Porous silicon for drug delivery applications and theranostics: recent advances, critical review and perspectives
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Porous silicon for drug delivery applications and theranostics: Recent advances, critical review and perspectives

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Abstract

Introduction: Porous silicon (pSi) engineered by electrochemical etching has been used as a drug delivery vehicle to address the intrinsic limitations of traditional therapeutics. Biodegradability, biocompatibility and optoelectronic properties make pSi a unique candidate for developing biomaterials for theranostics and photodynamic therapies. This review presents an updated overview about the recent therapeutic systems based on pSi, with a critical analysis on the problems and opportunities that this technology faces as well as highlighting pSi’s growing potential.

Areas Covered: Recent progress in pSi-based research includes drug delivery systems, including biocompatibility studies, drug delivery, theranostics and clinical trials with the most relevant examples of pSi-based systems presented here. A critical analysis about the technical advantages and disadvantages of these systems is provided along with an assessment on the challenges that this technology faces, including clinical trials and investors’ support.

Expert Opinion: pSi is an outstanding material that could improve existing drug delivery and photodynamic therapies in different areas, paving the way for developing advanced theranostic nanomedicines and incorporating payloads of therapeutics with imaging capabilities. However, more extensive in-vivo studies are needed to assess the feasibility and reliability of this technology for clinical practice. The technical and commercial challenges that this technology face are still uncertain.

Keywords: Porous Silicon, Drug Delivery, Theranostics, Imaging, Therapy, Toxicity.
1. Introduction

From the beginning of the 20th century when Paul Ehrlich postulated his visionary concept of a “magic bullet” right up until the modern concept of nanomedicine, intensive activity in fundamental research and medical translation has been carried out for the development of nanomedicines. Omni-capable therapeutic systems have the potential to diagnose disease, deliver drugs and repair diseased organs [1-3]. The term, nanomedicine, refers to drug delivery systems that employ nanometer-scale materials in the form of nanoparticles (of various shapes and geometries) as carriers to encapsulate the therapeutic agents to be delivered at a target site with controlled release rate [4, 5]. The ultimate aim of drug delivery systems based on nanocarriers is to selectively target disease-causing organisms while keeping the rest of the host organism spared to minimize side effects and toxicity associated with non-targeted therapeutic substances. Furthermore, these systems can also overcome other intrinsic limitations of conventional medicines by enhancing their biodistribution, solubility, selectivity and pharmacokinetics [6, 7]. However, the modern concept of nanomedicine goes beyond simple drug delivery and more sophisticated forms of nanomedicines have been envisaged. For instance, theranostic agents combine both therapeutic and diagnostic entities into a single drug delivery carrier. This revolutionary concept has opened a plethora of possibilities, not only to treat diseases but also to diagnose and understand the biological response and progression of living systems to different treatments [8, 9]. Drug nanocarriers with theranostic capabilities enable imaging of nanomedicines to localize and monitor drug release and assess the therapeutic efficacy by analyzing the response to a given treatment in-situ, using numerous imaging modalities. Many different types of theranostic nanomedicines have been developed [10, 11]. Typically, these nanomedicines are composed of nanocarriers such as polymers, liposomes, micelles, antibodies and nanoparticles. In many cases, these vehicles are endowed with therapeutic and
diagnosis capabilities by co-loading drugs and imaging agents. However, some of these vehicles can offer intrinsic theranostic capabilities based on the chemical and physical properties of the nanocarrier, without additional co-loaded agents. Among these, porous silicon (pSi) is considered an outstanding platform to develop advanced drug delivery systems due to its unique set of physical and chemical properties, which can be precisely tuned by engineering its geometric features, surface chemistry and porosity.

pSi produced by electrochemical etching of silicon was discovered by the Uhlirs in 1956 at the Bell Laboratories [12-15]. Years after, Gösele and Canham identified quantum confinement effects in pSi structures, opening new opportunities to expand the application of pSi in optoelectronics [16, 17]. The unique optoelectronic properties of silicon and its porous forms make this the material of choice for microelectronic systems, the discovery of its biocompatibility and biodegradability by Canham in 1995 spread its use to drug delivery applications [18, 19]. Canham demonstrated for the first time the in-vitro biocompatibility of pSi films featuring micro-, meso- and macro-porosity. His pioneering studies revealed that the biodegradability and biointegration of pSi structures could be modulated by the degree of porosity and the size of its pores. Canham’s works stimulated an increasing activity in the field of pSi for drug delivery applications and enabled new opportunities to use this material in medicine and related fields [20-22]. More recently, Sailor demonstrated for the first time that pSi-based nanoparticles (pSiNPs) can also act as imaging agents using a time-gated imaging approach by taking advantage of the pSi optoelectronic properties [23-25]. As a result, pSi is considered a unique and promising vehicle platform for theranostic nanomedicines.

Although some companies such as pSiMedica Ltd UK have initiated the commercial development of nanomedicines based on pSi for clinical therapies, it is worthwhile noting that the bench-to-bedside translation of pSi-based drug delivery systems into clinical
nanomedicines faces both technical and commercial challenges [20]. For instance, nanotechnology is increasingly reaching the investors’ attention and drug delivery is expected to grow strongly in the next decade [26]. Nevertheless, clinical developments require considerable financial support from investors and, as demonstrated recently, the investors’ enthusiasm for funding biotechnological companies is more focused on later-stage projects with demonstrated performance, which require extensive clinical trials and detailed long-term studies. Therefore, this technology will need to be developed in partnership with big pharmaceutical companies to be available in developed countries. Furthermore, clinical studies may undergo failure or delays and the unexpected long-term side effects associated with pSi are yet to be assessed in clinical practice. Another factor to consider is the increasing competition from established treatments and other nanomedicines with faster development. Despite these challenges, pSi offers a set of promising opportunities based on its unique properties.

This review presents the most recent advances in pSi-based drug delivery systems with an overview on the fabrication, properties and applicability of this nanomaterial for drug delivery and theranostic applications (Figure 1). We focus our attention on drug delivery concepts and systems with a critical overview of the advantages and intrinsic limitations of this technology. We provide detailed information on recent progress in the clinical translation of pSi-based systems for different therapies and medical treatments. Finally, we conclude this review with an outlook on the future challenges and trends in this exciting and dynamic research field.

2. Fabrication and Properties of Porous Silicon

2.1. Fabrication

pSi structures are produced by electrochemical etching of silicon wafers in hydrofluoric acid electrolytes based on organic solvents such as acetonitrile (CH₃CN), dimethylformamide
(C₃H₇NO) and ethanol (C₂H₆O) [13-15]. In this electrochemical process, the silicon wafer acts as anode with platinum wire typically used as cathode, the application of a current bias between anode and cathode results in the selective dissolution of the crystalline structure of silicon into a porous form. The structure of pSi, reported for the first time by Watanabe and Sakai in 1971, can be precisely engineered by the fabrication conditions (e.g. anodization current, type of silicon wafer, level of doping, illumination, patterning, electrolyte and temperature) [27]. Table 1 summarizes the most representative conditions and pore morphologies for pSi structures produced by electrochemical etching.

