Front. Chem. Sci. Eng. 2014, 8(3): 265–275
DOI 10.1007/s11705-014-1442-x

REVIEW ARTICLE

Barriers to advancing nanotechnology to better improve and translate nanomedicines

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Abstract Engineered nanomaterials and nanotechnologies promise many benefits to enhance both *in vitro* and *in vivo* performance. This is now manifest in the increasing number of reported biomedical products under development and testing that contain nanotechnologies as their distinguishing performance—enhancing components. In many cases, nano-sized materials are selected to provide a specific functional aspect that contributes to improved medical performance, either *in vitro* or *in vivo*. Nanoparticles are most commonly exploited in diverse roles in topical lotions and creams, solubilization aids, for *in vitro* and *in vivo* diagnostic and targeting agents in nanomedicines and theranostics. Despite fundamental scientific excitement and many claims to nanotechnology-based improvements in new biomedical applications, several fundamental and long-standing challenges remain to be addressed using nanomedicines to make clinically important progress. This review addresses several issues that must be fairly and objectively reported and then overcome to provide truly credible performance for nanomedicines.

Keywords nanotechnology, nanomedicine, drug delivery, therapeutic, targeted delivery

1 Introduction

Nanotechnology is now widely recognized as a transformative technology platform to improve many life science innovations. In less than a decade, nanotechnology has established itself as a central feature intended to enhance product performance in many applications, including food technology, cosmetics, surfaces, new materials and medical products. The term "nanomedicine" is broadly used to refer to the collective medical applications emerging for nanotechnology, although no standard definition or consensus on meaning currently exists. These include pharmaceuticals and drug delivery products, medical imaging, theranostics and diagnostics, implantable materials, sensors and devices, regenerative medicine, and combinations of technologies comprising several of these features into single platforms. To date, nearly 250 nanomedicine products are either approved for human use or still in human testing seeking such regulatory approvals [1]. Typically, nanomedicine's context specifies exploitation of nanoscale components to enable unique technological or performance gains not possible using larger component sizes. Of these possibilities, nanoparticles have been the primary initial focus for medical use, exhibiting versatility as diagnostic and imaging tools; nanoscale drug forms and targeted drug delivery systems; antimicrobial medical implants, dressings and textiles; bone substitutes and dental materials; coatings for implants; and tissue repair/regeneration composite structures. However, this list generally disregards biomedical innovations that might contain nanotechnology but where application of nanotechnology is not the primary enabling feature to that technology performance. Over 50 nanomedicine-qualified products are currently FDA (Food and Drug Administration)-approved for use [2–4], although some of these (e.g., superparamagnetic iron oxide nanoparticle (SPION) image contrast agents) have been withdrawn from the market. A recent comprehensive analysis [1] identified 789 current clinical trials seeking human data for nanomedicine applications or products addressing 141 unique applications and products (and many products co-associate with multiple trials). Thirty-eight of these were already approved products seeking approvals for new conditions or serving as active comparators for new products. Another 103 products were new investigational products. By combining clinical trial data with another 222 unique applications and products identified through extensive literature searches, these authors identified a total of 363 possible nanomedicine applications and products [1].

Received August 11, 2014; accepted August 20, 2014

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To date the largest deliberate human administration of nanomaterials (in contrast to nanomedicines) to human tissues by volume is as topical cosmetics, lotions and creams. Another major nanomaterials medical market is *in vitro* diagnostics aided by nanoparticle assay detection or analytical separation functions. This emphasis likely stems from the reduced regulatory burden and procedures required to get these products to market. Currently, the most activities in nanomedicine research and product development are found in new cancer treatments, imaging contrast agents, and biomarker detection [1,3,4]. The substantial efforts focused on nanomedicine development, especially in cancer diagnosis and therapies [1], and increased regulatory scrutiny with the associated clinical trials costs, have not yet translated into comparable progress or clinical impact. Costly development of these technologies to prove both safety and efficacy, and their high production costs associated with their complex biomaterials, designs and quality control all together produce risks associated with their possible failure at many stages of maturation. Thus, both financial and technical de-risking of these new technologies is essential to allow them opportunities to succeed. Importantly, nanotechnology is not an appropriate answer to addressing many medical unmet needs: it must be chosen judiciously where it is best suited to function and performance in context. Lastly, while convention and the US National Nanotechnology Initiative define nanotechnology as comprising components sizes less than 100 nm. In many applications, the driving nanotechnology is larger than 100 nm [1]. Again this definition is merely an arbitrary formality, one not abided by in FDA approval decisions or performance requirements that appeal more to drug function and safety, not necessarily its size or form. In this review, we summarize current issues for nanoparticle applications in nanomedicine and discuss the challenges for asserting their clinical utility.

