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Drug delivery systems using mesoporous silicon microparticles incorporated with nanoparticles

Abstract

This minireview summarized a part of the recent work of Professor Mauro Ferrari on the application of mesoporous silicon microparticles to celebrate the occasion of the establishment of a new company, BrYet Pharma. This article is focusing on the principle and application of injectable nanoparticle generators (iNPG) for drug delivery systems. Three main examples are described; deliveries of anti-cancer agents and peptide cancer vaccines using iNPG, and oral formulation of iNPG for inflamed intestinal mucosa. The iNPG drug delivery system provides protection, the versatility of loading, and the selective delivery of therapeutics, which enables prolonged efficacy, the simultaneous delivery of a combination of therapeutics, and the reduction of toxicity. The advantages of the iNPG drug delivery system are discussed with examples in this minireview.

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Introduction

Nanoparticle drug delivery systems are designed to improve the stability and solubility of the drug, facilitate the transport through membranes, and prolong the circulation time, thereby enhancing safety and efficacy. For the effective delivery of nanoparticles to target cells, many biological barriers must be overcome, including the endothelium, extracellular matrix, and cell membranes. For this purpose, multifunctional drug delivery systems have been constructed by integrating the nanoscale system with the microscale system.[1,2] This system consists of three elements: porous silicon microparticles, nanoparticles encapsulated into the silicon particles, and therapeutic drugs bound to the nanoparticles, each of which is designed to overcome the specific biological barriers.

The first component is silicon microparticles which are designed to navigate the vascular compartment and accumulate in the vasculature of the target tissue. The second component is nanoparticles which are designed to be released from the microparticles into the tissue interstitium, triggered by target tissue-specific microenvironmental changes (e.g., pH or enzyme changes) or degradation of the silicon particles (the first component), and permeate biological barriers present in the extracellular and intracellular compartments. The third component is small molecule therapeutics that are designed to be released in targeted cells and to avoid efflux by cell membrane transporters.

Several excellent reviews have been published on this multi-step vector-based (MSV) drug delivery method. One of them describes the manufacturing process, properties, safety, and targeting strategies of mesoporous silicon microparticles[3] and another summarizes functionalized mesoporous silicon microparticles.[4] The application of this MSV system was featured in a review describing the application of the system for cancer therapy evaluated in clinical trials and promising preclinical delivery systems of antitumor drugs.[5] In this minireview, examples of MSV drug delivery systems with a payload, i.e., doxorubicin (Dox) or docetaxel (DTX), are presented. In addition, an MSV peptide vaccine delivery system for cancer immunotherapy and an orally available MSV delivery system are described.

Injectable nanoparticle generator (iNPG) and its application for lung metastasized triple-negative breast cancer

Porous silicon can be applied to drug delivery due to its large surface area, tunable properties, biodegradability, and non-toxicity.[6] Selective drug delivery to target organs can be achieved by changing the shape of the silicon microparticles, and well-established semiconductor fabrication techniques allow for the precise generation of silicon microparticles of a specifically designed shape.[7] The biodegradation rate of mesoporous silicon microparticles can be controlled by modifying pore size, with larger pores resulting in faster degradation and thus faster release of drug molecules. Furthermore, the introduction of polyethylene glycol (PEG) to porous silicon microparticles can change the surface chemistry, as well as their degradation rate in vivo.[8]

One of the examples discussed here is an MSV drug delivery system for doxorubicin targeting lung-metastasized triple-negative breast cancer.[9] The porous silicon microparticle used for this study is designed to release polyglutamic acid-doxorubicin conjugate (pDox) nanoparticles. The size of pDox nanoparticles released from the porous silicon at the target organ is determined by the pore size of the silicon microparticles, thus the porous silicon microparticle has been named injectable nanoparticle generator (iNPG). Specifically, mesoporous silicon microparticles of 2.5 micrometers in diameter and 700 nm thick disks were used as the first component, which is shaped to accumulate in lung lesion tissue. Polyglutamic acid was used as the second component to which the hydrazide group was introduced as a linker for the third component, an antitumor drug, doxorubicin. Polyglutamic acid and doxorubicin were connected through a hydrazone bond. The overview of this MVS drug transport system is shown in Figure 1.