2.2. Physical Properties

pSi structures can be produced with a plethora of pore morphologies, sizes and geometries which can be engineered by electrochemical etching conditions and the pre- and post-treatments of the pSi, such as photolithography, wet chemical etching in alkaline etchants, thermal treatments, etc. pSi structures can be classified into three categories according to their pore size ($d_p$): namely; i) micro-pSi ($\mu$pSi – $d_p < 2$ nm), meso-pSi (mpSi – $2$ nm < $d_p < 50$ nm) and macro-pSi (MpSi – $d_p > 50$ nm).

3. Biocompatibility of Porous Silicon

When considering the use of pSi for medical applications the highest importance is placed on the safety and wellbeing of patients administered any pSi-based treatment. Hence, much work has been directed towards assessing and demonstrating the biocompatibility of pSi-based biomaterials. The biodegradation of pSi [18] is dependent on several factors including the acidity of the local environment and intrinsic properties such as size, porosity and chemical functionality [30-35]. However it has been demonstrated that pSi and its degradation products are non-cytotoxic both in-vitro and in-vivo [28,29]. In 1995 the first investigation of pSi biocompatibility showed the successful growth of hydroxyapatite on pSi substrates [18]. Soon after, Popplewell et al. [36] determined that orthosilicic acid, a product of pSi
degradation in the body, is readily absorbed, gastrointestinally and subsequently excreted via the urinary system. Additional research has shown that orthosilicic acid is linked to homeostasis and regulation of key processes in the body like bone mineralization, collagen synthesis, skin, hair and nails health atherosclerosis and immune system enhancement [36, 37]. These two factors demonstrated the potential biocompatibility of pSi and enable this material to be used for biomedical applications.

The performance of pSi as a biomaterial is dependent on being able to control and stabilize its surface chemistry [38]. Freshly prepared electrochemically etched pSi surfaces are hydride-terminated (Si-H) [39]. After fabrication and upon exposure to air and aqueous media the Si-H terminated surface is highly unstable and rapidly converts to a mixed oxide surface [39]. To promote a controlled surface chemistry with less variability, pSi surfaces can be intentionally oxidized with ozonolysis or thermal oxidation to result in stable Si-OH or Si-O-Si groups on the surface [39, 40]. Si-OH functionalized pSi surfaces are stable, hydrophilic and compatible with further silane chemistry. Thermal oxide layers (Si-O-Si) are more bio-inert than Si-OH surfaces whilst pSi surfaces can be passivated by thermal nitridization [41, 42], thermal carbonization (TC) or thermal hydrocarbonization (THC) [43, 44]. The benefits of the more intensive TC processing of pSi include improved electrical and thermal conductivity, combined with greater mechanical strength and robust nature of the Si-C surface functionality [45].

Once stabilized the pSi surface can be modified with different chemical functionalizations via techniques such as silanization and more recently hydrosilanization [46]. Si-OH terminated pSi can be further modified with chloro or alkoxy silanes in ambient or elevated temperature conditions [47]. This allows for the introduction of functional groups such as amines, isocyanates, methacrylates and PEG moieties as well as other functionalities available by conventional organic synthesis routes [48]. The chemistries introduced by silanization can
then be used for further modification via commercial or custom made cross-linking agents, some even allow for the direct conjugation to various biomolecules, to promote cell adhesion, protein absorption or even anti-fouling surfaces [48]. The readily formed Si-O-Si bonds undergo hydrolysis, making the surface moderately unstable in aqueous solutions [49]. More stable pSi surfaces can be fabricated via the derivatization of Si-H terminated pSi directly, via the hydrosilylation reaction. This reaction forms more stable Si-C bonds on the pSi surface, by exposing the Si-H surface to alkene, alkyne or aldehyde groups [37]. This hydrosilylation reaction can be promoted by numerous methods including thermal, chemical, photochemical, electrochemical and microwave methods [50]. Subsequent pSi functionalization can also be performed via click chemistry [51] methods or with various polymers via polymer grafting techniques. These techniques include “grafting from”, “grafting to” and “grafting-through” methods [52]. Modification of pSi with polymers can help to reduce brittleness, improve stability and also provides additional features such as pH [53], temperature [54] and ligand responsiveness [52]. Polymer modified pSi materials also display improved degradation control and drug release kinetics [52].

3.1. In-vitro, Ex-vivo and In-vivo Studies using Porous Silicon

3.1.1. In-vitro Studies using pSi

pSi has been extensively studied in-vitro since Bayliss et al. in 1999 [55]. The authors of this paper investigated the biocompatibility of two cell lines (Chinese hamster ovary and rat neuronal B50 cells) and in a follow up paper the authors demonstrated that cell growth was preferred on pSi in comparison to glass [56] (Figures 2a and b). Since the late 1990's pSi has been used widely for in-vitro experiments with various cellular responses differing based on the particle size and chemistry of the pSi surface [57-59]. Table 2 summarizes several in-vitro studies evaluating pSi and cell interactions. Recent in-vitro studies of various pSi formulations have found effects such as: concentration dependent cytotoxicity [60], particle
size and concentration dependent apoptosis and cell damage [61], enhanced cell proliferation and attachment [62], the ability to produce and suppress ROS formation [63] and the enhanced association of pSi and drug permeation to and across cell membranes [64, 65]. All these effects are caused by variations in the size, shape or surface chemistry of the pSi; highlighting the need for robust in-vitro assessment of the material before moving to further in-vivo and clinical studies.

3.1.2 Ex-vivo Studies using pSi

pSi can be intrinsically luminescent [66], conjugated with dyes [67] or other contrast agents [67-70] and these features can be exploited to image target tissues or organs either in whole animals or after resection from the animal [71]. However, most ex-vivo applications of pSi have been studied for tissue engineering [72] or sensing of biomolecules [66]. The ability of pSi to act as a support for cell growth [73] lends itself towards the ex-vivo regeneration of tissues and eventually organs [74] with the potential to be used to grow a patient’s own skin for later autografting or a donor’s cells for allografting onto burns or other serious wounds. Ex-vivo applications with pSi can benefit from manipulating the excellent optical properties of the underlying pSi for reporting the health of the cells [75] and for the reporting of the delivery of drugs from within the pSi scaffold [76]. Various biomolecules and even their metabolites can be detected using ultra-sensitive mass spectroscopy techniques [48].