2 Unmet drug delivery needs effectively and credibly addressed by emerging nanomedicine strategies

Little evidence to date suggests that nanotechnology is a "one-size-fits-all" strategy for improving medicines. In as much as it is appropriate to use nanotechnology to address certain select clinical needs, it is simply not appropriate for many others. Therefore, appropriate selection and matching of specific nanotechnology's strengths and capabilities with clinical challenges is complicated, and must be done with insight, intelligence and careful mutual assessments. Appropriate use of nanotechnology will dictate best commercial push versus the observed "hype pull" (i.e., the Gartner "hype cycle") that has led to exaggerated expectations for nanomedicine in the past [5]. Some nanotechnologies have already clearly made credible clinical impact in nanomedicines and some of these are described in this section.

2.1 Improving solubility for poorly soluble drugs

Some 40% of new drugs under development are characterized as "brick dust"—extremely poor water solubility. These poorly soluble compounds, while interesting as agents in cell cultures, are often a "no-go" for further therapeutic translational development. Nano-milling and nano-crystallization approaches to solid drug are now used to reduce drug solid particle size and increase solid surface areas to increase their dissolution rates. While this strategy is relatively new, it represents a major thrust in current nanomedicine. Importantly, for poorly water-soluble drugs in particular, the utility of this size-reduction strategy has been validated for over a decade [6–9]. Significantly, particle size-reduction strategies for drugs are not known to alter drug chemistry, but instead their physical presentation in solid dosage forms.

Several techniques are known producing nanometer-sized drug particles, with all commonly generating solid particles stabilized by either steric or ionic stabilization strategies. The general intent in size reduction for poorly water-soluble compounds exploits the exponentially increased surface area resulting for particles in nanometer sizes. The classic Noyes-Whitney predictions for dissolution predict that particle surface area is directly proportional to its rate of dissolution. The strategy is that drug particles reduced to nanometer size ranges dissolve more rapidly. Beyond increasing dissolution rates, kinetic solubility enhancement is also reported when the particle size reaches the nanometer size range. Both Ostwald-Freundlich and Kelvin equations correlate particle size and particle curvature with solubility [10–11]. Based on accepted thermodynamic and particle physical chemical principles, many nanosized drug particles exhibit distinctive fundamental properties compared to their respective bulk materials [12]. Current clinically approved drug products exhibiting performance issues resulting from intrinsic poor drug solubility will benefit from reformulation into nanosized dosage forms [13]. While enhancing bioavailability and therapeutic index through dosing changes, this strategy also provides opportunities for new intellectual property and extended patent life to old compounds.

2.2 Nanoparticles in topical suncreens and cosmetics

Nanotechnology use in direct human tissue contact was adopted early by the personal care and cosmetics products industry. Nano-sized cosmetic ingredients provide enhanced properties to topical lotions, creams and make-up, such as innovative and improved coloration, better solubility, texture, stability and transparency, deep stratum corneum skin penetration, sustained ingredient release and

better finish quality [14,15]. In sunscreen formulations, nanomaterials provide better UV protection and visual transparency [16]. Many types of nanoparticles have been applied in cosmetic development, including liposomes, nanoemulsions, nanocapsules, solid lipid nanoparticles, nanocrystals, nanosilver and nanogold, dendrimers, hydrogels, and buckyballs [14]. With an intrinsically lower regulatory barrier (and sometimes none at all) compared to drugs and medical products, many cosmetic products with these nano-ingredients have been brought to market and widely adopted in consumer lifestyles. A general consensus view is that dermal penetration of nanomaterials in normal skin is minimal [17]. However, cosmetic nanocarriers have been modified with enhancers with largely increased skin penetration ability. Nanoparticles are reported to reach liver cells through intact skin [18,19], implying a chance of induced further complications. Therefore, safety concerns seemingly remain unaddresses so that nanocosmetic regulation might better take these issues into consideration [15].

2.3 Nanoparticle image contrast agents

Several different types of nanoparticles have been developed and approved for clinical use to enhance *in vivo* magnetic resonance imaging (MRI) contrast. These particles include superparamagnetic iron oxide nanoparticles (SPIONs) [20,21] and gadolinium-based nanoparticles [22]. Yet, despite regulatory approvals, few, if any, SPION nanoparticulate MRI agents are commercially available for clinical use presently. SPIONs are an important nanomaterials example highlighting the challenging production, marketing, and liability issues remaining for commercializing nanomedicines even after regulatory approvals are gained. Nonetheless, clinical applications continue to be reported. Annexin V-CLIO nanoparticles are developed for detecting apoptosis by MRI [23]. These nanoparticles have also been investigated for noninvasive detection of clinically occult lymph-node metastases in small and otherwise undetectable lymph-node metastases in prostate cancer [24]. The study showed that for certain particular applications, nanotechnology might be the only way to make *in vivo* diagnostic information reliable and accurate.

