FEASIBILITY OF SILICON-BASED MESOPOROUS MATERIALS FOR ORAL DRUG DELIVERY APPLICATIONS

SUMMARY

This review article presents a general overview of fabrication parameters and characterisation methodologies for various mesoporous silicon (PSi) materials. Some new data considering drug loading, drug dissolution/release enhancement and biological compatibility (cellular viability/toxicity) of the PSi materials are also discussed. Typically, the mesopores (pore diameters in the range of 2–50 nm) are produced in the silicon wafers or particles by electrochemical etching in HF-solution. Subsequently, surface modification of the PSi is needed, since the hydrogen-terminated as-anodized surface is unstable and susceptible for oxidation. The etching process determines the pore architecture/morphology, whereas the type of the surface modification determines the interactions of the material with the surrounding environment. With regards to oral drug delivery applications, the most important functions for the surfaces are to provide high drug payloads and to control the release of the molecules in a desired way by enhancing (or prolonging) the dissolution behaviour. The surface chemistry plays also a key role in the cellular interactions and putative toxicity of the PSi particles. Overall, the presented results on the feasibility, fabrication, characterisation, loading and release (incl. modelling), and cellular compatibility and safety, clearly confirm the fact that the meso-/nanoporous silicon materials may indeed offer a distinct advantage in dissolution enhancement and precisely controlled oral drug delivery applications. Moreover, the results of the newly developed toxicity assays also suggest that the mesoporous microparticles could be utilised in oral drug delivery formulations.
INTRODUCTION

Progress of micro and nanotechnology during the last decades has impacted tremendously to the current research of biomedical applications. Controlled-release nanoparticles, quantum dots, targeted delivery and cancer nanotechnology are all intensively studied, and the results of their biomedical applications are very encouraging. However, many of these applications of nanotechnology are still far away from practical use, although the speed of the progress raises hopes of achieving completely new biomedical applications even in the near future (Salonen et al., 2008). Mainly these future prospects fall into areas like imaging and sensor technology, and in the case of drug delivery applications, improved cancer therapy and more efficient and user-friendly administration of other active pharmaceutical ingredients (APIs). Pharmaceutical industry has encountered problems in the development of new drug molecules as commercial products. Many potential molecules cannot be delivered in oral form due to their poor dissolution and/or pharmacokinetic properties, typically poor solubility and dissolution in the intestinal lumen, poor permeation properties in the GI tract, as well as high intestinal or hepatic first pass metabolism.

Mesoporous materials own unique and advantageous properties considering drug delivery applications. Small size of the pores (2–50 nm) confines the space of a drug and engages the effects of surface interactions of the drug molecules and the pore wall. Depending on the size and the surface chemistry of the pores, increased dissolution rate or sustained release of drugs can be obtained (Horcajada et al. 2004, Vallet-Regi 2006). Since the first paper by Vallet-Regi in 2001 (ibuprofen loaded into mesoporous silica material MCM-41), numerous articles have been published on these materials with modified pore size (Salonen et al. 2000) and chemical modifications of the surfaces (Munoz et al. 2003; Schwartz et al. 2005), in vitro studies, including calcification (Canham 1995; Canham et al. 1996a), cell adhesion and culturing (Bayliss et al. 1999; Chin et al. 2001; Low et al. 2006), neural networks (Sapelkin et al. 2006), protein adsorption (Collins et al. 2002; Karlsson et al. 2003), and biodegradability studies (Canham et al. 1999; Anderson et al. 2003). Also some in vivo assessments of tissue compatibility have been carried out (Bowditch et al. 1999, Rosengren et al. 2000). While the highly porous Si (p > 70%) dissolves readily in simulated body fluids (except in the simulated gastric fluid), PSi with porosity of < 70% is bioactive and slowly biodegradable.