3.1.3. In-vivo Studies of pSi

Studying the performance of pSi in-vitro and ex-vivo is completely different to in-vivo applications due to the complex, dynamic and responsive nature of a living animal model. The natural immune system that all animals possess is readily active to combat foreign objects. Immune responses typically elicited to the presence of foreign bodies, such as pSi, include enzyme degradation, the mononuclear phagocyte system (MPS), cell membranes, interstitial pressure and efflux pumps [77, 78]. Implantation of pSi in-vivo for extended
periods can result in inflammation around the implant and may also cause the formation of fibrotic capsules, meanwhile, the by-products of modified pSi degradation may also cause cascades of unpredictable events. Hence, in-vitro and ex-vivo results of pSi experimentation cannot be relied upon for conclusive performance evaluation of pSi in in-vivo systems. Furthermore, in-vitro and ex-vivo systems cannot ascertain crucial factors for example biocompatibility, biodistribution, resorption rate, or chronic/acute toxicity [77]. Low et al. [79] examined the potential of pSi membranes as implantable scaffolds by first oxidizing and aminosilanizing the pSi prior to implantation in the conjunctiva of rats (Figure 2c). Over 8 weeks the implants showed a slow degradation rate and a thin fibrous capsule with very little evidence of inflammation at the implant interface. There was no tissue erosion or neovascularization observed. This study demonstrated the use and potential of pSi as an implantable scaffold. Table 2 summarizes pSi-based materials in-vivo.

3.2. In-vivo Administration of pSi

Each pSi administration route; intravenous, subcutaneous, oral or other, will vary in efficiency and bioavailability. Hence, dramatically different biological responses could be observed when pSi is implanted/administered to different tissue, even for the exact same material [80].

3.2.1. Intravenous Injection

Intravenous injection (IV) allows for the systemic administration of pSiNPs, lending itself towards targeted applications. However, IV requires the specific functionalization of the pSi surface with the correct targeting ligands to direct the pSi and the payload it is carrying to the desired organ or site of pathology [74, 81]. Once adequately functionalized with targeting molecules, pSi can adequately hone to specific sites such as the stroma of pancreatic tumor mouse functionalized with the Ly6C antibody [82]. pSi and other materials administered intravenously are likely to suffer from opsonization, which occurs when the MPS entraps the
material [83]. Here, once again, the surface chemistry/properties and size of the particles play an important role in avoiding or promoting opsonization, clearance and or cellular uptake [84]. Other properties that have a significant effect on biodistribution include particle size, shape, surface charge and morphology. Typically, particles larger than 200 nm activate the complement system more efficiently and are therefore cleared faster than sub 200 nm particles.

### 3.2.2. Subcutaneous Injection

Particles injected subcutaneously (SC) are trafficked in a size-dependent fashion to the lymphatic system [85]. This size dependence occurs due to large particles being consumed by peripheral antigen presenting cells (APCs) and smaller particles being internalized by resident APCs after cell-free trafficking [85, 86]. Hudson et al. [87] demonstrated little toxicity of mesoporous silicates following subcutaneous injection in rats. However, intraperitoneal and intravenous injections caused either death or the animals needed to be euthanized. They reported the cause of the death was due to the formation of thrombus [87]. An in-vitro cytotoxicity study, determined that smaller particles were reportedly the most toxic, whilst surface chemistry also played a key role in determining toxicity [47]. Subcutaneous pSi delivery systems are a particularly attractive option for the delivery of newly developed peptide drugs, which possess relatively short half-lives [88]. Various pSi functionalizations (thermally oxidized pSi, undecylenic acid-modified thermally hydrocarbonized pSi and thermally hydrocarbonized pSi) have been investigated for sustained subcutaneous peptide delivery [88]. The continued study into the in-vivo cytotoxicity and biocompatibility of silicon structures are essential for ensuring their use in future biotechnological applications [89].

### 3.2.3. Oral Administration

Oral administration is the most common route of administration for pSi drug delivery
applications as it is convenient, safe and inexpensive. The biggest drawback with oral administration, however, is the presence of digestive enzymes and the low pH in the stomach. These two factors in combination with the mostly low permeability of the intestinal walls can lead to further delivery difficulties. Fortuitously, pSi particles possess more stability at acidic pH’s than alkali; hence, pSi is somewhat resistant to the gastric fluids of the stomach. Furthermore, pSi provides a small pore size to confine the drug in a completely tunable fashion \[21, 90, 91\] and coating pSi with enteric polymers permits tunable release at the desired pH \[42\].

3.2.4. Comparison of Biodistribution Following Various Administration Routes

Bimbo et al. demonstrated that pSiNPs distribute via different pathways after different administration routes \[92\]. Radiolabelling pSiNPs allowed them to observe that the pSi accumulated in the spleen and liver after IV delivery, but mostly in bone and the gastrointestinal tract when administered by SC and oral route, respectively. There are further studies, in small animal models, demonstrating that intraperitoneal injections result in absorption into circulation via peritoneum and are proposed to be as efficient as IV for general drugs \[93\] and even NPs \[94\]. Much more work is required to further our understanding of the complex biological reactions elicited by the immune system, when these porous nanomaterials are administered in-vivo via various routes, before we can safely move to regular and routine clinical trials.

3.2.5. Clinical Trials

Two products from pSivida Corp., MA, USA have been approved by the FDA; Iluvien® and Retisert®. Both are used for the treatment of ophthalmic conditions like Diabetic Macular Edema and Uveitis and are marketed by Alimera and Bausch and Lomb, respectively \[95\]. These two products are based on the Durasert™ technology, which is an injectable, non-erodible, intravitreal implant, developed by pSivida, these systems are primarily polymer
based sustained delivery solution. pSivida are currently developing the Durasert™ technology to deliver Latanoprost, this technology is licensed to Pfizer Inc. for the development stage and currently under Phase I/II clinical trials. Currently, pSivida is developing a controlled release ophthalmic delivery system based on nanostructured silicon, Tethadur, to provide the long-term sustained release of biologics to the eye. Additionally, other clinical trials have occurred for the non-commercialized BrachySil™, pSi with 32P radioactive isotope incorporated used for brachytherapy, from pSiMedica (a subdivision of pSivida) [96, 97]. BrachySil™ is injected into the tumor under a local anesthesia and delivers radiation (β-emission) through around 8 mm of tissue [98]. During the trial the toxicity was assessed by the nature, incidence and severity of adverse events and by hematology and clinical chemistry parameters. The preliminary data for the initial safety study presented at American Society of Clinical Oncology (January 2008), claimed improved disease control in 82% of patients treated with BrachySil™.