Activated iNPG consists of approximately 500~600 nanopores (20 ~ 80 nm) which are covered by covalently bonded positively charged amino groups. In theory, negatively charged pDox can associate with positively charged nanopores. In the environment at the lower pH, the charge of pDox is altered to be less negative and promotes dissociation from the positively charged environment. The particle size of released pDox aggregates from iNPG is approximately the size of the nanopores, which was observed by atomic force microscopy.[9]

The molecular weight of pDox used in this study is approximately 60K Daltons. Thus, the enhanced permeability and retention (EPR) effect observed in biopolymers with molecular weights above 50KD is expected to occur.[10] In other words, the pDox is expected to be distributed in tumor tissues at higher concentrations than in normal tissues.

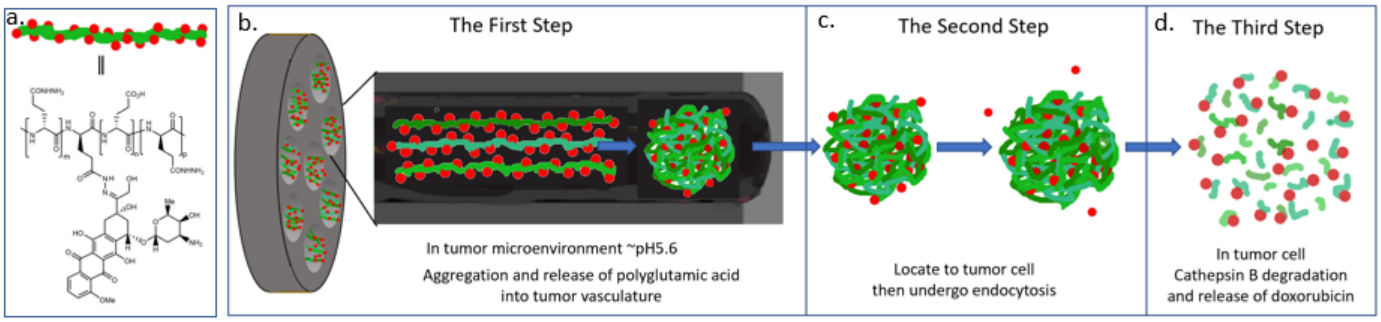

Figure 1 a. Chemical structure of pDox. b. iNPG-pDox composition, pDox prodrug encapsulation, pDox nanoparticle formation. c. Release of pDox from nanopores. d. Digestion of pDox and release of doxorubicin.

In the tumor microenvironment (about pH5.6), released pDox nanoparticles from iNPG undergo energy-dependent endocytosis by tumor cells.[11] The pDox nanoparticles incorporated into endosomes by endocytosis migrate along microtubules into the perinuclear space to form late endosomes which are eventually converted into lysosomes.[12] The pDox nanoparticles are degraded by the hydrolytic enzyme cathepsins. The hydrazone linker of the resulting fragments is hydrolyzed inside of the acidic lysosome to release doxorubicin which can avoid efflux by membrane transporters such as P-glycoprotein and multidrug resistance proteins. Doxorubicin migrates into the nucleus, binds to base pairs of DNA, inhibits topoisomerase II function, and alters chromatin structure, which induces apoptosis of cancer cells.

Doxorubicin is toxic to the myocardium, and its cardiotoxicity leads to treatment interruptions. However, by selective delivery to tumor tissues, cardiotoxicity can be suppressed and the dosage can be increased. In fact, the iNPG-pDox complex used in this study had a 10-fold greater distribution to the lungs than to the heart 24 hours after the injection. Furthermore, high concentrations of the drug were still detected in the lungs seven days after the injection, confirming the stability and persistence of the iNPG-pDox complex. Compared to free doxorubicin, lung accumulation increased 7-fold in tumor-bearing mice, and the median survival was evaluated for the control, doxorubicin, Doxil®, and iNPG-pDox complex groups and was 87, 98, 124, and 233 days, respectively.[9]

The biodistribution kinetics of the iNPG-pDox complex were quantitatively observed in a mouse model of lung metastatic breast cancer using positron emission tomography (PET-CT) and optical imaging and high-resolution microscopy, from the whole body to the organ and finally to tissue and cell levels. iNPG-pDox showed passive tumor tropism at five minutes after injection, accumulating significantly in tumor-bearing lungs. Subsequently, trafficking patterns of iNPG and pDox diverged and differed according to the size of the tumor. iNPG tended to migrate in the tumor-associated pulmonary blood vessels, while pDox nanoparticles were found to infiltrate tumor lesions. High-resolution confocal microscopy confirmed that the iNPG tends to localize to the pulmonary tumor-associated blood vessels while pDox nanoparticles infiltrated into the tumor lesions. Furthermore, significantly more iNPG accumulated in tumor-bearing lungs compared to healthy controls.[13]

