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## Overcoming transport barriers for interstitial-, lymphatic-, and lymph node-targeted drug delivery

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### Abstract

Despite drug formulation improving circulation times and targeting, efficacy is stymied by inadequate penetration into and retention within target tissues. This review highlights the barriers restricting delivery to the connective tissue interstitium, lymphatics, and lymph nodes as well as advances in engineering drug carriers to overcome these delivery challenges. Three-dimensional tissue physiology is discussed in the context of providing material design principles for delivery to these tissues; in particular the influence of interstitial and lymphatic flows as well as differential permeabilities of the blood and lymphatic capillaries. Key examples of materials with different characteristics developed to overcome these transport barriers are discussed as well as potential areas for further development.

### Keywords

drug delivery; interstitium; lymphatics; lymph node

### Introduction

Drug efficacy after administration is restricted by its capacity to appreciably accumulate at the desired site of action at bioactive doses in order to mediate its effect(s). The concept of drug targeting to enhance therapeutic benefit and minimize toxicities largely brings to mind the directed retention of drugs or imaging agents at and within cells and tissues of interest using affinity-based molecular interactions or, in the case of solid tumors, exploiting the

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leaky vasculature to facilitate their preferential accumulation. Innovation in carrier design has also improved the penetration of delivered agent deeply to within healthy and diseased tissues, as well as to tissue targets of emerging interest for their diagnostic and therapeutic utility – lymphatic vessels and the lymph nodes to which they drain.

This review describes the current state of interstitial-, lymphatic-, and lymph node-targeted drug delivery and gives perspective for their future directions. Its focus is on delivery strategy and carrier design and not related applications including cancer therapy [1], immunomodulation [2-4], or regenerative medicine [5], which have been reviewed in depth elsewhere. We also do not discuss sustained release formulations [6,7] or cell transplantation approaches [8], which clearly have significant promise, nor the significant delivery barrier of the mucosa. We discuss the emerging concepts pertinent to drug delivery to these tissues and design strategies that have facilitated advances in effective targeting.

## The interstitium, lymphatics, and lymph nodes as targets for delivery

The extravascular fluid, solute, matrix, and cellular environment within tissues constitutes the interstitium. Interstitial targets therefore include stromal, cancer, resident immune, and other cells as well as components of the protein- and proteoglycan-rich extracellular matrix (ECM). Accordingly, the interstitium is a central target in a plethora of diseases, including cancer, arthritis, and chronic wounds, amongst others.

The lymphatic vasculature transports interstitial fluid and solutes to lymph nodes in a unidirectional fashion, regulating tissue fluid balance, immune cell homing, and lipid transport from the gut. Both initial lymphatics and the larger collecting lymphatic vessels to which they drain are comprised of lymphatic endothelial cells connected to the extracellular matrix by anchoring filaments and underlying smooth muscle, respectively. Lymphedema, which is the loss of lymphatic function that results in tissue fluid accumulation and swelling, represents an underlying pathology in a variety of disease states. Restoration or prevention of the loss of lymphatic function is of major interest for management of malignancies, particularly of the breast, as well as for a variety of infectious diseases. In particular, therapeutic lymphangiogenesis to increase lymphatic function has been explored in transplantation applications since lymphatic transport also contributes to immune regulation [9\*,10].

Lymph nodes are lymphoid tissues comprised primarily of immune cells with a supporting stroma whose primary function is to orchestrate cell-cell contact in the mounting of adaptive immune reactions. They are thus central targets in immunotherapy applications, such as vaccines [11] or immune suppressive regimens [12]. Lymph node targeting may also have utility in improving the treatment of B and T cell malignancies. The lymph nodes draining solid tumors, referred to as sentinel lymph nodes, have well-appreciated diagnostic utility in staging disease since they are a common site of metastasis. Sentinel lymph nodes are also considered to be functionally involved in directing antitumor immune response and suppression [9\*,13\*]. Consequently, both chemotherapeutic [14] and immunotherapeutic [15\*\*] interventions targeting lymph nodes have been explored.

