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Recent advances in porous silicon-based therapeutic delivery

“With the incorporation of superparamagnetic iron oxide nanoparticles, direct imaging of the porous silicon particles *in vivo* using MRI is possible.”

Keywords: biopharmaceuticals • drug delivery • nanostructured • poorly water soluble drugs • porous silicon • theranostics

Porous silicon (PSi) is a nanostructured material that continues to receive extensive interest as a therapeutic carrier system. Consisting of a highly controllable network of nanoscale pores (typically 5–20 nm in diameter) this mesoporous biomaterial is ideal for use in the controlled delivery of therapeutics, owing to its excellent biocompatibility and potential for high loading capacity. The PSi surface can be readily modified, with thermal oxidation (TOPSi) and thermal carbonization (TCPSi) continuing to be the most common approaches. Alternatively, various other chemistries, such as Click, hydrosilylation and direct amination, can be used to achieve more specific functional surfaces [1]. A variety of nanostructured PSi materials have been investigated as therapeutic delivery vectors, including thin films, rods, nanoparticles (e.g., discoids) and microparticles for the delivery of therapeutics ranging from small molecules (e.g., mitoxantrone dihydrochloride [2], daunorubicin [3]) as well as biopharmaceuticals including both peptides [4,5] and nucleotides [6].

Here, we highlight recent advances in the development of novel multicomponent PSi composites as well as identifying novel therapeutic applications. We also review recent reports on the development of targeted PSi nanoparticulate systems for anticancer therapies, as well as studies improving our understanding of the PSi structure–activity relationship and their behavior both in model cell lines, as well as recent successful *in vivo* studies which demonstrate the potential of PSi as an oral therapeutic delivery system.

Composite nanostructured PSi systems

Although PSi provides a highly versatile therapeutic delivery platform in its own right, there is growing interest in preparing composite PSi-based therapeutic delivery systems to maximize potential opportunities and therapeutic benefits. In general, these composite systems consist of a simple polymer ‘coating’ of the PSi particles, providing triggered release or enhanced drug loading capacity by loading a drug–polymer complex into the PSi pores or the composite matrix includes additional components to provide additional functionality to the system, such as the incorporation of superparamagnetic iron oxide nanoparticles to enable direct imaging of the delivery system *in vivo*.

For example, Nan *et al.* [7] prepared hybrid TOPSi-poly lactide-co-glycolide microparticles for sustained ocular delivery of daunorubicin. In this system, the TOPSi acted as a drug reservoir while the poly lactide-co-glycolide outer coating provided controlled drug release over 70 days. Similarly, researchers have coated PSi with β -cyclodextrins to control the release of model drugs ciprofloxacin and prednisolone over more than 70 hours [8].

An interesting PSi nanocomposite was prepared by combining bovine serum albumin with PSi to achieve pH sensitive delivery of the anticancer drug doxorubicin (DOX) [9,10]. Initially a DOX–bovine serum albumin complex was prepared and subsequently loaded into the PSi pores using a solution partitioning method. A fivefold increase in DOX loading compared to loading DOX

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directly into PSi was observed. DOX release was triggered by acidic conditions (pH 5.5) and inhibited at neutral to basic conditions (pH 7.5 and 9.5).

Finally, there continues to be interest in developing so-called multimodal therapeutic delivery systems particularly to enable direct imaging *in vivo*, so-called theranostics. With the incorporation of superparamagnetic iron oxide nanoparticles, direct imaging of the PSi particles *in vivo* using MRI is possible. To-date, two methods for incorporating iron oxide into the PSi nanostructure have been investigated; direct deposition of preformed iron oxide nanoparticles [11,12] and the precipitation of iron oxide nanocrystals [13]. For example, Gizzatov *et al.* successfully demonstrated enhanced tumor accumulation of iron oxide-PSi composites, enabling much smaller iron doses (0.5 mg Fe kg⁻¹ animal) than currently used [12]. Nissinen *et al.* reported enhanced superparamagnetic behavior (high T₂ relaxivity) with their precipitated Fe nanocrystals, without sacrificing the high pore volume (hence therapeutic loading capacity) that makes PSi such an appealing delivery system [13].

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Targeted anticancer therapeutic delivery

There is considerable interest in developing targeted PSi-based therapeutic delivery systems, with several examples emerging in recent literature. Almeida *et al.* [14] prepared amine-modified hyaluronic acid functionalized PSi microparticles to target CD44 receptors that are overexpressed in breast cancer tumor cells. Interestingly, although no reduction in cell viability was observed over 24 hours (evaluated using an ATP-luminescence assay), upon incubation of the cancer cells with 100 µg ml⁻¹ for 24 hours, a statistically significant reduction in cell viability was observed. This poses the question of whether as we better target biological systems to enhance uptake of our nanoparticle-based delivery systems (compared to relying on passive targeting), will this correlate with increased toxicity?