Recently, as an approach enabling the simultaneous eradication of cancer cells and cancer stem cells (CSCs), it was reported that a platform consisting of gold nanoparticle-coated porous silicon microparticle (AuPSM) that was also loaded with docetaxel micelles (mDTX).[14] AuPSMs are stimulated with near-infrared light in tumor lesions, which promotes released mDTX and generates mild hyperthermia. Treatment of MDA-MB-231 and SUM159 TNBC cell lines with mDTX-loaded AuPSM and mild hyperthermia treatment reduces the mammosphere formation and the number of ALDH1+ cells compared to treatment with mDTX alone or mild hyperthermia treatment alone, which suggested simultaneous eradication of cancer cells and CSCs. Furthermore, in the orthotopic model with SUM159 and 4T1 cell lines injected in immunodeficient NSG mice, treatment with mDTX-loaded AuPSM and mild hyperthermia treatment markedly suppressed orthotopic tumor growth of SUM159 and metastasis of 4T1. The mechanism of the synergistic effect was explained as the result of enhanced sensitivity to mild hyperthermia by the downregulation of heat shock protein 27 by DTX.[14]

Application of iNPG to Peptide Vaccine Delivery Systems in Cancer Immunotherapy

Cancer immunotherapy is a therapy that manipulates the immune system to recognize and eradicate cancer cells. The recent discovery of immune checkpoint inhibitors that enhance antitumor immunity has revolutionized cancer immunotherapy.[15,16] Among cancer immunotherapeutic agents, cancer vaccines elicit specific immune responses to tumor antigens such as induction of tumor-specific T-cell responses. Cancer vaccine platforms are classified as a cellular, viral vector, or molecular (peptide, DNA, or RNA). The applications of cancer vaccines are expected to expand with the identification of cancer-shared antigens and mutation-derived neoantigens.[17] Tumor antigen peptides have been widely applied in cancer vaccine formulations because of their safety and accessibility; however, the immune response of peptide cancer vaccines is weak and transient. The improvement of the efficacy of peptide vaccines is the subject of current research involving the improvement of vaccine delivery systems, modifications of peptides, and combination with other therapies such as immune checkpoint inhibitors.[18] Many of such improved peptide vaccines advanced into the clinical trial. The status of recent clinical trials on therapeutic cancer vaccines including tumor antigen peptides is summarized in an excellent review by Cohen et.al. of UCSD.[19]

This article focuses on the delivery of molecular cancer vaccines, especially peptide vaccines. Organic materials such as liposomes and lipid-based particles, polymers such as polylactic-co-glycolic acid (PLGA), polylactic acid, poly β-amino esters, and polyethylene glycol (PEG), or inorganic materials such as gold (Au)[20] and porous silicon[21-22] have been used as delivery vehicles for molecular vaccines.

Among them, gold nanoparticles and porous silicon microparticles are highly biocompatible and easily modified in size, shape, and surface chemistry to fine-tune cellular responses. With this advantage,

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gold nanoparticles have been successfully applied to deliver peptide vaccines and showed anti-tumor effects and adjuvant effects. This adjuvant effect was postulated as the contribution of the peptide coated the gold nanoparticle.[23] On the other hand, porous silicon microparticles alone act as adjuvants and are suitable for the delivery of molecular cancer vaccines due to their high-loading capacity and biodegradability.[24]

In this minireview, two multi-step vector-based (MSV) peptide vaccine delivery methods are presented. This study used highly porous hemispherical silicon microparticles with an average diameter of about 800 nm, with about 50 nm nanopores crossing the particle surface vertically.[25] The silicon microparticles were loaded with dioleoyl phosphatidylcholine (DOPC) liposomes encapsulated with peptide antigens (tyrosinase-related protein 2 peptide, TRP2 peptide SYVDFFVWL)[26,27] and dual toll-like receptor (TLR) agonists (CpG and MPLA)[28,29] to form the vaccine MSV/TRP2-CpG-MPLA (MSV/TRP2-CM for short). Coadministration of TLR agonists is intended to induce TLR signal-induced immunostimulatory effects against B16 melanoma.[30]

To monitor the cellular uptake of the MSV/TRP2-CM vaccine and intracellular localization of the vaccine components, TRP2 peptide, CpG, and MPLA are each labeled with fluorescent probes. The fluorescent-labeled MSV/TRP2-CM vaccine was incubated with bone marrow-derived DCs (BMDCs) in vitro. Subsequent analysis of BMDCs revealed that TRP2 peptide, CpG, and MPLA are localized to the endolysosomal compartment, suggesting simultaneous delivery of the three components by DC phagocytosis of MSV/TRP2-CM.