## Drug delivery approaches and design for interstitial-, lymphatic-, and lymph node-targeting

We now consider the material design to achieve targeting of the tissues described above, focusing on the relevant tissue physiology as a guide for the engineering of the therapeutic or imaging agent formulation. We contemplate barriers to delivery including the extracellular matrix after injection in connective tissue such as skin or extravasation from the vasculature, uptake by cells resident in the interstitium, as well as the subcapsular sinus in the lymph node. Delivery challenges such as vascular permeability effects that divert agent from its intended target tissue are also discussed. Materials with different design principles and characteristics are being developed to overcome these transport barriers.

### The interstitium as a barrier and target

Delivery of therapeutic agents to the interstitium can be achieved by either direct injection in connective tissues or after extravasation from the vasculature post intravenous infusion. The extravasation process clearly presents a significant barrier to delivery and has been well-reviewed elsewhere [16]. We will instead primarily focus on penetration of the agent into the interstitium, which is restricted by the gel-like ECM that is composed of fluid, solutes, fibrillar proteins, and proteoglycans. Accordingly, macromolecular size, shape, and charge are major determinants of transport characteristics through both healthy and diseased tissues.

Quantification of molecular transport within living tissues has provided significant and nuanced insight into interstitial solute transport after direct injection [17,18]. For example, in mouse tail skin, transport of uncharged solutes larger than 70 kDa is hampered by the ECM (Figure 1), whereas below this molecular weight threshold, transport is dominated by size exclusion principles [17]. However, this behavior is likely altered between tissue types as a result of differences in ECM pore size [19] and composition [20]. Molecular rigidity and globular structure also restrict interstitial penetration (Figure 1), since size-matched nanospheres and proteins demonstrate reduced measured rates of interstitial convection relative to more flexible chain-like dextrans [17]. Electrostatic repulsion is also a potential mechanism reducing hindrance [17] by the negatively charged ECM [21], though computational approaches suggest this may actually restrict diffusion [22]. Importantly, following onto seminal work by Jain and colleagues measuring the diffusivity of albumin and immunoglobulin G *in vivo* [20,23], two-photon fluorescence correlation microscopy distinguished that transport within tumors is two-phase in nature, comprised namely by fast and slow diffusion that likely result from the aqueous and viscous components of the ECM [18]. Modification of the tumor ECM using hyaluronidase or collagenase reduced the extent of rapid and slow diffusion, respectively [18], indicating distinct functional roles for each ECM component on restricting intratumoral solute transport and antitumor activity of therapeutic drugs [24-26]. Furthermore, slow diffusion appears more pronounced with increasing molecular size [18]. Formulations that are somewhat stealth, so as to not adsorb to the interstitial biopolymer network [27], therefore have the potential for enhanced penetration through the interstitium.

Since intravenous infusion is the most common delivery strategy for anticancer therapy or imaging applications, a challenge is optimizing transvascular transport, tumor penetration, and agent circulation time, for which carrier design can have opposing effects on efficiency of delivery (Figure 2A). For example, anionic charge improves transvascular transport in tumors [28] while neutral charge is ideal for elongating circulation times [29]. Furthermore, whereas increasing size prolongs agent circulation time [30], transvascular transport [31] and interstitial penetration [30] are dramatically attenuated. To overcome the opposing effects of carrier size on intratumoral delivery and penetration after intravenous infusion, a multistage delivery system (Figure 2B) in which 100 nm nanoparticles that demonstrate improved circulation times [30] “shrink” to 10 nm quantum dots after extravasation via the proteolytic activity of matrix metalloproteinase within the tumor microenvironment has been proposed [32]. This system was able to overcome perivascular entrapment by the dense collagenous tumor ECM to facilitate deep interstitial tumor penetration. Since increased aspect ratios not only improve particle circulation times [33] but also increase nanorod diffusion through pores and porous media *in vitro* (Figure 1), another scheme utilized nanorods to improve intratumoral penetration *in vivo* after intravenous infusion relative to nanospheres of similar hydrodynamic radii and plasma half-lives [31].

