰ states, "One thing we know for sure: The path to a happy ending begins with the awareness that something is wrong, that changes are necessary, and that we the people have to be the agents of those changes" (p 348). One problem now is how nanotechnology is perceived.¹¹ The next generations of scientists will have to be the agents of these changes.

One of the changes to be made is to stop spreading inaccurate information. Findings made in this area, which may be true only under limited experimental conditions, are frequently inflated and overblown with the futuristic rhetoric by the press and the media. Such rhetoric may be necessary to attract the public's attention to nanotechnology and, thus, more funding, but these statements create unintended side effects. Researchers may be forced to create fiction-like stories for funding, instead of

proposing solutions to real problems. Many reviewers at funding agencies, without a clear understanding of the field, may demand something innovative over the existing technology. It will be extremely difficult, for example, to propose something better than making nanoparticles that "selectively lock onto only the cancer cells".

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History of Drug-Delivery Technologies. A brief overview of the history on controlled drug delivery provides some insight into how the current nanotechnology-based drug-delivery systems have evolved. As shown in Figure 1, the drug-delivery discipline is 60 years old. The first generation (1G) of drug-delivery systems was developed from the early 1950s to the end of the 1970s. During this time, the basic mechanisms of controlled drug release were established. Most of the drug-delivery formulations were for oral and transdermal administration, and thus, the duration of drug release ranged from 12-h (twice-a-day) oral formulations to 1-week transdermal formulations. Since then, numerous clinical products for oral delivery have been introduced to the market. The second generation (2G) from 1980 to 2010

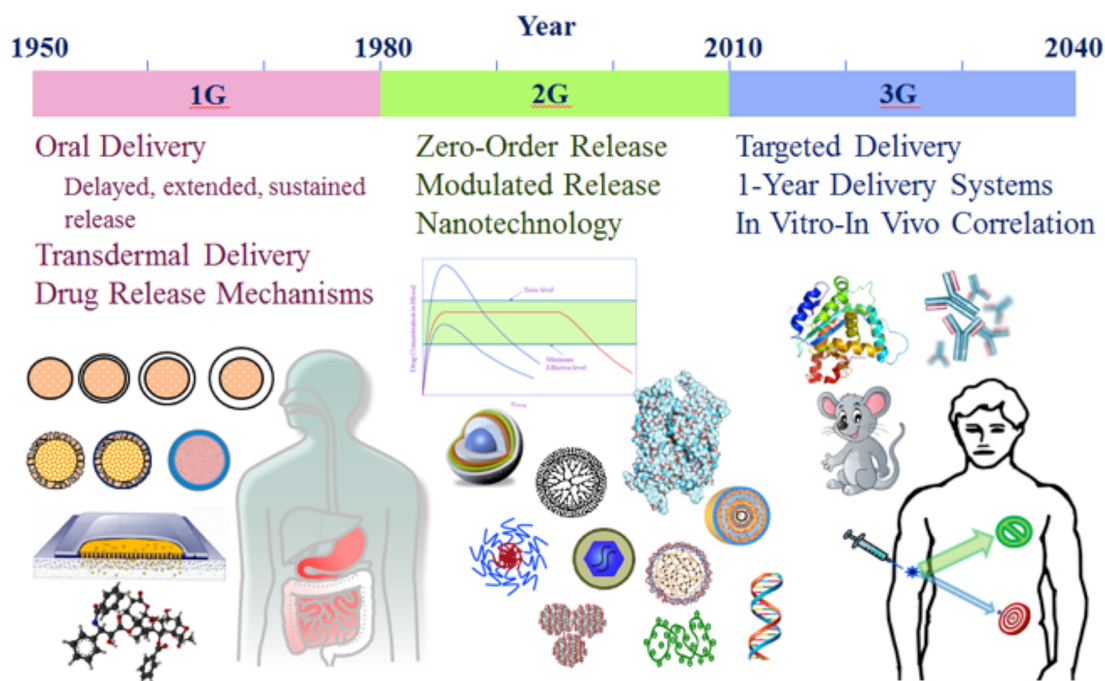


Figure 1. Evolution of controlled drug-delivery systems. Adapted with permission from ref 12. Copyright 2013 Springer.

was not as successful at introducing useful clinical systems. Extensive efforts were made to develop zero-order release techniques, which turned out not to be necessary. A dozen extended release depot formulations were developed, but this was minor compared with the thousands of oral controlled-release formulations available to patients. Other efforts on modulated (*i.e.*, self-regulated) drug-delivery systems, *e.g.*, glucose-dependent insulin delivery systems, have not been fruitful. This is mainly due to the difficulties associated with making an implantable closed system that has both glucose sensing and insulin release controlling abilities. At the turn of the 21st century, the National Nanotechnology Initiative initiated the current nanotechnology fever. During the fever, “new and innovative” often meant “nano and complicated”. The assumption was that the nanosized materials would have properties different and unachievable by microsized and larger materials. The assumption was thought to be reasonable, and thus, making something nano was all that was required at that time.