2.4 Nanoparticle-enhanced *in vitro* diagnostics and bioassays

Clinical *in vitro* diagnostic testing currently accounts for approximately 1%–2% of government health care expenditures worldwide, yet influences the majority (60%–70%) of healthcare decisions and therefore, ultimately also medical treatment costs. Clinical assays, often from complex patient-derived tissue, blood and physiological samples, are frequently tedious, insufficiently sensitive or reliable, and expensive based on current offerings. Nanotechnology has begun to enter this arena to improve assay conduct, automation, assay size and sampling amounts, multiplexed throughput, and performance in clinical milieu [25,26]. For example, the commercial Verigene® F5 Nucleic Acid Test approved by the US FDA is a genotyping *in vitro* diagnostic for identifying a single point mutation for the human coagulation Factor V gene from isolated DNA from patients' blood samples. The assay uses gold nanoparticles as optical probes that increase test sensitivity compared to organic fluorophore labels and reduce background noise. Additionally, gold nanoparticles are very stable, providing sustained reagent shelf-life.

Nanoparticles from biomedically familiar and biocompatible ingredients often exhibit magnetic or optical properties with promising utility in many biomedical imaging applications [17,27]. Once certified to (1) exhibit suitably low toxicity and (2) not interfere with intrinsic biological activity or assay activity, these nanomaterials properties are practically useful in assay formats as optical tags with improved resilience to photobleaching, as multiplexed multi-spectral labels for multi-wavelength detection in flow cytometry, lateral flow, or microarray analyte assays, and as convenient, sensitive (i.e., optically bright) labels for traditional biotechnology assays (e.g., Western blots, probe microscopies, Raman imaging, histology and polymerase chain reaction (PCR) methods) [28]. Most of these *in vitro* assay and diagnostics applications either require no regulatory scrutiny (i.e., direct to market technologies for *in vitro* research use) or reduced regulatory rigor as FDA Class I or even FDA Class II devices if their intent is to inform and guide human medical decisions. New nanotechnologies in this *in vitro* sector are therefore anticipated to more rapidly enter the marketplace than *in vivo* imaging and theranostics. Again, science and commercial products will exhibit different motivations timeliness and trajectories for respective development. Size reduction strategies offered by exploiting nanotechnologies certainly provides advantages to *in vitro* assays, including low sampling volume, multiplexed detection, multiple analyte capabilities, rapid time-to-answer, accurate flow, mass transport, mixing and thermal control, and ultra-sensitive detection opportunities [29,30]. Emerging nanoarray diagnostics have advantages over microarrays for better control of higher density surface-capture features, transport, kinetic control of target capture, more reliable signal generation, and much faster signal generation. Nano-wire and nano-cantilever methods provide high sensitivity and high sensor densities on a single unit to enable multiple-biomarker screening.

3 Current challenges facing nanotechnology translation to nanomedicine

Hype, over-used terminology, and inflated expectations for nanomedicine's capabilities aside, the practical realities for

advancing *in vivo* applications of nanotechnology toward clinical use face a set of technical and scientific challenges [31,32]. Despite interesting science, translating *in vitro* technologies to applications must assert clear performance advantages to adoption of select nano-component or miniature designs. By contrast, all *in vivo* biomedical applications of nanoparticles require direct absorption, inhalation, ingestion or injection into host tissues or circulation. Both require reagent stability, namely resistance to particle aggregation in complex biological media. Both require compatibility assessments as to acceptable toxicity profiles in their context of use: *in vitro* assays with biological components have vastly different requirements than nanomaterials *in vivo*. Possible dose-response and toxicity effects from these new nanomaterials on both isolated biological elements (e.g., enzymes, cells, proteins), tissues, and to human health is a critical prerequisite to translation. Currently, there is no successful predictive test strategy for *in vitro-in vivo* correlations to provide such risk assessments. Each nanomaterial must be evaluated separately under the conditions of use after validating materials purity and particle surface analytics [33]. Full *in vivo* profiling would then follow with absorption, distribution, metabolism and excretion (ADME) tests and physicochemical and toxicological characterization, involving both *in vitro* tests and *in vivo* animal studies. Figure 1 shows this conceptual design and performance evaluation. How these methods are performed is critical to understanding both risk and functional assessments of each nanomaterial's design. Few nanomaterials to date subscribe to a thorough, rigorous analytical protocol across *in vitro* and *in vivo* test beds [34–37]. The result is a frustrating and persistent gap between *in vitro* results and *in vivo* performance for poorly characterized nanomedicines that stymies ready translation [38,39].