Clearance from the interstitium also presents a major delivery challenge that diminishes efficacy by reducing agent residence at the site of action over the necessary timescales to achieve its desired effect(s) (Figure 2C). Thus diffusion and convection as a benefit versus detriment to interstitial delivery must be balanced. Perrault and Chan [34] proposed an elegant strategy to overcome this via serial intravenous infusion of poly(ethylene glycol) (PEG)-grafted nanoparticles bearing biotin followed by streptavidin-conjugated molecular contrast agent, both of which are highly diffusive and permeable to the tumor vasculature, thus facilitating rapid and extensive penetration within the solid tumor ECM. Upon colocalization, assembly occurs, favorably influencing retention within the tumor [34]. This approach leads to sustained high levels of intratumoral contrast agent within three hours post infusion that are on par with those seen 24 hours post infusion of the assembled macromolecular complex [34]. Another innovative approach developed to reduce agent clearance in a target-specific manner harnesses a novel nanoaggregation chemistry that selectively increases intratumoral retention *in vivo* to monitor apoptosis resulting from chemotherapy [35\*\*]. These engineered molecules undergo macrocyclization in the presence of caspase, resulting in the *in situ* formation of 170 nm nanoaggregates [35\*\*] (Figure 2D).

### Lymphatic targeting

Due to the physiology of the lymphatic system, typical intravenously administered molecules and nano-formulations do not result in appreciable delivery to lymphatics. A considerable portion of administered agent accumulates instead in the lung, kidney, and liver. Even in the case of tumors where accumulation can be significant by virtue of the leaky tumor vasculature, agent penetration is limited to the perivascular space [30]. As a result, direct injection techniques are typically utilized for lymphatic targeted delivery strategies [15\*\*,36]. Within the interstitium, fluid flow, which always drains to a lower pressure, is directed towards the lymphatics by virtue of their lower pressure relative to the

blood and interstitial tissue. The potential for infused solutes to be directed towards and drained through the loose junctions of initial lymphatics after injection within connective tissues is thus dependent on their capacity to be subjected to this convective transport and are therefore restricted by the barriers of interstitial penetration as discussed above.

Several key aspects of connective tissue physiology influence the feasibility of lymphatic and lymph delivery despite the focusing influence of interstitial flow on directing injected agent towards draining lymphatics. Since non-fenestrated blood capillaries are permeable to molecular species less than 5 nm in hydrodynamic diameter [37], they are rapidly cleared after injection [38], reducing their potential for lymphatic exposure. Molecular species roughly 5 nm or larger, on the other hand, are largely impermeable to the blood capillaries within healthy tissues [38] and can therefore only be cleared by lymphatic drainage mechanisms. Furthermore, since the pore size of the ECM limits the movement of macromolecular species through the interstitium, infused nanoformulations ~100 nm or larger become entrapped [36] and are thus poorly suited to lymphatic-targeted drug delivery applications. Lymphatic targeting is therefore optimized by agents or formulations roughly tens of nanometers in hydrodynamic diameter, for example synthetic formulations such as ~30 nm Pluronic-stabilized poly(propylene sulfide) nanoparticles [36]. Lymphatic partitioning of drug after subcutaneous infusion can also be improved by PEG grafting, thereby increasing drug hydrodynamic radius. With increasing PEG molecular weight, grafted polylysine dendrimers demonstrate decreased absorption into the blood and a significant increase in dendrimer recovery in lymph [38]. Moreover, incorporation into PEGylated polylysine dendrimers can additionally increase the fraction of administered doxorubicin dose with lymph exposure from roughly zero for the free drug to 40% of injected dose [39]. PEGylation also increases passive lymph node targeting of cationic liposome-formulated vaccines [40].