Similarly, PSi also has interesting potential for use in immunotherapy applications. Recently, Serda *et al.* [15] successfully demonstrated increased uptake of PSi functionalized with toll-like receptor 4 ligands monophosphoryl lipid A or lipopolysaccharide by murine bone marrow derived dendritic cells (DCs). DCs are well-known activators of our adaptive immunity system, where they actively remove foreign bodies by

processes such as pinocytosis, endocytosis or phagocytosis, leading to excretion of proinflammatory cytokines and chemokines, and are of interest as an anticancer therapy. In this work, uptake of toll-like receptor-4 functionalized PSi microparticles by antigen presenting cells resulted in the DC mediated production of several cytokines, including IL-1β, IL-6 and TNF-α.

From using model cell lines to *in vivo* studies

The most challenging stage of development for new therapeutic delivery systems remains the demonstration of their performance *in vivo*, particularly because of the costs involved with such studies and the ethical considerations around animal testing. There remain very few published studies that demonstrate PSi fate and performance *in vivo*. Various delivery routes have been considered for PSi systems, including oral, intravenous, subcutaneous and intravitreal products. To date, the only PSi-based drug delivery products that have been successfully commercialized in US and European markets are Iluvien® and Retisert® (pSivida Corp., MA, USA) for the treatment of Diabetic Macular Edema and Posterior Uveitis, respectively, by intravitreal injection of fluocinolone acetonide.

With ongoing concerns over toxicity of nanomaterials, the short- and long-term fate of human cells after internalization of PSi nanoparticles has been investigated [16]. In this work human umbilical vein endothelial cells were exposed to hemispherical mesoporous microparticles (~3 µm in diameter). PSi microparticle uptake was observed within 60 minutes of exposure, and the internalized microparticles were subsequently passed on to daughter cells via cell proliferation. PSi microparticle internalization did not reduce cell viability, even at extremely high PSi:cell ratios (100:1). Live animal (mice) imaging was used to assess the biodistribution of the PSi microparticles upon intravenous administration over 168 hours. Not surprisingly, the liver and spleen were observed to be major accumulators of the PSi microparticles, while the lungs and heart also exhibited elevated Si levels, particularly in the first 2–4 hours after dosing. This was a particularly thorough and detailed study, which broadly confirmed earlier observations of a lack of toxic response to PSi exposure.

Recently, Huotari *et al.* [17] investigated the influence of PSi surface chemistry on controlling the loading, release and *in vivo* delivery of Glucagon-like peptide 1 (GLP-1) from anionic TOPSi/TCPSi and cationic amine terminated PSi. The results of the study were mixed, with the cationic PSi found to increase GLP-1 loading (three to fourfold) compared with the anionic TOPSi/TCPSi; however, while all GLP-1 loaded PSi samples decreased blood glucose after subcutaneous

injection in male mice, the cationic PSi did not provide prolonged blood glucose levels as was observed for the TOPSi/TCPSi. Along similar lines, Rytönen *et al.* developed an amine-functionalized TCPSi system for the delivery of splice correcting oligonucleotides. Splice correcting oligonucleotides were loaded (loading capacity approximately 14%) into the TCPSi using a solvent partitioning method, and the delivery system efficacy was demonstrated using HeLa pLuc 705 cells. It was determined that a cell penetrating peptide (NF51) was required as the transfection rate of the PSi nanoparticles was lower than that of the routinely used commercial Lipofectamine 2000; however, the PSi-based system was significantly less cytotoxic.

The usefulness of PSi in the delivery of poorly soluble drugs is well known, due to the geometrical confinement of the drug within the nanoscale dimensions of the pores, typically in the range of 10–20 nm [18,19]. This confinement prevents the drug molecules from forming a crystalline structure, rather, it remains in a stable amorphous form. Based on this approach, Wang and co-workers successfully demonstrated the use of TOPSi for oral delivery of the poorly soluble drugs indomethacin and celecoxib using a fasted rat model. In the case of both molecules, improved maximum plasma concentrations along with a decreased time

taken for maximum plasma drug concentration ($t_{\max}$) was observed compared with either the pure drug or the current commercial product [18,20].

Future perspective

PSi provides an incredibly versatile platform for the delivery of a wide variety of small molecule to peptide and nucleic acid based therapeutics. Currently, there is a significant effort toward developing multicomponent/matrix-type systems to provide additional control of loading and delivery of therapeutics. Recent cell studies have demonstrated the safety of PSi upon internalization while several *in vivo* studies using rats have provided some of the first evidence of PSi's ability to enhance poorly soluble drug uptake after oral dosing.

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