3.3. Drawbacks and Limitations, Approaches to Overcome Inherent Disadvantages, Advantages, Recent Advances and Future Trends

In-vitro studies are an ideal tool to gain an overall understanding of the biocompatibility or cytotoxicity of a biomaterial [69, 99]. Typical important cell behaviors assessed include viability, proliferation and phenotypic change [100-102]. However, in-vitro tests do not effectively represent complex in-vivo environments. More controlled in-vitro assessments enable faster reproducible testing platforms for biomaterials [69]. Consequently, it is very important to perform in-vitro studies prior to in-vivo test and any clinical assessments. Conventional in-vitro, cell viability assays examining biomaterials can highlight the release of toxic agents in specific biological fluids and indicate if a material is suitable for a specific physical location [100]. The most severe cellular effects are often observed if a biomaterial is releasing toxic agents that produce apoptosis and necrosis [65, 100, 102]. Compatibility
issues can arise when pSi is placed into conventional cell-based assays. Once again, the adequate control of the pSi surface is crucial to inhibit the generation of ROS and avoid these unwanted interferences [49]. Thus far extracellular ROS generation by pSi appears to be dependent on the protein concentration in the assay environment [103], indicating that the effect will vary both in-vitro and in-vivo. Despite the recent developments in pSi-based drug delivery systems and advantages like tunable pore size, particle size and surface chemistry, high loading capacity, ability to protect sensitive payloads, intrinsic photoluminescence (PL), biocompatibility and biodegradability and many others, the key limitations remain unresolved. The challenges in the field of pSi particles based nanomedicine include fast dissolution under physiological condition, redox activity, low PL quantum yield, poorly understood in-vivo characteristics, etc., all of which need to be overcome before commercialization of pSi nanoparticles based therapeutic systems.

In summary, pSi particle accumulation and bioavailability will depend on the administration route and particle size, there are now many studies demonstrating the susceptibilities of various cell types [104-106]. Future in-vivo studies with various pSi particle preparations need to be watchful of the toxicity and preferential accumulation in different organs of the host.

4. Porous Silicon for Drug Delivery Applications

4.1. Porous Silicon as a Platform for Drug Delivery Applications

The key properties that make pSi attractive for drug delivery applications are its highly controllable pore size, morphology, surface chemistry, biocompatibility and biodegradability. The pores of pSi can host large quantities of drug molecules and even concentrate them to up to 400 times compared to the same volume of pure therapeutic solution due to ultra-high surface area and huge pore volume [107]. This was demonstrated by loading bevacizumab (avastin) into pSi and the concentration factor was defined as the ratio of the mass of the
avastin in the pSi film per unit volume to the mass of drug in the initial loading solution per unit volume. In addition, loading of a drug inside pSi pores prevents crystallization providing a better control over drug availability and dissolution profile [21, 108]. pSi particles are able to protect sensitive proteins from degradation and enzymatic denaturation in the stomach [109]. Furthermore, the photonic properties of pSi substrates, imparted during the electrochemical fabrication process, have been demonstrated for reporting the release of the therapeutic payload [107].

4.2. Porous Silicon Films for Implantable Drug Delivery Solutions

pSi-based photonic crystals can be generated simply by varying the current-time profile during the electrochemical etching process [14] and demonstrate a linear correlation between the optical response and concentration of the released payload (dexamethasone) [110]. A similar optical structure was also used to load and release avastin, an antibody used for cancer treatment and certain ophthalmic diseases [107]. The ease of fabrication and modulation of pore morphology of pSi was utilized by Vaccari et al. to fabricate a bi-layered structure with a macroporous layer (2 µm) covered by a nanoporous layer (200 nm) [111]. This bi-layered porous structure was loaded with doxorubicin (DOX), an anticancer drug, by immersion loading. A biphasic release profile was obtained with a burst of DOX released for 5 h followed by a slower release. This structure was also tested in an in-vitro cell study using human colon adenocarcinoma cells (LoVo and HT29) with no significant anti-proliferative. Perelman et al. demonstrated a pH triggered system based on pSi thin films for the release of vancomycin [112]. The pSi carriers were loaded with vancomycin using immersion loading followed by pore capping by serum albumin protein (BSA) at pH < 4. The pH trigger mechanism is based on the pSi isoelectric point 4 with adsorption of BSA at pH < 4 and desorption at pH > 4.

4.3. Porous Silicon Microparticles for Local, Oral and Ophthalmic Delivery Solutions
pSi films can be detached from the underlying bulk silicon by simply setting the etching conditions to the electropolishing regime. The key advantage of the lifted-off pSi membrane is that it preserves the structural and morphological features of a pSi chip with the ability to be broken into smaller fragments of macro- or micro-dimensions by sonication [14]. pSi microparticles (pSiMPs) are limited to local, oral, or ophthalmic delivery solutions due to size constraints. pSiMPs have used to deliver payloads like poorly soluble hydrophobic small molecules drugs (e.g. furosemide, ethionamide, ibuprofen, griseofulvin, indomethacin) [113-115] and proteins (e.g. insulin, serum opsonins, bovine serum albumin, glucagon-like peptide-1) [116-118]. Besides, the loading and release of therapeutic payloads pSiMPs have also been tested for their bioactivity and compatibility [62]. Another study by Wu et al. reported the degradation of redox activity drugs inside pSi can be prevented by either surface modification, thermal oxidation, or thermal carbonization [119]. Salonen et al. systematically compared the loading and release of five different small molecules (three poorly soluble drugs; ibuprofen, griseofulvin and furosemide and two water soluble drugs; ranitidine and antipyrin) of varying hydrophobicity from thermally oxidized and carbonized pSiMPs [113]. The loading varied between 9 % and 45 % for thermally carbonized pSiMPs, while the release rate was found to be dependent on the dissolution behavior of the loaded drug with the pSiMPs delaying the drug release. Bullpit et al. reported significantly improved bioavailability of immunosuppressant drug cyclosporine A after loading into the pSiMPs [20]. After a single subcutaneous injection of cyclosporine A loaded into pSiMPs, the blood concentration of cyclosporine A in CD rats was increased by approximately 280 % compared to the control group, in which equal mass of cyclosporine A was injected in simple phosphate buffered saline. The pores of pSi act as containers that can house a sensitive payload and protect it from harsh environments, which is why it has been employed for delivery of proteins and peptides through oral delivery. Delivery of insulin is most widely studied from
pSiMPs, where pSi has been shown to enhance the bioavailability of insulin while protecting it against the low pH and digestive environment of the stomach [120]. Sreshtha et al. loaded insulin into chitosan modified thermally hydrocarbonized pSiMPs and tested for permeation across a CaCo2/HT-29 cell monolayer [121]. They observed a 20-fold increase in the amount of insulin permeated and 7-fold increase in the apparent permeability (Papp) value in comparison to the pure insulin across the CaCo2/HT-29 cell monolayer. In a recent study, the same group used thiolated and cell penetrating peptide modified pSi particles for oral delivery of insulin (Figure 3a and b) [109]. Similar results were reported by Pastor et al. using chitosan coated pSiMPs [120]. Besides insulin, other proteins like Cry5b (an anthelmintic protein), which was shown to be active in eradicating stomach worm, C. elegans, effectively even after incubating the Cry5b loaded pSiMPs in simulated gastric fluid (SGF). (Figure 3c).