In addition to achieving lymph accumulation via transluminal transport, delivery of therapeutic or imaging agents directly to the lymphatic endothelium is of interest. Directed targeting has been explored by affinity interactions facilitated via antibody-mediated recognition of lymphatic endothelial cell markers Lyve-1 [41] and podoplanin [42]. Emerging evidence supports the concept that lymphatic endothelial cells actively scavenge protein antigens *in vivo* [43,44\*\*] in both inflammatory [43] and steady-state conditions [44\*\*] (Figure 3A). The ramifications of this activity have largely been explored for its immunological consequences [43,44\*\*], however, these data and *in vitro* observations of synthetic nanoparticle uptake by lymphatic endothelial cells [44\*\*] support the concept that endogenous uptake mechanisms may be exploited for delivery of drugs to lymphatic endothelial cells. If and the extent to which solute scavenging by lymphatic endothelial cells is a detriment to penetration into lymph and delivery to lymph nodes and whether this can be overcome using formulation design has yet to be determined.

### Lymph node targeting

Direct lymph node injection has been proposed for transplantation applications [45\*] and shown promise for immunotherapy [46], but requires laborious and invasive surgical manipulations impractical for most drug delivery scenarios. Since lymph nodes are bathed in

lymph drained from the periphery in a steady state fashion, design principles optimizing drug targeting for penetration through the interstitium and lymphatics to reach lymph using more conventional and less intrusive drug administration techniques, such as subcutaneous injection, are attractive for lymph node delivery (Figure 4). Indeed, since interstitial flow always follows a decreasing pressure gradient toward the lymphatic capillary and subsequently to the draining lymph node, such an approach for targeting the lymph node after direct injection in the periphery is highly facile yet robust: the particles must mainly be small enough so as to not be entrapped by the tissue interstitium, yet large enough not to be cleared into the circulation after injection. This corresponds to roughly 10-100 nm in hydrodynamic radius and is optimum at an ideal size of 20-40 nm [36,38,39,47]. This targeted delivery strategy is also advantageous by enabling dose sparing to help minimize the potential for undesirable off-target effects and/or toxicities [48\*\*]. This principle has been explored for lymph node targeting using both synthetic materials, including nanoformulations comprised of Pluronic-stabilized poly(propylene sulfide) [36,49], polymer dendrimers [38,39] and poly( $\gamma$ -glutamic acid)-*L*-phenylalanine ethylester [47], as well as biologically derived biopolymers, such as hyaluronic acid [50] and albumin [48\*\*]. Such approaches have been used to improve lymph node delivery and bioactivity of co-formulated small molecules [15\*\*,39], oligonucleotides [15\*\*,48\*\*,51], as well as proteins and peptides [11,48\*\*,52]. Of particular interest is an innovative, recently described “albumin hitchhiking” approach based on adjuvant and antigen conjugation to albumin-binding lipids to improve response to vaccination by coopting albumin drainage out of the interstitium to lymph nodes [48\*\*]. Additionally, we recently reported the benefit of toll-like receptor ligand formulation into 30 nm polypropylene sulfide) nanoparticles in improving drug delivery to sentinel (tumor-draining) lymph nodes for cancer immunotherapy [15\*\*].

Once arriving in the lymph node, lymph-borne solutes are filtered at the subcapsular sinus depending on size [53] and opsonization [54] (Figure 4). A fraction of lymph solutes will penetrate into the lymph node follicle and cortex; the remainder will leave via the efferent lymph to reach the blood [49]. Larger sinus-penetrating molecules or particles are taken up by subcapsular macrophages and transferred to B cells [53-55], whereas smaller molecules (less than roughly 3-5 nm in diameter) can penetrate more deeply into the lymph node to enter the B- or T-cell zones through conduits [53] to be sampled directly by B cells [53] or taken up by dendritic cells [56]. Drug and macromolecular formulation therefore have the potential to play a significant role in regulating the distribution within the lymph node as a function of macromolecular size [36] and complement deposition [52].

