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Review

Multi-stage delivery nano-particle systems for therapeutic applications ☆

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ABSTRACT

Background: The daunting task for drug molecules to reach pathological lesions has fueled rapid advances in Nanomedicine. The progressive evolution of nanovectors has led to the development of multi-stage delivery systems aimed at overcoming the numerous obstacles encountered by nanovectors on their journey to the target site.
Scope of review: This review summarizes major findings with respect to silicon-based drug delivery vectors for cancer therapeutics and imaging. Based on rational design, well-established silicon technologies have been adapted for the fabrication of nanovectors with specific shapes, sizes, and porosities. These vectors are part of a multi-stage delivery system that contains multiple nano-components, each designed to achieve a specific task with the common goal of site-directed delivery of therapeutics.
Major conclusions: Quasi-hemispherical and discoidal silicon microparticles are superior to spherical particles with respect to margination in the blood, with particles of different shapes and sizes having unique distributions *in vivo*. Cellular adhesion and internalization of silicon microparticles is influenced by microparticle shape and surface charge, with the latter dictating binding of serum opsonins. Based on *in vitro* cell studies, the internalization of porous silicon microparticles by endothelial cells and macrophages is compatible with cellular morphology, intracellular trafficking, mitosis, cell cycle progression, cytokine release, and cell viability. *In vivo* studies support superior therapeutic efficacy of liposomal encapsulated siRNA when delivered in multi-stage systems compared to free nanoparticles.
This article is part of a Special Issue entitled Nanotechnologies - Emerging Applications in Biomedicine.

1. Introduction

Nanotechnology pertains to synthetic, engineerable objects which are nanoscale in dimensions or have critical functioning nanoscale components, leading to novel, unique properties [1,2]. These emergent characteristics arise from the material's large surface area and nanoscopic size [3]. Nanotechnology now occupies a niche as a burgeoning and revolutionary field within medicine known as nanomedicine, particularly within the field of oncology [1]. One of the potential benefits of nanomedicine is the creation of nanoparticle based vectors that deliver therapeutic cargo in sufficient quantity to a target lesion to enable a selective effect. This is a daunting task for all drug molecules, owing to the highly organized array of "biological barriers" that the molecules encounter [3–6]. The human body presents a robust defense system that is extremely effective in preventing injected chemicals, biomolecules, nanoparticles and any other foreign agents from reaching their intended destinations. Biobarriers are sequential in nature, and therefore, the probability of reaching the therapeutic objective is the product of individual probabilities of overcoming each barrier [7,8]. Sequentially, with respect to intravascular injections, these comprise: enzymatic degradation; sequestration by phagocytes of the reticulo-endothelial system (RES) [9,10]; vascular endothelia [11]; adverse oncotic and interstitial pressures in the tumor [12,13]; cellular membranes, or subcellular organelles such as the nucleus and endosomes [14,15]; and molecular efflux pumps [16]. Without an effective strategy to negotiate these barriers, new or current therapeutic agents based on enhanced biomolecular selectivity may yield sub-optimal utility, simply because they reach the intended targets in very small fractions, with only 1 in 10,000 to 1 in 100,000 molecules reaching their intended site of action [7]. Due to this narrow therapeutic window, marginal tolerability and considerable mortality ensue [17]. Transport through different compartments and across biological barriers can be

Abbreviations: MDS, multi-stage delivery system; PEG, poly (ethylene glycol); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; HMVEC, Human MicroVascular Endothelial Cell; HUVEC, Human Umbilical Vein Endothelial Cell; APTES, 3-aminopropyltriethoxysilane; Qdots, quantum dots; RES, reticulo-endothelial system

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enhanced by optimization of particle size, shape, density and surface chemistry. These parameters dominate transport in the bloodstream, margination, cell adhesion, selective cellular uptake, and sub-cellular trafficking.

An early obstacle for intravascularly administered therapeutics is the endothelial wall which forms the boundary between the circulatory system and tissue specific microenvironments. Specific adherence of delivery vectors to diseased vasculature provides a key to conquering this early barrier, as does the hijacking of cells bound for the inflammatory microenvironment of the lesion. In order to efficiently overcome various biobarriers multiple levels of targeting, spatial release of secondary carriers or therapeutics, simultaneous delivery of independent systems or systems with a synergistic impact should be considered. The purpose of this review is to present the multi-stage concept of drug delivery and to summarize the experimental techniques and research findings that have transpired from this area of research.

2. Nanovector taxonomy

A variety of nanocarrier-based drug delivery systems with different compositions, geometry, and surface modifications are under various stages of investigation [1,18], producing an enormous collection of nanoparticles with a large array of possible combinations. Fig. 1 illustrates three main categories of nanovectors that have been described based on their functions and capabilities [1,19,20]. First generation nanovectors are the most elementary, and home to diseased sites by passive mechanisms such as the enhanced permeation and retention (EPR) effect, or more specifically, extravasate through gaps in tumor neo-vasculature. Avoidance of uptake by the RES is through functionalization with neutral polymers, such as poly(ethylene glycol) (PEG) [21–23]. Interactions between the aqueous environment and the hydrophilic polymers permit extension and mobility of the polymeric chains [24]. Derivatized nanoparticles adsorb plasma components more slowly [25] based on steric repulsion forces [26], negating opsonin driven uptake of nanoparticles by phagocytic cells, and enhancing blood circulation time. Advantages of nanoparticle-based carriers include improved delivery of water insoluble drugs, prolonged circulation half life, and reduced immunogenicity [27,28].