3.1 Oral nanomaterial bioavailability

Gastrointestinal barriers represent a formidable challenge for therapeutic fluxes of particles [40]. Strategies to improve both passive and active transport of particles across the gut wall are an active focus [41,42]. Most published reports confirm that despite many surface and morphological changes, oral bioavailability of insoluble nanomaterials is consistent with that long-known for oral penetration of microparticles: both are generally very poorly bioavailable [43]. While absolute efficiencies of gastric absorption and systemization reported for various nanoparticles in different oral preclinical models vary, their bioavailability is consistently less than 10% frequently much less than this. A recent highly accurate biodistribution study using radioactive gold nanoparticles showed less than 1% oral bioavailability from gastric instillation in rats, regardless of particle size or surface charge [44]. Hence, while (nano)particle uptake from the gut certainly has been shown, that this particle flux and oral bioavailability can be (1) general enough across a broad patient population, and (2) sufficient and sustained to produce reliable therapeutic dosing has not yet been shown.

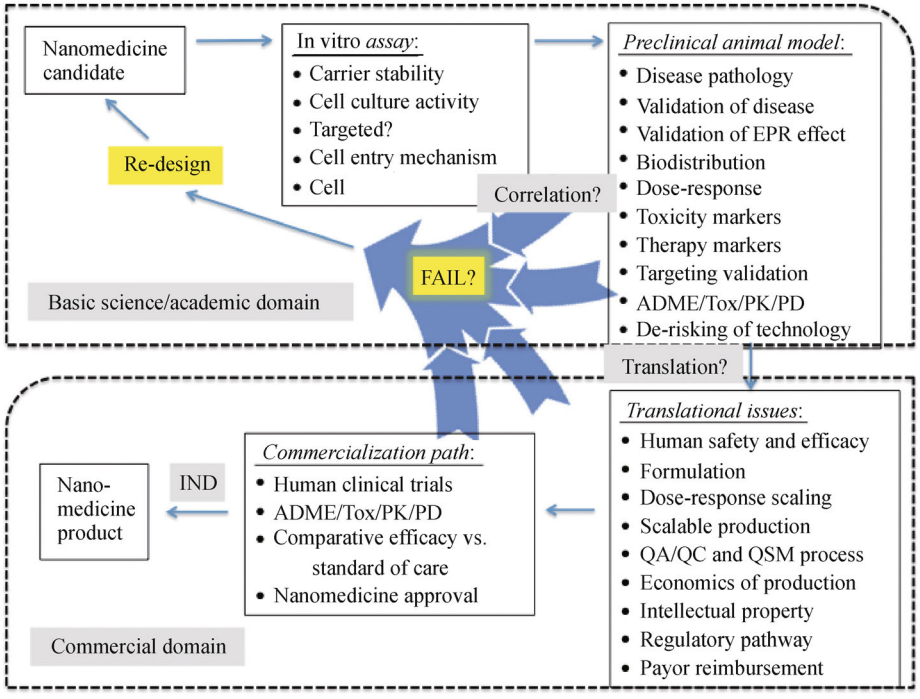

Fig. 1 Nanomedicine product development task set from conception through basic science assessment to preclinical proof-of-concept and then to translation for clinical use via commercialization

3.2 Circulating lifetimes of systemic nanoparticles

Most systemically injected nanomedicines have circulating half-lives of less than 24 h and many less than 6 h [38]. When injected intravenously, particles are cleared rapidly from systemic circulation, predominantly by the liver Kupffer cells and spleen (marginal zone and red pulp) macrophage populations [45]. Larger particles, 200 nm and above, are more efficient at activating the human complement system and are hence cleared faster from the blood by Kupffer cells than their smaller counterparts [46]. Regardless of steric stabilization, charge, shape or size, circulating particles accumulate rapidly in the liver and spleen and followed by filtration through the kidneys and lung capillary beds. Particle filtration efficiencies in non-target organs including the liver, spleen and lungs is generally greater than 90% and often 95% [47,48]. This means that only 5% of the injected bioavailable dose produces the observed therapeutic outcome at the target site. Most studies emphasize this 1%–5% disease targeting while neglecting the important information provided from analyzing the 90%–99% untargeted nanomedicine dose.

Enhancing drug circulating life-times (i.e., creating long-circulating vehicles) to improve accessibility to disease sites has been a research focus for improving nanomedicines for decades [47–49]. Improved lifetimes have been most frequently achieved using grafted polymeric steric stabilizers (commonly polyethylene glycols to date) [50]. These hydrophilic polymer grafts are thought to minimize non-specific serum protein opsonization and corona formation, limit particle-cell and particle-particle aggregation, and inhibit scavenging uptake by the host mononuclear phagocyte system (MPS) [47]. This is shown in Fig. 2.