The complex siphoning taking place in the interstitium and lymph node subcapsular sinus plays a critical role in the rate, extent, and distribution of agent accumulation within lymph nodes that may influence drug efficacy, required dose, and associated toxicities. For example, Roozendaal et al. [53] found that when administered simultaneously in the periphery of immunized animals, small molecules are taken up by lymph node B cells more rapidly than larger ones (Figure 3B). Though faster, small molecule uptake by lymph node B cells is not as efficient as that of larger molecules [53]. Small molecules also penetrate more deeply into lymph nodes as the result of enhanced shunting through lymph node conduits relative to larger molecules [53]. These data support the concept that despite

nanoformulations of an intermediate nano-scale size being ideal for lymphatic partitioning after injection in the periphery, small molecules have increased potential to accumulate deeply within the lymph node. This model might be utilized to optimally target lymph node-resident cells via controlled release strategies of co-formulated drug such as multi-stage release strategies that improve intratumoral drug penetration [32].

In addition to lymph node drug delivery guided by passive convection, several successful schemes rely on exploiting mechanisms of endogenous cell-mediated trafficking. This principle is based on the activity of antigen presenting cells, which reside in most tissues performing immunological surveillance and migrate via lymphatics in homeostatic and inflamed conditions to lymph nodes to present antigen to resident T cells [57]. Conventional as well as more recent engineered vaccines have relied on this active trafficking mediated by antigen presenting cells after uptake of administered formulation in peripheral tissues. The utility of particle size [58] and shape [59], functionalization with dendritic cell binding moieties including mannan [60] or antibodies against C-type lectin receptors such as DEC-205 (CD205) [61] and DC-SIGN (CD209) [62], as well as attraction by delivery of chemotactic stimuli [63], to enhance uptake by these migratory cells has been demonstrated. The timing, extent, and tissue distribution of lymph node delivery facilitated by active cell-mediated trafficking is significantly altered relative to passive delivery approaches [58] and may influence potency and quality of therapeutic response.

## Conclusion

Consideration of three-dimensional physiology and the corresponding fluid mechanical microenvironment within interstitial, lymphatic, and lymph node tissues provides a means to improve the design of drug formulations for enhanced delivery. The full potential of existing and even orphaned drugs may be improved by reformulation to optimize delivery to their target site of action, which in the case of lymphatics and lymph nodes as well as the tumor interstitium, are not efficiently dosed with conventional drug formulations and administration regimens. Moreover, effective targeting can spare dosage and reduce potential toxicity. Overall, engineering design to overcome several key transport barriers has addressed many of the challenges in drug delivery to improve potency and therapeutic efficacy.