The second category of nanovectors is comprised of delivery systems with an additional functionality [29–33], including: (a) targeting of the disease site through ligands that specifically bind to receptors uniquely or over-expressed in the tumor microenvironment; (b) advanced functionalities, including co-delivery of multiple therapeutics or imaging agents, or triggered or controlled release of therapeutic agents. More sophisticated than their predecessors, the second generation of nanovectors represents a progressive evolution of first-generation nanovectors.

The third generation of nanovectors represents a paradigm shift in the strategy to overcome numerous obstacles encountered by nanovectors on their journey to the target site. Since no single agent can conquer the plethora of barriers that exist, these nanovectors are comprised of diverse families of nanoparticles nested into a single vector to achieve collaborative interactions. These carriers, or Logic Embedded Vectors (LEVs) [8] are therapeutic, multi-component constructs specifically engineered to avoid biological barriers, in which the functions of biorecognition, cytotoxicity and biobarrier avoidance are decoupled, yet act in efficacious, operational harmony. As an example of this therapeutic strategy, one can envision a vector which effectively navigates through the vasculature based on its geometry, attaches to the diseased vascular site through specific surface recognition and releases different nanoparticle payloads that simultaneously and synergistically extravasate, reach tumor cells and deliver their active agents at optimal concentrations to selectively eliminate malignancy with minimal side effects. This concept describes the multi-stage delivery system that will be extensively reviewed in this paper. By definition, third generation nanovectors have the ability to perform a time sequence of functions through the use of multiple nano-based components that synergistically provide distinct functionalities.

In addition to the multi-stage delivery system, an example of third generation nanoparticles is biologically active molecular networks called “nanoshuttles,” which are self-assemblies of gold nanoparticles within a bacteriophage matrix. Nanoshuttles combine the hyperthermic response of the gold to near-infrared external energy with the biological targeting capabilities of the 4C-RGD sequence presented by the phage [32,34]. These nanoshuttles collectively accommodate enhanced fluorescence, dark-field microscopy, and surface-enhanced Raman scattering detection.

Another example of a third generation nanovectors is the “nanocell,” based on a disease-inspired approach to therapy. [35]. Utilizing the combinatorial chemotherapy approach, researchers have developed a nested nanoparticle construct that comprises a lipid-based nanoparticle enveloping a polymeric nanoparticle core called a “nanocell.” A conventional chemotherapeutic drug, doxorubicin, is conjugated to a polymer core and an anti-angiogenic agent, combretastatin, is then trapped within the lipid envelope. When nanocells accumulate within the tumor through the EPR effect, the sequential time release of the anti-angiogenic agent, and then the cytotoxic drug, causes an initial disruption of tumor vascular growth and effectively traps the drug conjugated nanoparticle core within the tumor to allow eventual delivery of the cancer cell killing agent.

Silica and silicon-based delivery systems represent the final example of third generation nanovectors. Mesoporous silica nanoparticles have been developed to co-deliver doxorubicin and Bcl-2 siRNA by encapsulation of doxorubicin inside the pores and complexation of siRNA in a dendrimer shell [36]. The goal of this nanodevice is to simultaneously deliver an anticancer drug as an apoptosis inducer and siRNA molecules as suppressors of membrane pumps that mediate multidrug resistance. This multi-component nanodevice was able to significantly enhance the cytotoxicity of doxorubicin by decreasing the IC_{50} 64-fold.

Mesoporous silicon devices include our multi-stage system [37]. Based on well-established semiconductor microfabrication lithography techniques, which allow for exquisite control of size, shape, and porosity, in concert with active biological targeting moieties, these vectors are intended to deliver large payloads of nanoparticles and higher order therapeutic and imaging agents to the tumor site. The “stage one” mesoporous silicon microparticles are designed based on mathematical modeling to exhibit superior margination and adhesion during their navigation through the systemic circulation. Stage one particles shoulder the burden of efficiently transporting, shielding, and controlling the rate of release of the nanoparticle payload. The encapsulated nanoparticles, called “stage two” nanoparticles, can be any nanovector construct within the approximate diameter range of 5–100 nm. The multi-stage drug delivery system is an example of LEVs which strategically combine numerous nano-components aimed at delivering single or multiple component nanovectors to the tumor site. The stage one particle is rationally designed to have a hemi-spherical or discoidal shape to enhance particle margination within the blood, as well as interactions between particles and endothelia, with a goal of maximizing the probability of active tumor targeting and adhesion [38]. In addition to improved hemodynamic properties and active biological targeting utilizing nano-components such as aptamers and phages, as will be discussed below, the stage one particles may also present specific surface modifications to avoid RES uptake. Following recognition of tumor vasculature and firm vascular adhesion, a series of nanoparticle payloads may be released in a sequential order dictated by diffusion from expanding or newly opened nanopores. Factors governing nanoparticle release include stage one particle degradation rates, polymeric coating, and stage two design strategies (e.g., environmentally sensitive cross-linking techniques with pH, temperature, and/or enzymatic triggers). We have observed that the degradation rate of the porous silicon particles is proportional to its porosity, and can be tuned from hours to days without surface functionalization. The versatility of