Regarding the fixation of TRP2 peptide on BMDCs, MSV/TRP2-CM was able to maintain the antigenic TRP2 peptide for more than 96 hours, while free TRP2-CM and Lipo/TRP2-CM disappeared in 24-48 hours. This strongly suggests that TRP2 peptide in silicon particles is protected from rapid degradation by hydrolytic enzymes. Furthermore, the co-delivery of CpG, MPLA, and the peptide vaccine with silicon microparticles enhanced antigen-specific T-cell responses. In the B16 lung metastasis mouse model, MSV/TRP2-CM vaccine significantly suppressed the number of B16 lung metastatic nodules.[30]

A similar study used the STING agonist cyclic2',3'-GAMP (cGAMP) which induces type I interferon expression in DCs and promotes the maturation and migration of DCs. The combination of TRP2 peptide, TLR9 agonist (CpG), and cGAMP were encapsulated in DOPC liposomes, then the liposomes were loaded into MSV to form an MSV/TRP2-CpG-cGAMP vaccine. MSV/TRP2-CpG-cGAMP is effective in inhibiting the growth of lung metastatic melanoma. Other tumor antigen peptides such as Her2-specific p66 antigen peptide TYVPANASL or gp70 antigen peptide SPSYVYHQF were incorporated into the MVS in the same formulation protocol. The resulting vaccines showed efficacy against Her2-positive breast cancer or subcutaneous colorectal cancer in their respective murine models.[31]

Oral formulation of iNPG targeting inflamed intestinal mucosa

Mesoporous silicon has been successfully applied to the oral formulation of various drugs, such as small molecules[32] and peptides.[33] The main target of this delivery system is the intestines since mesoporous silicon is resistant to low pH and degrades at higher pHs. The MVS system was applied to deliver anti-inflammatory steroid, budesonide, to inflamed intestinal mucosa.[34] This system consists of mesoporous silicon microparticles loaded with budesonide encapsulated poly-lactic-glycolic acid (PLGA) nanoparticles. The silicon microparticle protects budesonide-encapsulated nanoparticles from the acidic gastric environment and enables them to reach the inner mucus layer of the intestine due to the optimized size and weight of the silicon microparticle, allowing efficient delivery and prolonged release of budesonide. The efficacy of the MVS loaded with budesonide-encapsulated PLGA was tested with an in vitro model of inflammatory bowel disease and it was found that silicon microparticles accumulated at the inflamed area and restored barrier function. By monitoring a major tight junction protein, ZO-1, with confocal analysis, the process of recovery and reorganization of the tight junction was traced. Within two days of treatment, most of the ZO-1 structure was recovered although the barrier function was not fully recovered. After four days of treatment, the full recovery of the IBD in the Caco-2 model was observed and the barrier function was comparable to the healthy tissue.[34]

Conclusion

We have discussed multi-step vector-based (MSV) drug delivery systems, iNPG targeting lung metastatic breast cancer, vaccine delivery systems targeting DCs, and oral delivery systems targeting intestinal tissue. With the MSV delivery system, delivery of siRNA,[35] and topical delivery of growth factor[36] were developed and demonstrated efficiency and effectiveness.

To overcome biological barriers that cannot be addressed by nanoparticle delivery systems alone, a multifunctional drug delivery system that integrates nanoscale and microscale was introduced, which provided high flexibility in carrying multiple molecules to the same target simultaneously. Silicon microparticles can be loaded with various small molecules and/or nanoparticles such as proteins, PLGA, quantum dots, or liposomes. We hope that further knowledge and innovations make this MSV system safe, functional, and effective so that the MVS drug delivery system creates new therapies for unmet medical needs.

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None.

Conflicts of interest

All authors declare that there is no conflicts of interest.

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