<table>
<thead>
<tr>
<th>GRADE OF SILICON</th>
<th>INDUSTRIAL USE</th>
<th>PURITY (%)</th>
<th>COST ($/kg)</th>
<th>GLOBAL PRODUCTION (tonnes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wafer</td>
<td>Electronics</td>
<td>99.99999</td>
<td>1000</td>
<td>5,000</td>
</tr>
<tr>
<td>Electronic</td>
<td>Si crystals</td>
<td>99.99</td>
<td>10-100</td>
<td>19,000</td>
</tr>
<tr>
<td>Solar</td>
<td>Solar cells</td>
<td>99.99</td>
<td>10-50</td>
<td>26,000</td>
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<tr>
<td>Chemical</td>
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<td>Steel</td>
<td>97-99</td>
<td>1-5</td>
<td>1,000,000</td>
</tr>
</tbody>
</table>

Table 1. Industrial attributes of different Si grades.
The very low porosity Si and macroporous Si are quite bioinert materials similar to Si. In addition to the porosity, the bioactivity of PSi depends also on the pore size (Canham et al. 1996b). As the costs of raw materials play an important role with regards to production profits, also within pharmaceutical industry, Table 1 presents some characteristic numbers for different silicon grades that can be used to produce PSi. Frequently in laboratory tests, when no large or pilot scale production has been realized, the grade utilized is the one with the highest purity and cost (wafers). When scaling-up is performed, the grade typically used for solar cells can be used without compromising the quality of the pharmaceutical/medical PSi product. Furthermore, silicon ingots can be directly (without making wafers from them) used for etching PSi, which further reduces the production costs.

In this review, we will focus on fabrication and (bio)pharmaceutical characterisation of various mesoporous silicon (PSi) materials. The PSi materials are regarded “top-down” materials on the contrary to the synthesized mesoporous molecular sieves, like “bottom-up” silica materials, which refers to the self-assembly of silicon oxide by means of polymeric templates determining the structure obtained. Porous silicon has some advantages compared to the silica-based materials, but also some disadvantages, like a wider pore size distribution, which will be described and discussed in this review. In addition, some new data considering drug loading, drug dissolution/release enhancement and drug-PSi biocompatibility (cell viability, cell toxicity) issues will be presented.

**Fabrication of the various mesoporous silicon particles**

The fabrication of mesoporous silicon (PSi) for drug delivery purposes can be divided in two phases. First, the pores are produced in the silicon wafers or particles by electrochemical or stain etching. The technique denotes the systems as ‘top-down’ nanomaterials contrary to mesoporous molecular sieves (e.g. MCM-41, SBA-15 and TUD-1), which are ‘bottom-up’ materials synthesized via the self-assembly of silicon oxide by means of polymeric templates determining the structure obtained. Regarding PSi, a subsequent surface modification is needed, since the hydrogen-terminated as-anodized surface is unstable and susceptible for oxidation. The etching process determines the pore architecture/morphology, whereas the type of the surface modification determines the interactions of the material with surrounding environment. The surface modification can comprise the functionalisation of the surface, e.g., for enhanced, prolonged or targeted drug delivery.

![Figure 1. A simplified scheme of the set-up to produce a porous layer on Si by electrochemical anodization.](image)
purposes. However, for oral drug delivery the most important functionalities of the surfaces are to provide high drug or therapeutic agent payloads and to control the release of the molecules in a desired way by enhancing (or prolonging) the dissolution behaviour. The surface chemistry plays also a key role in the toxicity of the PSi particles, since, as such, PSi degrades mainly into monomeric orthosilicic acid (Si(OH)4), which is the natural form of Si in environment and vital for normal bone and connective tissue homeostasis (Anderson et al. 2003).