pSiMPs have been extensively exploited for intravitreal delivery [122-124]. A key study reported intravitreal distribution and clearance after ocular injection of pSi and oxidized pSiMPs [125]. The results revealed that both pSi and oxidized pSi are non-toxic in rabbit retina and degrade into silicic acid over time. Hou et al. loaded Daunorubicin (DNR) for intravitreal delivery for inhibition of VEGF and PDGF that are responsible for neovascularization [124]. The study showed that oxidized pSiMPs delivery system can extend DNR vitreous residence from a few days to three months. In another study, the same group compared the DNR release from oxidized pSiMPs loaded by physical adsorption and covalent attachment. Covalent attachment method not only provided better loading (> 67 µg/mg) in comparison to physical adsorption (~ 27 µg/mg) but also showed better dissolution profile in-vivo with DNR still detected after more than three months in rabbit eyes (Figure 3d and e). Wang et al. used similar pSiMPs for intravitreal delivery of dexamethasone (DEX), a glucocorticoid [126]. They showed that in-vitro DEX release lasts up to 90 days in
comparison to only 10 days for free DEX. The in-vivo study showed no sign of ocular toxicity in rabbit eyes (at 3 mg) after intravitreal injection and drug level of $107.23 \pm 10.54$ ng/mL (at 2-week mark), which is well-above the therapeutic level. The most interesting development for the use of pSiMPs for intravitreal delivery of therapeutics include their self-reporting ability based on the changes in the photonic properties over time due to degradation, which can be correlated to the drug release [123, 127]. In a pioneering study, the Sailor group demonstrated that it is feasible to visualize the pSiMPs and their photonic signature in-vivo in rabbit eye, which was used to assess the degradation state of the particles. An extension of this study focused on rugate pSi particles loaded with two model drugs, rapamycin and DEX, loaded by physical adsorption and covalent attachment, respectively. These drugs were tested to monitor the feasibility of drug release from the pSi particles through a color fundus camera imaging [123]. pSi particles with adsorption loaded rapamycin displayed violet color, which was negatively correlated to the rapamycin released into the vitreous. DEX was covalently loaded onto the fully oxidized pSi rugate particles that appeared yellow in vitreous and decreased over time with a strong correlation with the DEX detected from the vitreous samples.

4.4. Advantages and Disadvantages of Porous Silicon Drug Delivery Platforms

The countable advantages of pSi for drug delivery include: (i) confinement of the drug molecules into the pores to prevent crystallization; (ii) protection of the sensitive payloads from harsh conditions; (iii) release kinetics can essentially be tailored to suit the requirements by tuning the pore morphology; (iv) high drug loading (up to 60 % by mass) and concentration (up to 400 times); (v) surface chemistry tunability to control the drug loading, release, dissolution and biocompatibility; (vi) surface chemistry to attach targeting moieties to pSi particles to deliver the payloads specifically to diseased organs; (vii) the electrochemical etching systems are commercially available with variable degree of
automation (AMMT systems, Germany) and is utilized by companies like TruTags inc. and Spinaker Biosciences. It is worth noting that pSiNPs have shown great potential for systemic drug delivery, but localized and implantable drug delivery systems based on pSi are inherently limited by the intrinsic instability of pSi under physiological conditions. For oral delivery systems based on pSi, most studies have only investigated the release and dissolution characteristics under in-vitro conditions. Therefore, it is essential to further carry out in-vivo investigations to fully understand the release characteristics and dissolution of the pSi carrier. A recent example of important differences between in-vitro and in-vivo release characteristics of pSi substrates was reported by Wu et al., who demonstrated that pSi can load and deliver the protein payload (Cry5B) to eliminate stomach worms under in-vivo conditions [128]. However, during in-vivo testing it was observed that pSi particles were cleared out in the faeces before they dissolved and released their payload. Therefore, novel methods are required to specifically tune the release profiles of different payloads under different conditions to obtain better in-vitro to in-vivo correlation. The limitations of pSi for local and implantable systems might be overcome by utilizing oxidized pSi, whereas oral delivery of therapeutic will require better designing of pore morphology, structure and surface chemistry. For self-reporting ophthalmic delivery, the key issue has been angular dependence of the photonic properties of pSi particles and the roughness of the surface resulting in increased scattering. One of the key future directions for pSi-based therapeutic agents would be as multivalent pSi systems, which not only deliver therapeutics with desired kinetics but also help in imaging based on inherent long-lived photoluminescence that can be used for both continuous-wave and time gated photoluminescence based imaging.

5. Porous Silicon for Sensing and Theranostics

5.1. Introduction

pSi can be used to detect biomedically relevant species from small molecules such as
metabolites, to large molecules such as DNA, proteins and even whole organisms such as viruses and bacteria. There are some extensive reviews discussing sensing with pSi available in the literature [129,130]. The most attractive properties of pSi for biosensing applications are its high surface area [131], made-to-measure pore structure [132,133] and exceptional optical characteristics.

5.2. Types of sensing systems based on porous silicon

Optical biosensing with pSi is the most common format of pSi biosensors [134]. These optical pSi sensors originally consisted of thin films [135], but have advanced to structures such as microcavities [136,137], rugate filters [110,138], Bragg mirrors [139] and superlattices [140,141]. These sensors use small but monitorable changes in the optical reflectivity of pSi to enable detection of virtually any biological analyte including: proteins, enzymatic activity, DNA, viruses and even whole bacteria [129,130,132]. pSi-based sensors have also been used in the detection of hazardous organic molecules and explosives [142,143].