Clearly, improved methods to provide simultaneous stabilization of particles in blood while improving circulation times and accessibility, either passively or actively to disease sites are clearly required to improve nanomedicine dosing to target sites. Even small changes in the on-site to off-site particle distributions would have dramatic impacts on therapy versus adverse drug side effects (i.e., liver and kidney toxicity). One study reported attachment of polymeric nanoparticles to the surfaces of red blood cells, enhancing their circulating lifetimes as long as the attachment was stable [51]. It is unclear from such a “piggy-back circulation strategy” how these particles reach their intended target site without a programmed release mechanism. Other emerging strate-

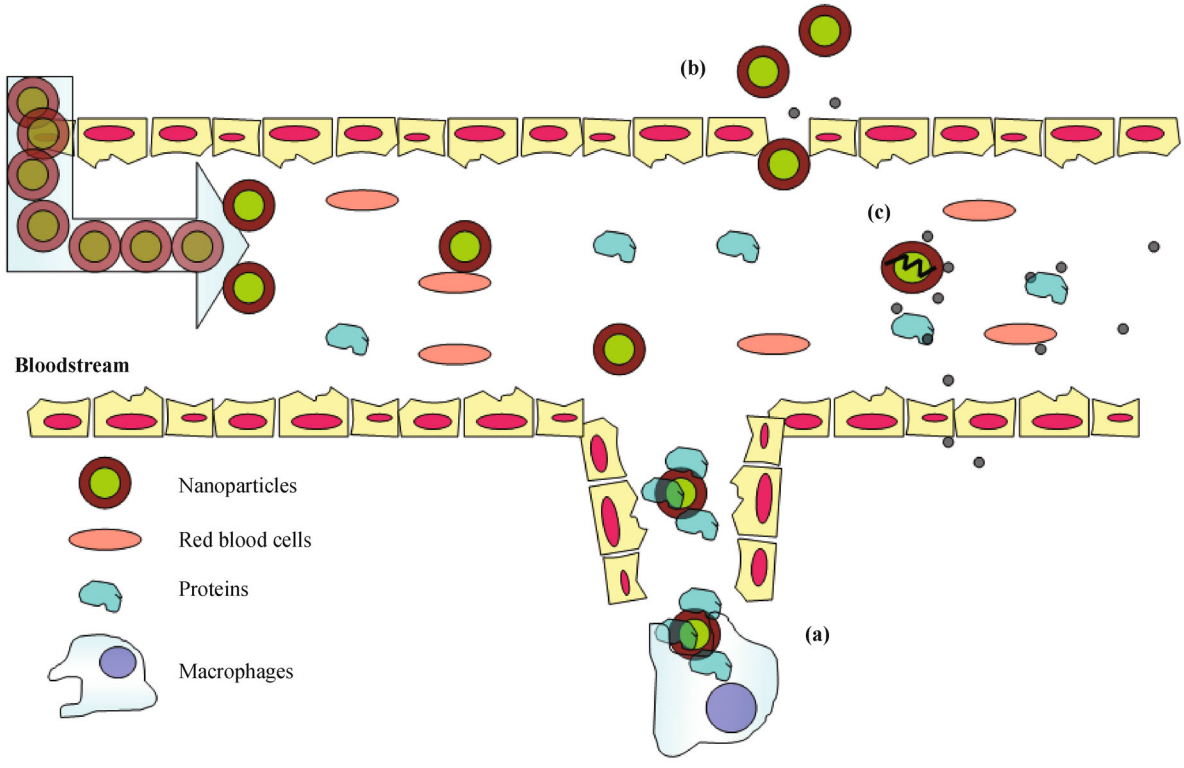

Fig. 2 *In vivo* nanoparticle fate in systemic circulation after intravenous injection that determine circulation lifetime and efficiency of tumor delivery. (a) exposure to blood causes plasma proteins and lipids to rapidly adsorb onto particle surfaces (opsonization). These coated plasma proteins (the corona) can promote macrophage-mediated uptake, particularly by the host MPS system; (b) if the EPR effect is present, nanoparticles can exit the vasculature by passing through the vascular endothelial layer via open tight junction pores and fenestrae to accumulate in tumor interstitium adjacent to matrix, actually entering the tumor bed (see Fig. 3); (c) nanoparticles release drug payloads to blood components in the blood stream that can then diffuse into the tumor bed or flow downstream

gies include surface grafting with self-recognition peptides [52] and erythrocyte membrane surface mimics [53].

3.3 Targeting nanomedicines: fact and fiction

Most nanomedicines either approved for clinical use or in development are intended to become systemically bioavailable, that is, circulate widely within the vascular and lymphatic transport systems to find their intended sites of action. Lacking innate modes of self-propulsion to direct their movements, nanomedicine particles are held hostage to passive transport mechanisms within the systemic circulation: diffusion, partitioning, entrainments in blood flow (hydrodynamic phenomena). Their movement and transport in the body is therefore largely stochastic: particles can only move where these passive mechanisms take them.