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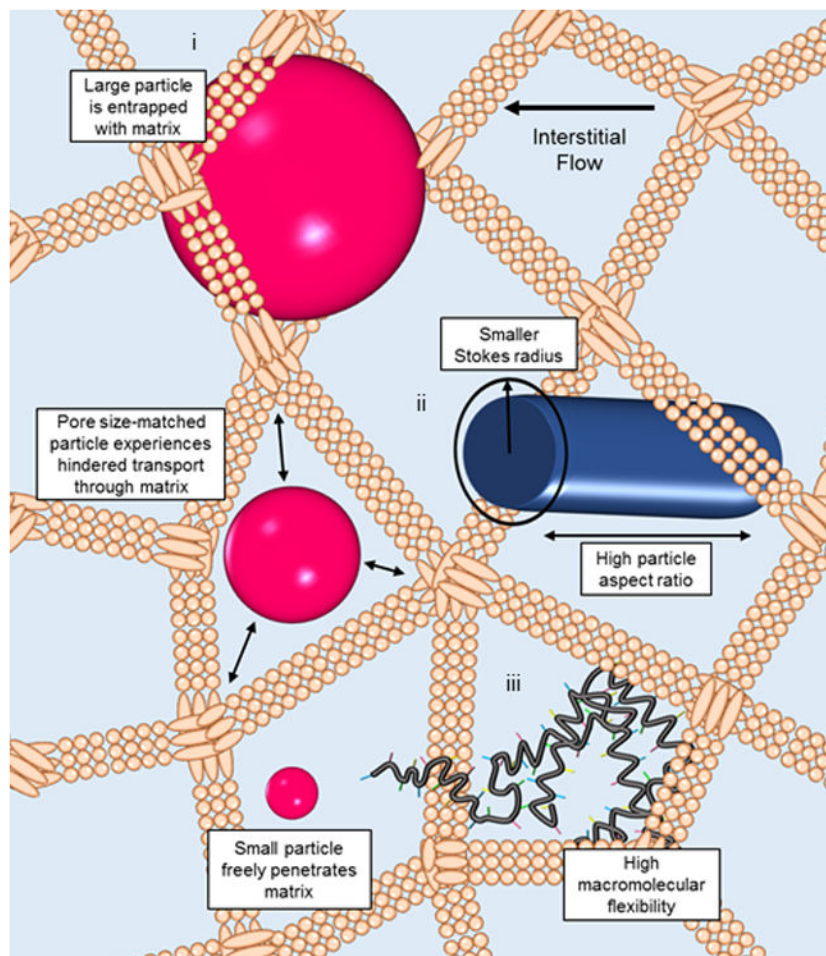
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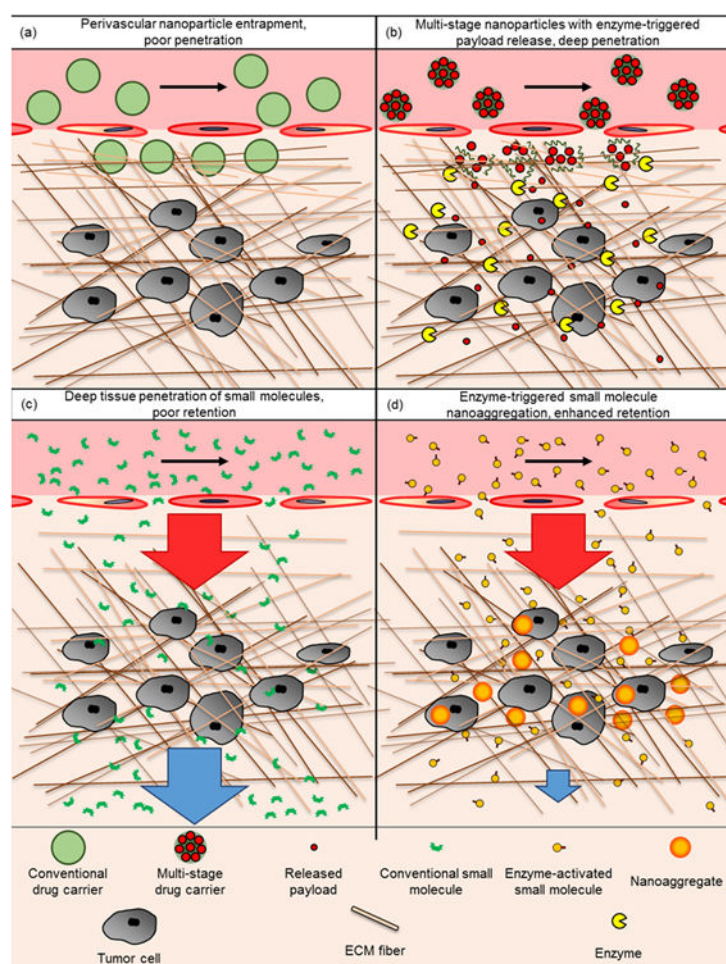
### Highlights

- Multistage delivery systems for interstitial penetration and retention in tumors.
- Drug focusing to lymphatics and lymph nodes via interstitial and lymphatic flows.
- Lymph node drug targeting using nanoparticles or “albumin hitchhiking”.



