PERSPECTIVE

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To cite this article: Jin Qian et al 2017 Nanotechnology 28 122501

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Self-assembled nano-balls released from multistage vector for cancer therapy

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Abstract
The efficacy of cancer drugs is often compromised due to the existence of biological barriers such as nonspecific distribution, hemorheological flow limitation and endothelial extravasation, impaired delivery across tumor cell membranes and tissue, and multidrug resistance. To overcome these obstacles, Xu et al developed an injectable nanoparticle generator platform to negotiate with the biological barriers and enable self-assembly of nano-balls in situ in order to maximize drug accumulation inside the tumor tissues and hence the therapeutic efficacy. This perspective aims to elaborate the designing strategy, and discuss the mechanism of action of the new drug and the potential for future development of nanoparticulate drugs.

Keywords: injectable nanoparticle generator platform, self-assembly, nano-balls, cancer therapy, multistage vector

Despite years of efforts spent on research and development for cancer treatment, cancer still remains a major cause of human deaths in the world. One of the most important factors that limit the efficacy of conventional chemotherapeutics is the existence of biological barriers hindering the accumulation of drugs in tumors. They include nonspecific distribution, hemorheological flow limitation and endothelial extravasation, impaired delivery across tumor cell membranes and tissue, and multidrug resistance (MDR) [1]. Currently, study on nanoparticle-based drug delivery is extending beyond the confines of convention so as to rationally design entities tasked with overcoming biological barriers specifically. While site-specific delivery of therapeutics will remain a distant reality unless nanocarrier design takes the majority of the biological barriers that a particle encounters upon intravenous administration into account. Also, it is questionable that a single material or component would be able to execute multiple strategies for obtaining a desirable biodistribution. Rather, a platform that integrates various components may have greater potential to perform several functions. Recently, an innovative design has been reported by Xu et al, to overcome multiple biological barriers for cancer drug delivery [2]. They reported an injectable nanoparticle generator (iNPG) that can negotiate with sequential biological barriers and release self-assembled nano-balls in situ, resulting in superior therapeutic efficacy in multiple tumor models (figure 1).

As specific biological barriers are encountered at different phases throughout the drug delivery process, several multistage drug delivery platforms have been designed, including the nanocell [3], the amplifying system [4] and the multistage vector (MSV). Based on the concept of MSV, Xu et al fabricated iNPG as a novel platform encompassing three different components: first stage of porous silicon microparticle, second stage of nanoparticles, and therapeutic agents, and each of them was designed to overcome continuous biological barriers with several strategies to achieve tumor accumulation of chemotherapeutic agents [2].
Geometrical targeting, particularly by modulating particle size and shape, has been applied as a strategy to achieve preferential accumulation of nanoparticles in specific organs of human body. In some early studies, spherical particles were commonly designed and modified for medical applications [5]; however, spheres did not display optimal transport properties. Hydrodynamic forces arise with the drag force along the flow direction and the torque, which will detach the spherical particle and dislodge it away from the substrate because the spheroid is fixed with respect to the flow [6]. Consequently non-spherical particles have been explored to achieve better delivery outcomes. Van Dilen and colleagues fabricated non-spherical particles by using ion beam radiation to transform spherical silica particles into oblate ones [7]. Comparing to spherical type, oblate particles have larger volumes with the same probability of adhesion, and can carry a larger number of payloads such as contrast agents, increase the therapeutic efficacy or the imaging resolution. Whilst, some researches have shown that nanoparticles possessing traditional spherical geometries exhibit minimal lateral drift and are less likely to migrate to vessel walls and establish contact/binding points with endothelial cells [8, 9]. Thus, the design of non-spherical particles could prolong the retention of drugs by resisting the hemorhelological flow limitations, and consequently raise both the bioavailability and the therapeutic efficacy.