In addition, (nano)particle retention at any tissue site depends upon frequency of encounter with that site, hydraulic permeability and hydrodynamic transport via flow that facilitate particle exit from the circulatory flow fields, and two-body forces at that site. In a non-uniform fluid shear field, a particle experiences heterogeneous fluid velocities around it and the resultant force from this non-uniform flow field causes the particle to move across the flow field. If the particle is not spherical, its center of mass and hydrodynamic centers are not coincident so the particle experiences imbalanced fluid forces and will move in a non-uniform manner. Many vascular conduits have laminar and even turbulent flow fields and are fenestrated, and extravascular, acellular extracellular matrix is a porous mesh of crosslinked macromolecules. Once transported by random flow and diffusive forces to a fenestrated vascular wall or to extracellular matrix, particles can become entrained in their porous network, removing them from flow and depositing them where intermolecular attractive forces determine their retention and residency. The interactions and relative contributions of these passive transport phenomena define passive targeting for nanomedicines: random particle encounters using stochastic processes to enable tissue site retention and particle removal from fluid flow [54]. Further addition of specific ligands to nanomaterials to improve intermolecular forces, engagement, and site-specific retention post-transport is termed active targeting. But active targeting is short-range: that is, targeting ligands can only physically extend across space at a nanometer-range, while the intermolecular forces that govern their avidity, affinity and specificity with a target molecule are sub-nanometer in range. Particles with active targeting strategies must therefore use passive mechanisms to first provide requisite transport and entrainment close to their intended site of binding. Only then can active targeting ligand engagement facilitate retention at the specific target site, and only if intermolecular forces favor binding in the local environment during the residence time of the particle at that site. Both thermodynamics and kinetics play important roles in active targeting to tissue sites.

It is important to note that both passive and active targeting processes with particles are saturable [54,55]. That is, the passive removal of suspended particles from flow depends on the initial removal characteristics of the filtering bed (i.e., fenestrated endothelium, extracellular matrix) and subsequent effects of particle deposition and retention within the initial outermost exposed porous tissue layer on subsequent particle encounters and retention efficiencies. Initial particle retention within exposed pores can cause particle-pore "jamming" and also alter the local hydrodynamic parameters, reducing both transport to and retention within the pores for further particle populations that might randomly encounter the site [54,55]. As more particle deposition occurs within tissue pores adjacent to the flow field, the relative porosity of the tissue bed is naturally changed, and with it the local hydrodynamics and the hydraulic sieving properties of the bed. Consequently, mean interstitial porous flow velocity near the tissue bed increases, reducing the probability of particle surface encounter, retention, and deposition for suspended particles. Additionally, higher interstitial pore velocities may induce a fraction of the deposited particles to detach and return to systemic flow.

In addition to ubiquitous passive deposited particle saturation by jamming and resulting hydrodynamic transport alterations in the tissue bed directly adjacent to the vascular or lymphatic flow field [48,54], active targeting processes also saturate based on ligand-target binding. In contrast to what might be inferred from the abundant literature popularizing this strategy, active targeting is simply not a matter of pairing a high-affinity binding entity for a disease target onto a particle and injecting it. In practice, active targeting is not simply an exploitation of classic lock-key ligand-binding principles. Active targeting can in fact yield less site-specific nanomedicine accumulation than for passive analogs, and be plagued with the same non-specific filtration issues [47,48]. Additionally, intrinsic tumor heterogeneity leads to heterogeneity in tumor site expression of specific receptors, both temporally and spatially in tissues of therapeutic interest, makes reliable "active targeting" very difficult to establish [47]. Targeting specific disease ligands often has failed to show much therapeutically relevant influence on nanomedicine tumor accumulations in animal models [56], despite showing increased agent internalization in animal models [57]. Reliable evidence showing substantial active targeting *in vivo* despite *in vitro* cell line support is presently scant [47].

Strategies for active targeting should consider the physiology of the local disease site, the context of site-specific delivery and, sometimes, the actual disadvantages of using high-affinity ligands for delivery in certain cases.

In the context of solid tumor targeting, target-specific design parameters have been clearly described [47,48,58]. Nonetheless, recent clinical success for an actively targeted antibody therapy [59] has re-ignited unreasonable expectations for general extensions for targeted nanoparticle approaches, despite the enormous differences in vehicle physical properties and transport mechanisms in tumor sites [47,48,58,60,61].

The enhanced permeation and retention (EPR) effect has been the basis for explaining passive targeting for macromolecules and particles to disease sites with pathology produces inflammation and loosening of tight junctions in vascular endothelium [58]. As first reported 40 years ago [62,63], micrometer-sized gaps between normally tight endothelial cells permit transport of large molecules and particles typically unable to traverse the vascular wall. This provides a passive route of therapeutic transport specifically enhanced in sites of inflammation, mostly observed in solid tumors to date, and recognized early as an avenue for enhancing drug delivery [64]. The EPR effect has been observed primarily in select preclinical animal disease models to date in solid tumor sites [65,66].