White light interferometry is commonly used to sense the changes in optical properties upon the binding/infiltration of an analyte into the pSi pore structure. The infiltration of various molecules into the pSi layer causes a change in the refractive index allowing for label-free detection [135]. These highly sensitive shifts can be exploited to sense down to the sub-picomolar range and are generally considered more sensitive when blue-shifting rather than red-shifting [130]. However, these biosensors can suffer from Effective Optical Thickness (EOT) drift and interferences can occur when analysing complex matrices, such as bodily fluid [130]. The minimization of these effects has been overcome by the development of pSi-based sensors with multiple layers, that can act to separate/exclude biomolecules of certain sizes [144]. The further development of Bragg reflectors, microcavities and sinusoidally etched rugate filters have allowed for the tailored improvement of biomolecule
sensing [130] as well as enabling applications such as smart-Petri dishes for live cell monitoring [76] and analyte sensing in whole blood [145]. Whilst superlattices, or spectral barcodes, have enabled discrimination between analytes such as rat and bovine serum albumin [110]. Photoluminescent (PL) based biosensing is also possible since pSi shows intrinsic PL at room temperature [16]. However, this method is not used as regularly as white light interferometric techniques, due to the greater error in measuring PL changes compared to wavelength shifts and the vulnerability of PL surfaces to quenching by a wide range of species [130]. However, recent work has focussed on the enhancement of luminescence using pSi microcavities and achieving limits of detection in the order of 150 nM [146].

5.3. Surface Chemistry Modification of Porous Silicon for Selective Sensing of Biomolecules

To design a selective sensor, pSi must be successfully covered with the correct sensing molecule. There exist many different methods for covalently attaching molecules such as peptides [147], proteins [148], enzymes [149], antibodies [81] and DNA [150] to pSi. Generally, moieties linked to the pSi surfaces react with either amine or thiol groups contained in the biomolecule being targeted. These species typically include isocyanates [47], epoxides [151] and N-hydroxysuccinimide [72] among many others [152]. When considering immobilizing biomolecules one must consider not only the surface coverage and stabilization but also the retention of the biological activity of the linked species [153]. Recent advances have also allowed for the dual functionalization of pSi with two different chemistries both laterally [47, 72] and vertically [154, 155]. To pattern laterally uses photolithographic techniques and has been used for both silanization [47] and hydrosilylation [72]. Vertical dual functionalization is performed by exploiting the wettability of the pSi when functionalizing [154, 155].

5.4 In-vitro and In-vivo Biosensing
Typically, most sensing applications with pSi thus far have been performed in-vitro. This however is not conducive to translation into implantable devices as the current optical sensing method, namely white light interferometry, requires an unobstructed path to the pSi surface where the interactions are taking place [130]. Similarly, it is evident that pSi sensors that work under controlled conditions often begin to fail when challenged with the milieu of components in biological samples [130]. In-vitro sensing with pSi is beneficial for applications where sampling is relatively straightforward, for example glucose biosensors for diabetic chronic wound healing [156], however, optical sensors fall well short when required in-vivo for very low concentration biomolecules such as Troponin, which only spikes in the heart muscles shortly before cardiac arrest [157]. The need to develop pSi-based sensors into implantable and easily interrogatable devices seems far off at this point in time. However, with the development of technologies such as wireless telemetry [158,159] it does seem likely in the future that this transition will occur.

5.5. Theranostics Based on Porous Silicon Platforms

Theranostics is the combination of both diagnostics and therapy in one. The plethora of pSi formats (i.e. films, membranes, micro- and nanoparticles) as well as physical modifications (i.e. pore size, particle size, thickness and optical properties) and chemical/secondary modification available, opens the potential of pSi in theranostics [153]. Further investigations into factors such as residence time, specific disease targeting and on demand payload delivery are still required to optimise pSi for theranostic applications. Studies with pSi using a combination of diagnosis (or targeting) and treatment include works by Secret et al. [81] and Chiappini et al. (Figures 4a and b) [160]. These studies investigated the use of multistage delivery vehicles fabricated with coronas of targeting molecules around the pSi particles. The ability to combine modified pSi with secondary NPs allows targeting or activation via external stimuli. Consequently, combinations using magnetic and luminescent pSi [161],
Quantum Dots (QDs) in pSiMPs [162], photoluminescent pSiNPs [71] and radiolabelled pSi [92] are now beginning to appear in the literature. Some theranostic pSi systems exploit the photoluminescent properties of pSi and are photosensitizers, capable of generating singlet oxygen [162]. Other systems combine pSiNPs, bacteriophage and super paramagnetic iron oxide nanoparticles (SPIONs) into the one device allowing for the delivery of the particles with a therapeutic and an imaging payload to tumors [163]. These types of theranostic particulate systems are capable of targeting diseased tissue both in-vitro and in-vivo. These systems could potentially be used for a myriad of applications including cardiovascular disease and cancer therapy. Additionally, these pSi-based materials do not require surgical resection after implantation or injection due to the biodegradation of the pSi scaffold into silicic acid.

5.6. Drawbacks and Limitations of Porous Silicon in Theranostics

There are a multitude of barriers present in biological systems, which can potentially interfere with the theranostics applications of pSi in the body. These include: enzymatic degradation, phagocytosis by the reticulo-endothelial system (RES), vascular endothelia, interstitial pressure, cellular/nuclear/endosomal membranes and molecular efflux pumps [164]. All these systems can prevent particulate drug carrier systems from reaching their target site of action at therapeutic levels [165]. Hence, to deliver more of the payload to the required site it is important to control the size, shape, density and surface chemistry of the particles, properties which, help regulate the effects such as cell adhesion, cell uptake and flow in the bloodstream [166]. To overcome some of these limitations pSiNPs systems can be specifically designed with various geometries [82] or surface chemistries/modifications [167]. There are three generations of NP formulations:

The first are passive particulate systems, which reach their intended site by exploiting passive mechanisms like the Enhanced Permeation and Retention effect (EPR). These first-generation
NPs can be coated with molecules such as PEG to avoid RES uptake [167], or opsinization [168] whilst also enhancing their circulation time [169]. Second generation particulate systems incorporate additional functionality that allow for the targeting of individual disease sites through the binding of specific ligands to unique markers at the site of pathology [81] or secondary functionalities that allow the delivery of various therapeutic agents, imaging or triggered/controlled release [153, 163]. Third generation systems are comprised of multiple components, which are designed to completely avoid the above-mentioned biological barriers. These functions are often timed so that the payload is released at the ideal time-point and in the ideal location [163]. This release can be triggered by either an internal stimuli or external stimuli such as heat, RF, or light [153].

In summary, pSi properties and functionality are open to modification and able to be completely tailored with a diverse range of pSi-polymer or pSi-biomolecule hybrid architectures. This allows for the generation of a wide range of materials with well-defined optical and mechanical properties, as well as degradation and drug release profiles. The ability to perform dual operations, such as, imaging and drug delivery simultaneously, will lead to more advanced theranostic applications in the future. Potentially allowing for the specific delivery of controlled amounts of drugs on demand in response to an appropriate external or more beneficially a natural biological signal. In the future, smart active biomaterials will emerge and be commercialized that are able to perform functions such as targeting specific disease states, respond to in vivo stimuli in that disease environment and finally perform an appropriate action, such as delivering its drug payload or sequestering toxins.