Another strategy to improve the biodistribution of nanoparticles is to use surface ligands that bind to specific biological targets associated with pathological tissue. However, binding interactions between surface ligands and biological targets can be interfered in the presence of a dense protein corona that forms upon the nanoparticles exposing to biological fluid [10, 11]. It has been shown that

Figure 1. Sequential steps in drug transport and release of the active ingredient including vascular transport, tumor accumulation of iNPG-pDox in sites of interest due to innate tropism and association with diseased endothelia, in situ self-assembly of pDox nanoparticles, cellular internalization, and intracellular transport of pDox.
binding capacity of transferin-functionalized nanoparticles to target receptors decreases with the increasing concentration of protein [12]. Since transport of systemically injected nanoparticles predominantly depends on blood flow, the main function of this strategy is to increase retention as well as cellular uptake of nanoparticles [13]. Although the results of molecular targeting have been less impressive than initially anticipated, this approach is likely to yield promising effects when combined with other strategies. Besides, the presence of targeting ligands would potentially enhance receptor-mediated transcytosis, so as to facilitate the penetration of biological barriers [14].

With the aforementioned strategies, Xu et al designed porous silicon microparticle-based non-spherical iNPG to realize local delivery of therapeutic agents. The accumulation of particles on the surface of tumor blood vessels rather than blood vessels in the normal tissues is mainly due to the different blood flow hydrodynamics between healthy and disease tissues [15, 16]. Moreover, the porous silicon microparticle was chemically conjugated on the surface with 3-(aminopropyl)triethoxysilane (APTES), which endowed particles with a positive charge [17], produced stable linkages between amino groups of silicon microparticles and functional linkages, and increased the binding to biological targets. In Xu et al’s research, histological analysis of tumors showed accumulation of iNPG-polymeric drug (pDox) in tumor tissues, and TEM analysis of tumor tissues confirmed tight attachment of the silicon carrier constructs to tumor microvessels [2].

However, localized strategies alone would not produce a superior therapeutic effect, as the anthracycline drugs function in nucleus by intercalating the genomic DNA. In general, delivering the active drug to perinuclear region is difficult, especially for those drugs can be mediated by efflux pumps, which also easily leads to MDR. As a result, the therapeutic efficacy of doxorubicin (Dox) is enormously limited by MDR because it is a substrate of multiple efflux pumps such as p-glycoprotein and MDR protein 1 distributed on the membrane of cancer cells [18]. Considering the ‘enhanced permeability and retention (EPR)’ effect in solid tumors [19], Xu et al applied self-assembly as a novel strategy to form the pDox nanoparticles in order to facilitate Dox entry into the nucleus of cancer cells [2].

A direct method to implement this strategy is to conjugate Dox with hydrophilic synthetic polymers, which may result in an increased cytotoxic effect comparing to free Dox therapy [20]. Xu et al used poly (L-glutamic acid) as a biodegradable material to conjugate with Dox to prepare the pDox, which could self-assemble into nano-balls in aqueous environment. As the pDox was constituted of hydrophobic parts (Dox) and hydrophilic parts (L-glutamic acid), once exposed to aqueous environment, its hydrophilic part tends to cover the hydrophobic part and generally form into nanoparticles. The self assembled nanoparticles has shown enhanced efficacy owning to targeted localization in tumors and active cellular uptake [21] by extravasating into the tumor interstitium through leaky vasculature [22, 23], or alternatively through transcytosis.