EPR-based tumor and tissue disease site leakiness is attributed to unnatural openings in endothelium rather than fenestrations found in normal tissues. This local vascular wall hydraulic permeability and extravasation mechanism is slow and passive, certainly contributing to local flux, delivery, vehicle partitioning and availability of therapeutics via this passive accumulation route. However, beyond claims to cures in animal disease models, the operable efficacy of the EPR effect in improving nanomedicine delivery and therapeutic outcomes in humans is not evident to date. Many reasons have been offered to explain the lack of translation of EPR-exploited therapeutic efficacy from animal models to humans [58,65,66]. Both physiological and transport properties of the disease site and the physical and chemical attributes of the nanomedicine construct amenable to exploiting the EPR effect must be considered. As these critical parameters are site-, disease- and delivery vehicle- specific, there is “no-one-size-fits-all” nanomedicine solution to address varieties of disease. Vehicle properties critical to EPR performance and passive targeting pathways include particle surface charge, steric stabilizers, particle size and shape, aggregation states, deaggregation and degradation rates, matrix transport rates, and ligand binding affinities if bearing active targeting components [58,65,66]. That these properties must be carefully and uniquely designed for each nanomedicine on a case-by-case therapeutic context seems increasingly important. Ultimately, the EPR effect may not be a generally exploitable transport phenomenon useful for all nanomedicines [47,66]. Claims to its specific utility in assessing nanomedicine efficacy require experimental validations of operable EPR mechanisms in producing therapy [38,47,48].

4 Nanoparticles for cancer therapy: Opportunities and challenges

Despite many years of advocacy, research and development, only a few nanomedicines are in clinical use, and these suffer from many of the challenges described above, including high MPS scavenging, short circulation lifetimes, low fractional dose to disease site, aggregation issues, and poor targeting [65]. Additionally, nanocarriers that display promising efficacy in cell culture models or in accepted animal disease models do not show this same success in clinical trials [47]. This unsatisfactory outcome makes their commercial translation rather risky, costly and a tenuous ambition for pharma companies. Despite strong, compelling unmet clinical needs in clinical oncology, the risks associated with translating many nanomedicines remain unattractive for a large industrial sector. Reasons for lack of translation consistency from bench to bedside include: 1) a general distrust on the reliability of academic research and its quality and reproducibility [67,68]; 2) difficulties translating particle dosing determined for *in vitro* cell culture studies to *in vivo* models of tumors; 3) lack of validation of cancer cell lines, contaminated cell lines, irrelevant culture and assay conditions [69,70]; 4) lack of standards and standard methods to facilitate valid inter-study comparisons; and 5) glaring inequities between preclinical animal model results and clinical studies in humans [38,65,71]. In fact, only rodent models are used for *in vivo* tumor studies and most still lack of validation [65]. Most rodent tumors are generated ectopically with a large injection of humanized tumor cells and develop a large mass over relatively short periods of time compared to human tumor generation [65]. This process produces tumors in rodents that if allometrically scaled would be kg-size in humans, and with substantially different physiology. The current small rodent end result is not comparable with human tumor development. Yet, no large animal tumor models currently exist that might better replicate scaling, pathology and physiology issues relevant to human conditions.

Nanotechnology’s link to developing nanomedicine as a most favored approach to improve cancer therapeutics is readily apparent [72]. The opening paragraphs of many publications using nanoparticles for cancer therapy mention the EPR effect as the justification for their approach. However, the EPR effect has only reliably been shown in ectopic tumor models in rodents (*vida supra*) without much human validation, and most of that using intrahepatic arterial (not systemic intravenous!) infusions of drugs. The current scientific community’s extension of the EPR effects is largely from rodent tumor model data using unnaturally generated ectopic solid tumors where inflammation and pathology is dramatic and unrealistic compared to human tumors. The EPR effect is the exception rather than the rule for passive particle targeting and therefore

should be not be assumed to be operative in any experimental model. Additionally, extrapolation to human therapeutic potential is without much scientific or clinical support [58] and unjustified as a presumed mechanisms until actually proven operative in treating human tumors [38,65].

From a nanomedicine formulation perspective, control of solid-state drug polymorphs may be more difficult with small crystalline particles or solid nanoparticle formulations that require complex, multi-step chemical and formulation methods, quality control validation processes and extended storage. For example, active targeting often requires increasing compositional complexity The associated increases in yields, process chemistry scaling issues, unit costs, quality assurance/quality control (QA/QC) tasks and regulatory obstacles are formidable enough to preclude most industrial interest in taking the formulation forward to human trials (Fig. 1). Nanomedicine's impact will be found in successful translation; one-off academic prototypes may find an audience in high profile journals but their real test of value is in how many patients get treated. Such translation must anticipate the practical reality of economical therapeutic production, ready product validation and quality systems management in an actual product life cycle [73]. Many nanomedicines do not yet subscribe to this reality.