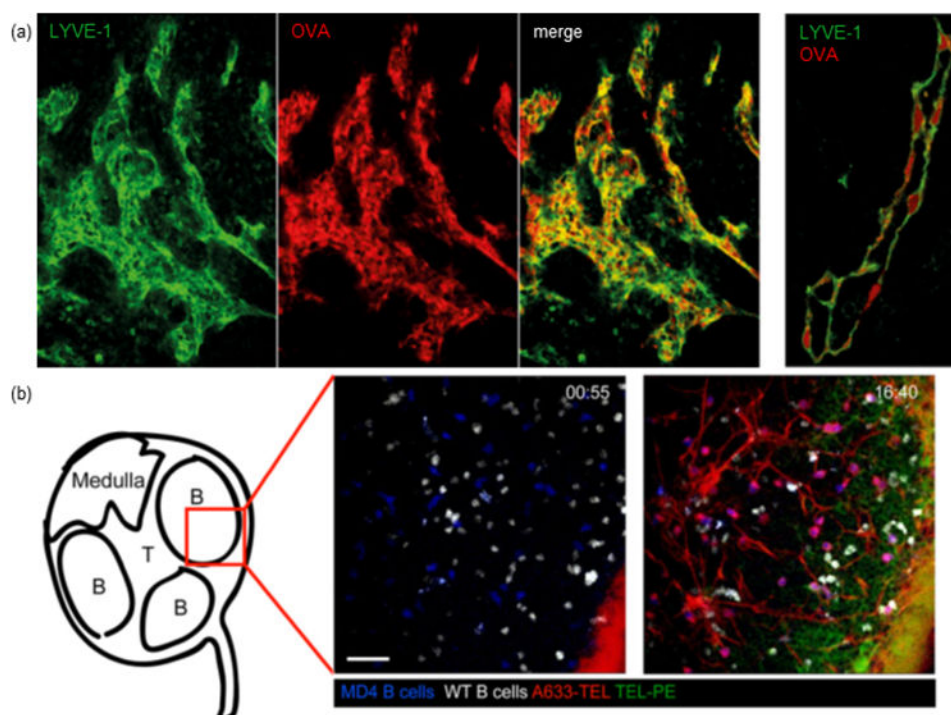
**Figure 1.**

Within the interstitium under the influence of slow interstitial fluid flow, macromolecular transport is restricted by the extracellular matrix (ECM). i) Large (>70 kDa) but not small molecular species become entrapped by the gel-like ECM [17]. At similar hydrodynamic radii, penetration through the ECM is increased by ii) high particle aspect ratios, which correspond to a smaller Stokes radius along one carrier dimension [31], and iii) flexibility of the macromolecular assembly, such as that exhibited by biopolymers [17].