As it is inefficacious to conjugate drugs to polymers if the therapeutic agents could not be released properly, the linkage between polymer and drugs seems to be another key point, so the pH-sensitive cleavable linker should be taken into account. pH-sensitivity is widely used as a trigger stimulus for targeted drug delivery since its variations in the human organism are significant. However, delivering drugs to cancer cells is a difficult task because the pH-sensitivity must be achieved for only a very small variation from 7.4 in blood stream, to 6.8 in cancer cells and 5–6 in intracellular space including the exosomes and endosomes. This difficult task is met by drug delivery systems that rely mostly on two strategies: destabilization of the micelles due to polarity change and subsequent release of the core entrapped drug, or hydrolysis of an acid-sensitive bond that was
used to conjugate drugs to the polymer. The micelle destabilization strategy generally is either based on polyacrylates and vinyl polymers that have poor biocompatibility, or poly (amino acids) that is mostly susceptible to enzymatic biodegradation, resulting in poor controlled release in vivo [24]. Thus it is a better choice to use an acid-sensitive bond to conjugate drugs to the polymer. For the past few years, pH-sensitive cleavable linkage has been vastly applied in conjugating Dox with micelles [25], and the release profiles of Dox from micelles showed a strong dependence on the environmental pH values, that is, the Dox release rate increased in the acidic medium. In a recent study, copolymer conjugates bearing Dox partly bound via a pH-sensitive hydrazone and partly via enzymatically degradable amide bonds were synthesized and their effect was compared with each other [26]. In contrast to conjugation with the amide bond-bound Dox which requires the presence of lysosomal enzymes to release Dox, the polymer-drug conjugate via a hydrazone bond released Dox by pH-sensitive hydrolysis, which was significantly faster in a buffer of pH 5.0 than pH 7.4. Moreover, it was shown in the latest research that the drug release profile can be changed artificially on the basis of different hydrolytic cleavability of the ester and hydrazone bonds [27]. In Xu et al’s work, hydrazone bond was adopted as a pH-sensitive cleavable linker to conjugate Dox with poly (L-glutamic acid), enabling Dox to be released in the perinuclear region with an acidic environment [2]. Thus, the drug molecules would be far away from the cell surface MDR proteins, which is beneficial for overcoming MDR and consequently enhancing the therapeutic efficacy. In addition, the shield of self-assembled nanoparticles that prevent the drug from exposure to the binding sites of trans-membrane efflux pumps is another reason for the MDR reversing property of iNPG.

Furthermore, the modified porous silicon also contributes to the mechanism of self-assembly. Apart from the potential influence of pH value, the particle size of self-assembled nano-balls produced by Xu et al was influenced by the pore of silicon vector [2], indicating the importance of the vector fabrication. As illustrated in one study, non-spherical porous silicon with various diameters, heights, pore sizes, and porosity were fabricated [28], which enabled a better control of the property of self-assembled nanoparticles. Besides, as the degradation and subsequent drug release of porous silicon is promoted in response to reactive oxygen species [29] present in high levels in tumor tissue [30], the therapeutic efficacy of released nanoparticles would be enhanced.

Combined with above-mentioned strategies, iNPG was provided with multiple functions to deliver Dox. Once injected, the non-spherical particle would preferentially bind to diseased vasculature and protect the cargo from degradation. As the silicon material degrades, pDox is exposed to aqueous environment and consequently self-assembles into nanoparticles, which not only enhances the cellular uptake through leaky vasculature and transcytosis, but also circumvents trans-membrane efflux pumps. When the self-assembled nanoparticles are transported to the perinuclear region, the pH-sensitive linker between Dox and polymer would be cleaved, and then Dox is released from the hydrophilic shield to nucleus (figure 1).

Apart from the strategies Xu et al has used above, there is another novel targeting strategy used in conjugation with the MSV. Based on biomimicry to improve the effect of geometrical targeting, it can be further exploited through imitation of biological surfaces. It is reported that MSVs coated with cell membranes derived from leukocytes could reduce immunological recognition and clearance by the mononuclear phagocyte system and resident macrophages in the liver and spleen [31]. And stealth polymers as polyethylene glycol have been used to reduce particle opsonization so as to decrease macrophage uptake and prolong blood circulation time [32–34]. Such cell-mimicry properties allow delivery
vehicles to promptly respond to dynamic changes in the microenvironment, which is a promising area in the further study of MSV.

On a whole, the porous silicon, pH-sensitive linker, and poly (L-glutamic acid), together constituted into the MSV, which incorporates multiple strategies to enhance the delivery of chemotherapeutics Dox for cancer treatment. Their controllable fabrication and various modifications may endow nanoparticles with versatile applications to deal with different situation of specific tumors. With each of the single strategy well developed, rational incorporation of all of them may have multifold therapeutic effect, which provides a novel way to overcome multiple biological barriers for effective cancer drug delivery. And in the years to come, this kind of novel platform may be used to deliver other drugs to treat different tumor models.

Acknowledgments

Our lab was supported by the ‘Shu Guang’ project supported by Shanghai Education Development Foundation and Shanghai Municipal Education Commission (15SG39) and the Shanghai Pujiang Program (16PJ044).

Conflict of interest

The authors declare that there is no conflict of interest.

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