Nanoparticle transport and through-barrier movements have been reported both *in vitro* and *in vivo* for nanomedicine penetration through cell membranes and various tissue barriers (i.e., intestine, vascular wall, brain, stroma, etc.). Particles can penetrate biological barriers. However, the statistical argument aside, the actual critical therapeutic question is whether these particles fluxes are sufficient and reliable across many disease barriers, patient phenotypes and across diverse particle chemistries to produce therapeutically important effects without toxic side effects from mega-dosing. This difficult functional question remains to be answered been shown [38].

Additionally, when particle delivery to tumor sites is shown, quantifying fractional dose of these particles that actually penetrate into tumor matrix beyond the vascular wall is not well understood. The important therapeutic and mechanistic differences of directing nanomedicines "to a tumor" versus "into a tumor" (Fig. 3) has been raised to question the current operative philosophy [58] and unjustified as a presumed mechanism until actually proven operative in treating human tumors [38,65].

5 Conclusions

Nanotechnology has dramatically changed approaches to drug development, vehicle designs and combination or multiplexed capabilities on a single injected payload. Versatile new opportunities in creating innovative platforms for new therapies, medical imaging and diagnosis, especially for cancer treatment are possible and many interesting but largely academic developments will continue to be reported. However, this revolution in nanotechnology has not yet become a revolution in nanomedicine, at least clinically. Rational retrospective analysis of what has been produced and what has succeeded and what has failed could yield huge benefits, rather than just pushing forward with the same compromised design paradigm [73]. Poor rates of technology translation from research to treatment regimens are beginning to draw ire from peers and payors. The nanomedicine community stands to lose credibility unless

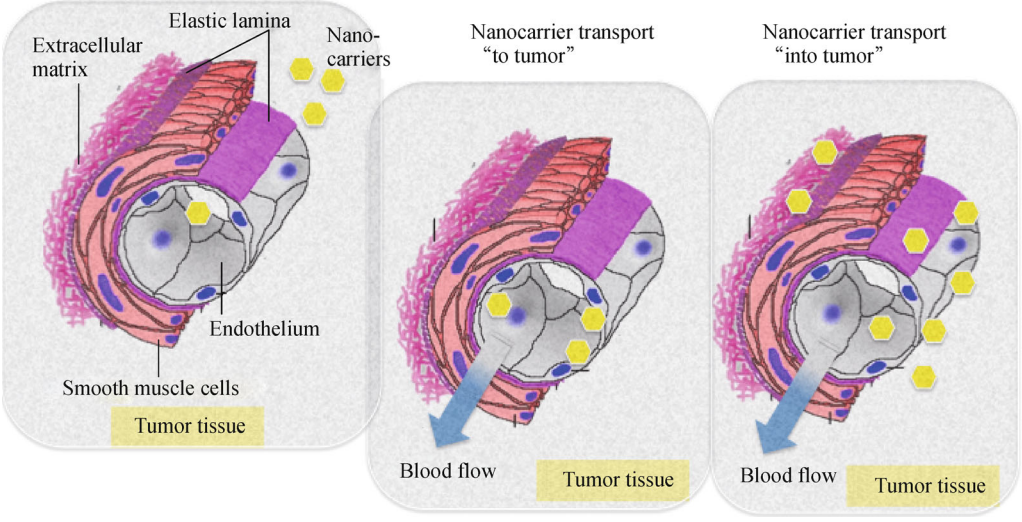

Fig. 3 Nanocarrier transport via the systemic vasculature and blood flow: contrast of nanomedicine transport "to the tumor" with carrier retention only in the vascular wall versus nanomedicine transport "into the tumor" where the nanocarrier penetrates through the vascular wall and into the tumor's extracellular matrix (Adapted from http://www.siumed.edu/~dking2/crr/cvguide.htm#vessels with permission)

a rational road-map forward is produced to make clinical impacts. Lack of significant therapeutic improvements in drug efficacy and difficulties and complexities of nanomedicine regulatory approval processes compared to the parent original drug form are identified as key factors slowing nanomedicine development [74]. The current inability to accurately evaluate and screen newly designed systems for efficacy and toxicity with reliable, inexpensive *in vitro* methods does not provide the desired industrial goal to discern "drug candidate failure faster" in order to reduce costs and risks of further development [75].

Lastly, limiting hype, exaggerated claims and overstated experiment results are also desired goals to provide objectivity of the real status of nanomedicines [31,32]. Blinded optimism lacks scientific credibility and undermines future work. Identifying and solving critical problems to translating nanomedicines is the challenge. Only when clean, reproducible data consistently elucidate the barriers, challenges and successes can design, optimization and more targeted development of improved nanomedicines proceed toward patient benefits.

Acknowledgements DWG is grateful to the George S. and Dolores Doré Eccles Foundation (USA) for support.

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