6. Conclusions and Future Perspectives

pSi is a promising platform for the development of a broad variety of nanomedicines and drug delivery systems, which could address some of the limitations of conventional therapies
and existing medical treatments. pSi structures can be produced by well-established fabrication process developed during the last decades and a multitude of pSi-based structures can be fabricated with high precision and tuned chemical and physical properties. Furthermore, although the long-term toxicity of pSi in clinical practice is yet to be established, the significant amount of in-vitro and in-vivo studies on pSi structures are displaying promising evidence of the biocompatibility of this material. pSi structures have a unique set of physical and chemical properties, including high porosity, controllable dimensions, tuneable surface chemistry, high loading capacity, biodegradability, biocompatibility and biointegration. These properties make it possible to develop advanced and highly versatile drug nanocarriers and delivery systems, where the release of therapeutics can be engineered with precision per the demands of specific medical treatments. As a result of its optoelectronic properties, pSi structures are excellent candidates for theranostics and photodynamic therapies, which is an advantage over existing systems based on the combination of imaging and therapeutic agents.

Although these studies have demonstrated promising and outstanding advances in pSi technology for drug delivery and medical applications and some companies have started to explore the commercialization of pSi systems, it is worthwhile stressing that this technology still faces both technical and commercial challenges for its ultimate clinical translation from bench to bedside. Therefore, more extensive fundamental research assessing the toxicity and side effects associated with pSi systems and their performance in terms of clinical efficiency will be needed before this technology becomes feasible and reliable. However, based on the evidences shown in the studies reviewed here, it is reasonable to conclude that there is considerable potential for pSi to become an alternative nanomedicine platform for therapies.

7. Expert Opinion
pSi technology brings new opportunities with realistic potential to be translated into clinical therapies because of its unique properties (e.g. high porosity, high loading capacity, controlled releasing performance, biodegradability, biocompatibility, biointegration and self-reporting and imaging features). These factors make pSi a promising candidate for a new generation of nanomedicines. Despite these fundamental and applied advances, it is worthwhile noting that pSi technology still faces important technical and commercial challenges. Although an extensive research activity in the last decade has aimed at demonstrating the in-vivo and in-vitro performance of pSi systems for different applications and therapies (e.g. oncology, theranostics, imaging, orthopedics, tissue engineering, etc.), the side effects, long-term toxicity and performance of this technology are yet to be demonstrated throughout exhaustive clinical trials. From a commercial point of view, it must be pointed that drug delivery technologies achieve the highest value after commercialization or when they are close to regulatory approval. pSi technology is still in its early-mid phase of clinical development, which is not the optimal stage for valuation and capturing the investors’ interest. Clinical trials can experience delays and failure and strong financial support is required during this stage of development. Therefore, pSi technology will need to be aligned with pharmaceutical sectors to stimulate the investors’ support and to penetrate markets in developed countries through partnership. Furthermore, the increasing competition in the biotechnological sector with the presence of fast-developing nanomedicines and well-established medical treatments are factors that could reduce the market size for pSi technology. Nevertheless, in contrast with other drug delivery technologies, pSi has potential broad applicability, making it an attractive technology for targeting a significantly wide range of niche markets (e.g. cancer, ocular diseases, orthopedics, tissue engineering, photodynamic therapies, diagnostics, etc.). pSi systems offer unique concepts and approaches that are distinctly different from existing competitive technologies such as liposomes and polymers.
These factors make pSi technology a highly promising platform for boosting the production of a new range of nanomedicines and advanced therapeutics. Furthermore, given its optoelectronic properties, pSi technology can be easily integrated into implantable and biodegradable electronics, which is perfectly aligned with existing bionic devices currently used to treat medical conditions such as mental health disorders.
Article Highlights

- Electrochemically and chemically engineered pSi structures are envisaged as a unique platform for drug delivery and theranostics because of its chemical and physical properties and structural versatility.
- Fabrication of pSi by electrochemical or chemical etching of silicon provide a means of engineering a unique set of porous structures based on this material, which can find broad applicability in biomedical applications.
- pSi structures have a set of unique optoelectronic properties as well as biocompatibility and tuneable biodegradability, making this material a promising candidate for the development of advanced biomedical devices and systems for drug delivery, photodynamic therapy and diagnosis.
- The performance of pSi for different biotechnological applications has been demonstrated by a multitude of in-vitro and in-vivo studies aiming to explore the capabilities of this material for drug delivery, diagnosis and imaging, complex biochips systems and tissue engineering.
- Although some companies have initiated the commercial development of nanomedicines based on pSi, the bench-to-bedside translation into clinical nanomedicines faces both technical and commercial challenges.
- These clinical developments will require considerable financial support from investors and extensive clinical trials and detailed long-term studies before this technology becomes feasible and reliable.
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Declaration of Interest

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** of considerable interest.


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* First identification of quantum confinement effect

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* First demonstration of bioimaging using porous silicon
* First observation of porous silicon structure

* First demonstration of carbonization stability in porous silicon
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Table 1. Compilation of the most representative electrochemical etching conditions used to produce porous silicon structures and details of their morphological features.

<table>
<thead>
<tr>
<th>Silicon Wafer</th>
<th>Current Density (mA cm$^{-2}$)</th>
<th>Doping Density (cm$^3$)</th>
<th>Pore Morphology and Porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1·10$^{17}$</td>
<td>1·10$^{18}$</td>
</tr>
<tr>
<td>p 300</td>
<td>Sponge-like</td>
<td>Narrow long pores</td>
<td>Wide long pores</td>
</tr>
<tr>
<td></td>
<td>80%</td>
<td>with dendritic branches</td>
<td>with high wall roughness</td>
</tr>
<tr>
<td>30</td>
<td>Sponge-like</td>
<td>Sponge-Like</td>
<td>Medium long pores</td>
</tr>
<tr>
<td></td>
<td>70%</td>
<td>60%</td>
<td>with dendritic branches</td>
</tr>
<tr>
<td>3</td>
<td>Sponge-like</td>
<td>Sponge-Like</td>
<td>Narrow long pores</td>
</tr>
<tr>
<td></td>
<td>60%</td>
<td>40%</td>
<td>with dendritic branches</td>
</tr>
<tr>
<td>n 300</td>
<td>Wide long pores with low wall roughness</td>
<td>35%</td>
<td>Wide long pores with medium wall roughness</td>
</tr>
<tr>
<td>30</td>
<td>Medium long pores with dendritic branches</td>
<td>10%</td>
<td>Narrow long pores with dendritic branches</td>
</tr>
<tr>
<td>3</td>
<td>Sponge-like</td>
<td>Sponge-like</td>
<td>Sponge-like</td>
</tr>
<tr>
<td></td>
<td>10%</td>
<td>20%</td>
<td>30%</td>
</tr>
</tbody>
</table>
Table 2. Summary of the most representative in-vitro, ex-vivo and in-vivo studies using porous silicon-based materials.