Convenient Misconceptions. In the area of targeted drug delivery, nanotechnology fever was fueled by an observation of the behavior of nanoparticles in tumors in mice, known as the enhanced permeation and retention (EPR) effect.¹³ The EPR effect is considered to be responsible for increased delivery of nanoparticles to targeted tumors in mouse experiments. This notion evolved into an idea that only nanoparticles have the EPR effect. Careful analysis of the original data, however, indicates that albumin and IgG are actually better in accumulating at the tumor site.¹⁴ It is also thought that PEGylated nanoparticles increase their blood circulation times, which in turn may enhance the EPR effect.¹⁵ Thus, it has been widely assumed that PEGylated nanoparticles having the EPR effect will result in an enhanced tumor-killing effect, and therefore, the problem of targeted drug delivery to tumors was partially solved. The reality is that these assumptions have produced numerous research articles but have made no significant advances in translation into patient treatment.¹⁶ These convenient misconceptions have to face the inconvenient truth.

Inconvenient Truth. Nanoparticle formulations, as compared with solution formulations, increase the drug concentration around a tumor by 100–400% (Figure 2 circle). These increases are phenomenal by any measure. What is missing here, however, is the big picture showing the full story on drug delivery. It should be understood that >95% of the administered nanoparticles end up at sites other than the targeted tumor (Figure 2); this fact has been largely overlooked.¹¹ Clinical applications of Taxol, Taxotere, Abraxane, and Genexol show that the latter two nanoparticle formulations have similar performance to the first two, which is based on solution formulations. The amount of a drug delivered to the target tumor may be about the same for different formulations. Taxol, Abraxane, and Genexol deliver paclitaxel, while Taxotere delivers docetaxel, a derivative of paclitaxel. Nanoparticles may provide an alternative way of making aqueous solution formulations for intravenous administration of poorly soluble drugs without using undesirable excipients, such as polysorbate 80 or cremophor EL.¹⁷ This is a great use of

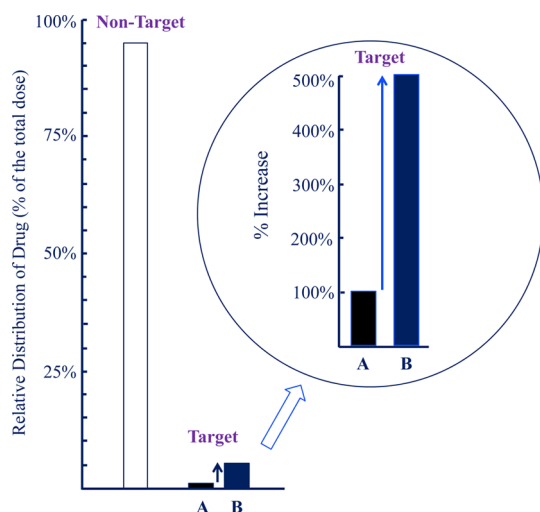


Figure 2. Relative distribution of a drug at a target tumor site by (A) conventional solution formulation and (B) nanoparticulate formulation. The majority of the administered drug ends up at nontarget sites, but the 5× more efficient delivery of the drug by nanoparticles can be exploited for maximizing drug efficacy. Adapted with permission from ref 12. Copyright 2013 Springer.

nanoparticle approaches. It is simply different than the widely believed notion that nanoparticles would be far superior to nonparticulate solution formulations.

OUTLOOK AND FUTURE CHALLENGES

Turning the potential of nanoparticle systems into clinically useful formulations requires setting up clear, realistic goals. The challenges in targeted drug delivery using nanoparticles can be overcome through understanding the limitations of nanoparticle approaches and maximizing the existing capabilities of nanoparticle formulations.

Exploit the 5% Reaching the Target Tumor. Nanoparticles go to target tumors simply as a result of blood circulation. Thus, the percentage of the administered drug reaching the tumor is similar regardless of the formulation type. The nanoparticles remain around the tumor longer, because they do not diffuse back into the bloodstream as easily as dissolved drug molecules. This results in more accumulation of the drug near the tumor site. Assuming 5% of the total administered nanoparticles can end up at the tumor site, one can make a nanoparticle system a clinically useful formulation. Currently, the drug loading in

most nanoparticles is not high, usually around 10%. If the drug loading can be increased by a factor of 5, it is the equivalent of delivering 25% of the total administered nanoparticles with 10% drug loading. For example, instead of loading a drug into liposomes or polymer micelles, one can use the drug nanocrystals themselves, which deliver 100% of the drug.¹⁸ The surface of the nanocrystals may need to be modified by polymers or proteins for enhancement of their affinity to cells or their stability.¹⁹ The percentage of the drug may decrease, but the majority of the total weight will be the drug. This approach, of course, delivers more drugs to other tissues, too, and this is where the reduced toxicity by nanoparticle approach is important. It is necessary to develop nanoparticle formulations having significantly reduced side effects by controlling the drug release depending on the environment. The drug delivery field can be advanced rapidly by making nanoparticles with high drug-loading capacity and the ability to control the drug release.