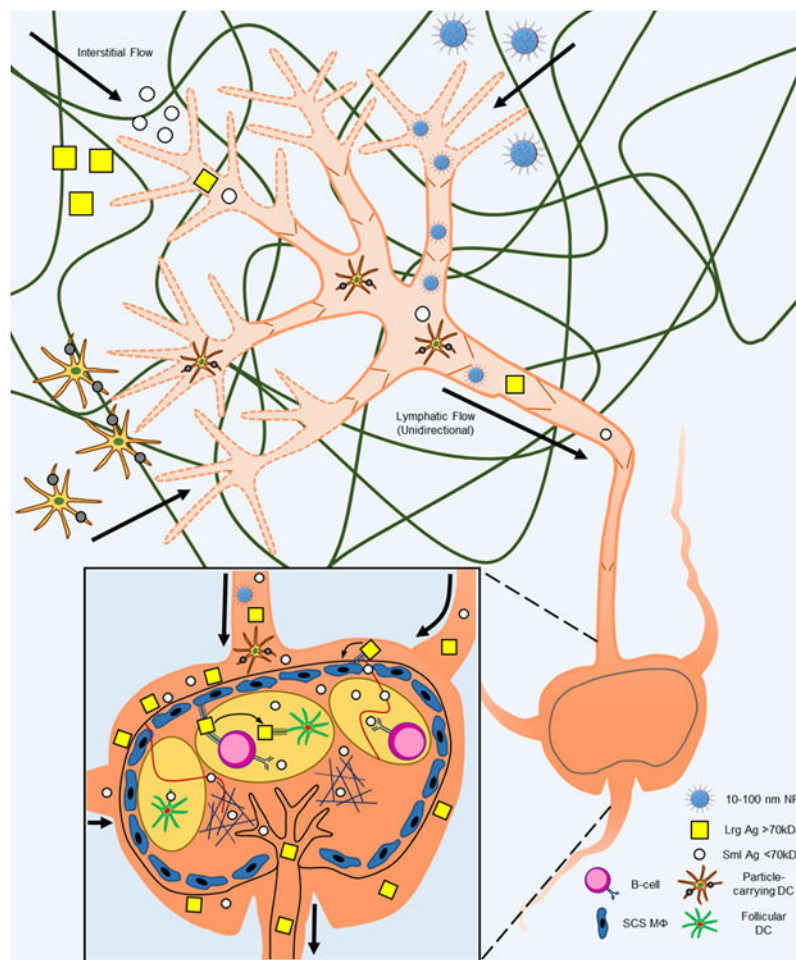


**Figure 2.**

Multistage carrier design innovations overcome the opposing influence of macromolecular/particle size on interstitial penetration and retention. (A) Nanoformulation sizes ( $\sim 100$  nm in diameter) optimum for intratumoral accumulation by the enhanced permeability and retention effect become trapped perivascularly as the result of remodeling of the tumor interstitium extracellular matrix, resulting in poor tumor penetration of encapsulated drug. (B) Release of payloads of smaller hydrodynamic radii from multistage delivery systems as the result of matrix metalloproteinase 2 activity within the tumor microenvironment significantly improves tumor penetration [32]. (C) Conventional small molecules penetrate rapidly and efficiently into the tumor interstitium but are poorly retained. (D) Engineered small molecules that aggregate as a result of caspase-3 activity into larger nanoaggregates are retained specifically within the tumor [35\*\*]. Arrow orientation and size represent direction and magnitude of molecular flux. Red and blue represent molecular transport inward and outward from tumor, respectively.



**Figure 3.** Emerging principles of *in vivo* molecular transport in the lymphatic system. (A) Lymphatic endothelial cells scavenge proteins *in vivo*. Left, brachial mouse lymph node showing lymphatic endothelial cell (Lyve-1, green)-associated AF647-conjugated ovalbumin (OVA, red). Right, intracellular localization of OVA (red) within lymphatic endothelial cells (green) of a Lyve-1+ lymphatic vessel. Reproduced with permission from [44\*\*]. (B) Proteins are distributed within lymph node B cell follicles after injection at different rates and locations as a function of molecular size. Intravital images of the distribution of 14 kDa (A633-conjugated turkey egg lysozyme (TEL), red) and ~250 kDa (phycoerytherin-conjugated TEL, green) protein-conjugates in a B follicle after footpad injection (white, wildtype B cells; blue, MD4 transgenic B cells). Top right corner indicates time post injection (min:sec). Scale bar, 30  $\mu$ m. Reproduced with permission from [53].



**Figure 4.**

Principles of lymph node targeting including the major transport barriers of the interstitium and the lymph node subcapsular sinus. Antigen presenting cells such as dendritic cells take up large particles (particle-carrying DC) and actively migrate to lymph nodes. Synthetic carriers such as nanoparticles (NP) 10-100 nm in diameter (optimum for penetration of the interstitial extracellular matrix) as well as protein antigens are carried from the interstitium by interstitial and lymphatic flows to draining lymph nodes. Lymph-borne solutes are distributed within lymph nodes in a size-dependent fashion. For example, large protein antigens (Lrg Ag) are taken up by subcapsular macrophages (SCS MΦ) and transferred to follicular dendritic cells (DC) either directly or as mediated by B cells. Small protein antigens, however, are efficiently taken up by follicular conduits and directly transported to B cell follicles or the T cell cortex. Thick arrows, direction of flow. Thin arrows, direction of antigen transfer to and between lymph node-resident cells.