<table>
<thead>
<tr>
<th>Form of pSi</th>
<th>Surface modification</th>
<th>In-Vitro</th>
<th>In-Vivo</th>
<th>Cell type</th>
<th>Animal Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Films</td>
<td>Fresh</td>
<td>X</td>
<td>-</td>
<td>Rat neuronal (B50)</td>
<td>-</td>
<td>[55]</td>
</tr>
<tr>
<td>Films</td>
<td>Fresh</td>
<td>X</td>
<td>-</td>
<td>Rat neuronal (B50)</td>
<td>-</td>
<td>[56]</td>
</tr>
<tr>
<td>Film</td>
<td>Oxidised</td>
<td>X</td>
<td>-</td>
<td>Hepatocytes</td>
<td>-</td>
<td>[57]</td>
</tr>
<tr>
<td>Film</td>
<td>Undecylenic acid and poly(styrene)</td>
<td>X</td>
<td>-</td>
<td>Hepatocytes</td>
<td>-</td>
<td>[74]</td>
</tr>
<tr>
<td>Films</td>
<td>Fresh, Ozone, Amine, PEG, Collagen and FBS</td>
<td>X</td>
<td>-</td>
<td>Rat pheochromocytoma (PC12)</td>
<td>-</td>
<td>[62]</td>
</tr>
<tr>
<td>Film</td>
<td>Oxidised, dodecyl, undecanoic acid and oligo(ethylene) glycol</td>
<td>X</td>
<td>-</td>
<td>Hepatocytes</td>
<td>-</td>
<td>[58]</td>
</tr>
<tr>
<td>Films</td>
<td>Amine, PEG</td>
<td>X</td>
<td>-</td>
<td>Human neuronal (SK-N-SH)</td>
<td>-</td>
<td>[47]</td>
</tr>
<tr>
<td>Film</td>
<td>Peptide</td>
<td>X</td>
<td>-</td>
<td>Rat mesenchymal stem</td>
<td>-</td>
<td>[148]</td>
</tr>
<tr>
<td>Film</td>
<td>Oxidised</td>
<td>X</td>
<td>-</td>
<td>Human mesenchymal stem</td>
<td>-</td>
<td>[59]</td>
</tr>
<tr>
<td>Membrane</td>
<td>Oxidised, Amine</td>
<td>X</td>
<td>X</td>
<td>Human lens epithelial</td>
<td>Rat</td>
<td>[79]</td>
</tr>
<tr>
<td>MP</td>
<td>Amine +/- PEGylation</td>
<td>X</td>
<td>-</td>
<td>THP-1 monocytes</td>
<td>-</td>
<td>[34,35]</td>
</tr>
<tr>
<td>MP</td>
<td>Oxidised, Amine</td>
<td>X</td>
<td>-</td>
<td>J774A.1 macrophages</td>
<td>-</td>
<td>[69]</td>
</tr>
<tr>
<td>MP</td>
<td>Amine</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>Mouse</td>
<td>[75]</td>
</tr>
<tr>
<td>MP</td>
<td>18F-THCPSi, 18F-THCPSi and 18F-TOPSi</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>Rat</td>
<td>[70]</td>
</tr>
<tr>
<td>MP</td>
<td>THCPSi</td>
<td>X</td>
<td>-</td>
<td>HepG2, Caco2, RAW264.7 macrophages</td>
<td>-</td>
<td>[115]</td>
</tr>
<tr>
<td>NP</td>
<td>THC</td>
<td>X</td>
<td>X</td>
<td>RAW264.3 Macrophage</td>
<td>Rat</td>
<td>[92]</td>
</tr>
<tr>
<td>NP</td>
<td>TOPSi</td>
<td>X</td>
<td>-</td>
<td>RAW 264.7 macrophage, Caco-2</td>
<td>-</td>
<td>[60]</td>
</tr>
<tr>
<td>NP</td>
<td>THC</td>
<td>X</td>
<td>X</td>
<td>B lymphocytes (Raji), T-cells (Jurkat), monocytes (U937) and RAW 264.7 macrophage</td>
<td>Rat</td>
<td>[36]</td>
</tr>
</tbody>
</table>
Figure 1. Diagram showing the fabrication process for materials based on pSi structures, with the electrochemical etching of silicon wafers (pSi films), lift-off of the film (pSi membrane), sonication (pSi microparticles – pSiMPs) and further sonication (pSi nanoparticles – pSiNPs).
Figure 2. (a) SEM images of hydroxyapatite crystallites on pSi surface exposed to simulated plasma for (top) 25 and (bottom) 30 days (adapted from [55]). (b) Cross-sectional SEM of B50 cells grown on pSi surfaces after 72 hours (top) and optical image of Fura 3 dye B50 cells superimposed on the pSi substrate image (bottom) (adapted from [56]). (c) Thermally-oxidised, aminosilanised pSi membranes implanted under the rat conjunctiva, shown immediately after implantation (0) and at three (3), six (6) and nine (9) weeks (adapted from...
Figure 3. (a) A schematic of the two strategies of thiolation of porous silicon used by Shrestha et al. for oral delivery of insulin ([109]). (b) In-vitro insulin release curve obtained by the particles prepared by the thiolation methods shown in (a) (adapted from [109]). (c) Merged fluorescence/differential interference contrast (top panels) and fluorescence (bottom panels) images of *C. elegans* incubated with rhodamine-labelled pSi nanoparticles. A: Untreated young adult with the relevant anatomical features indicated. B: Worm incubated with 0.4 μm rhodamine-labelled pSi particles for 2 h (rhodamine label can be seen throughout the lumen of the intestine). C and D: Higher magnification images of pharynx and anus regions of worms treated under the same conditions as in B (adapted from [127]). (d) Fundus photographs of the rabbits, monitored for 5 weeks post-injection of the pSi particle formulation. The particles show up as dark or predominantly violet colored features in the images ([123]). (e) Drug released and evolution of color classes as a function of time in-vivo for the pSi particles based self-reporting formulation injected into rabbit eyes at day 0 (adapted from [123]).
Figure 4. (a) Immunofluorescence microscopy image of the pSiNPs targeting motor neurons cells growing on astrocytes (left) and flow-cytometry images of DOHH-2 cells and Jurkat cells, incubated with pSi-Rituximab labeled with FITC (right) (adapted from [81]). (b) High-throughput fabrication of pSiMPs by combining lithography and etching (left) and in-vivo images of pSiMPs conjugated with C5.5 Alexa dye (right) (adapted from [160]).