Once the tumor site is reached, nanoparticles need to be cleared from the site after releasing the loaded drug. If the empty nanoparticles remain at the same site due to the low clearance rate, they may

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present a physical barrier for delivery of additional nanoparticles that are freshly administered. Extravasated liposomes 90 nm in diameter were observed to remain near the blood vessels even after 1 week.²⁰ The study of *in vivo* degradation of nanoparticles, made of PLGA (L:G = 50:50, $M_w = 44\,000$ Da) sized 200 and 500 nm, indicates that more than 1/3 of the administered nanoparticles remain not degraded after 1 week.²¹ Thus, nanoparticles need to be designed to undergo timely clearance from or degradation at the target site. Currently, little attention has been paid to this property.

Entering the Tumor Cells. For a drug to be effective, it needs to enter the tumor cells. Thus, improving the cellular interaction, leading to cellular uptake, is another necessary innovation. In an attempt to maximize interactions with the cell, a new nanocage approach was developed. In this issue of *ACS Nano*, Professor In-San Kim and his group describe a nanocage they designed that displays a high affinity to cell receptors.²² Specific peptides identified by phage display were genetically fused onto the surface of cage proteins. Symmetrical assembly of the cage proteins forms clusters of the peptides in bunches. The resulting peptide bunches on

the nanocage synergistically increase the affinity of the peptide ligands, leading to substantial increases in therapeutic efficacy. If such a nanocage can be grafted to the surface of drug nanocrystals, the therapeutic effect will be enhanced considerably.

The high affinity of nanoparticles to the cell surface may have the added benefit of increasing the intratumoral distribution of the nanoparticles. Extravascular transport, and thus the tumor-targeting efficiency of nanoparticles, depends on the nature of targeting ligands attached to the nanoparticle surface.²³ Receptor-mediated transcytosis can facilitate extravascular transport of nanoparticles, leading to enhanced nanoparticle delivery to solid tumors. It overcomes the barrier to efficient dispersion of nanoparticles in tumor interstitium. The efficient delivery of nanoparticles into the tumor interstitium exposes tumor cells to lethal doses and makes them less susceptible to the development of resistance. The presence of agonists on the nanoparticle surface may not improve the delivery from blood circulation to the target site, but can enhance subsequent extravascular transport.²⁴

Dealing with Biological Issues. Even if nanoparticles are designed to have high affinity to the tumor cell surface, the actual interaction between the two occurs when the tumor cells express the receptors. It needs to be understood that not all tumor cells express receptors. More importantly, tumor cells may not have overexpressed receptors at the time of nanoparticle arrival. The heterogeneity in tumor cells themselves and temporal receptor overexpression are not an easily addressed problem.²⁵ Thus, nanoparticles with the ability to control drug release depending on environmental conditions become even more important.

Time To Be Realistic. A few clinical studies were done to test the newly developed nanoparticle formulations. For example, a thermo-sensitive

liposome formulation, which showed excellent efficacy in mouse models,^{26,27} was tested for its efficacy in clinical studies. Patients were treated with heat before administration of the low temperature-sensitive liposome formulation. The result was not as good as expected and did not meet the goal of demonstrating evidence of clinical effectiveness.²⁸ For this approach to be successful, it may require fine-tuning of the procedure to maximize the usefulness of the liposome properties. Apparently, the optimal condition found in small animal studies was not optimal in a clinical application. The differences in size and other variables between small animals and humans may require changes in the time and duration of heat exposure. Enormous resources required for clinical studies, however, prevent repeated clinical experiments. This necessitates development of improved animal and *in vitro* models that can provide better predictions on nanoparticle efficacy in humans. It is difficult, as well as unnecessary, to develop a single model that represents all aspects of human physiology. It will be more than sufficient if each model can predict one or more aspects of nanoparticle behavior in humans. Microfluidic devices can test the effectiveness of nanoparticles to extravasate from the blood vessel into the surrounding tissues and subsequent clearance from the site.^{29,30} A three-dimensional tumor spheroid model can be used to examine how effectively nanoparticles interact with the cells on the surface and achieve intratumoral distribution.³¹

One thing that nanoparticle scientists need to realize is that clinical application of any formulation requires approval by the FDA or its equivalent overseas. The safety and efficacy of new formulations must be proven through controlled clinical studies. Pharmaceutical and biotechnology companies prefer using excipients that have already been used in clinical products

In this issue of *ACS Nano*, Professor In-San Kim and his group describe a nanocage they designed that displays a high affinity to cell receptors.

approved by the FDA. In this way, there will be little concern about the safety and toxicity of the excipients themselves. This brings another constraint in developing clinically useful nanoparticle formulations. By understanding the many limitations and constraints in developing clinically useful formulations, nanoparticle scientists can have a better perspective in their pursuit of finding the next generations of drug-delivery systems. Achieving “the next big thing” starts with being realistic now. There simply needs to be an understanding that overcoming the enormous difficulties involved in clinical applications of nanoparticles requires more than just rhetoric and pretty pictures.³²

Conflict of Interest: The author declares no competing financial interest.

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