The most frequently used method to fabricate PSi is electrochemical anodization of Si in hydrofluoric acid (HF) solutions. Anodization is controlled either by anodic current density or voltage; the constant current method is preferred as it allows better control of the porosity and thickness, and better reproducibility. In the simplest setup to anodize PSi, a strip of Si and a cathode material are dipped into HF solution and an etching current is applied between these two electrodes (Fig. 1). The porous layer is formed on the surfaces of the Si strip (positive anode), typically platinum is used as the cathode, and the fabrication cell is made of HF-resistant material. Dilute HF solutions are generally used as electrolytes where ethanol or another surface tension reducing agent is added to reduce the formation of hydrogen bubbles and to improve electrolyte penetration in the pores. The resultant uniform PSi layer warrants typically also for illumination during the etching (Halimaoui 1995). Different fabrication methods have been developed for optimized PSi fabrication (Salonen and Lehto 2008; Kolasinski 2005; Foll et al. 2002; Splinter et al. 2001).

The properties of PSi, such as porous layer thickness, porosity, pore size, pore volume and pore shape are strongly dependent on the fabrication conditions. In the case of anodization, these conditions include HF concentration, chemical composition of the electrolyte, current density, wafer type and resistivity, its crystallographic orientation, temperature, etching time, electrolyte stirring, illumination intensity and wavelength, etc. The complete control of the fabrication is complicated, but also provides a great potential to produce different types of porous material suitable for various applications.

The dopant type affects the pore diameter so that normally with n-type Si larger and straighter pores are obtained than with p-type Si (Levy-Clement 1995). However, the dopant concentration of Si and the current density have also major effects on the pore morphology as depicted in Figure 2 for the p-type Si wafer. The resistivity of the wafer decreases as the dopant concentration increases resulting in increased average pore size and decreased specific surface area. In the highly doped substrates, like in n+- and p+-type PSi, the average pore size is 6–20 nm and the specific surface area 100–300 m²/cm³. The pores are orientated perpendicular to the initial surface of the wafer and, in many cases, the pores are cylindrical with smooth pore walls that are not interconnected. However, depending on the current density and the composition of the electrolyte, branched, firtree-type pore structures can be produced on the highly doped wafers. Decreasing the HF concentration usually increases the diameters of the pores formed, and the pores become smoother and straighter. Generally, increasing the current density (i.e., anodization potential) has the same type of effect on the pores (Halimaoui 1997; Zhang 2001; Salonen et al. 2000).

In oral drug delivery, powdered materials are normally used. After etching, the porous layer is detached from the wafer by abruptly increasing the current density and free standing, as-anodized porous films are obtained. The porous film can be converted into porous powder with specific particle sizes by milling and subsequent sieving. If the average pore size of the powdered material is to be increased, this can be realized at this stage by thermal annealing in inert atmosphere, which causes the coarsening of the PSi structure (Björkqvist et al. 2006). The material must be functionalized after the comminution (or annealing), since new surfaces are produced during milling, and the new surfaces have to be also chemically modified to obtain homogenous material.

The as-anodized PSi is hydrogen terminated consisting of Si—H, Si—H₂ and Si—H₃ hydrides. The simplest way to stabilize the as-anodized PSi is the partial oxidation performed at quite mild environment (ca. 300°C, normal air conditions) for a few hours. The treatment causes a so called back-bond oxidation of PSi, where oxygen atoms
selectively attack the back-bonds of the surface Si atoms instead of replacing hydrogen atoms (Kato et al. 1988; Salonen et al. 1997). Due to the oxidation, the surface turns from hydrophobic to hydrophilic. Performing the oxidation above 600°C, a drastic drop in the specific surface area has been observed (Herino et al. 1984) due to the structural expansion caused by oxidation. Several other oxidation techniques have been reported (see references in Salonen and Lehto 2008).

Thermal hydrosilylation of alkenes or alkynes on the PSi surface offers a way to replace the Si—H bonds with Si—C bonds maintaining the hydrophobic surface due to the hydrogen attached to carbon (Stewart et al. 2000; Boukherroub et al. 2001; Lees et al. 2003). A more effective way to produce corresponding surfaces is the use of gaseous hydrocarbons to thermally hydrocarbonize the surfaces. The technique utilizes the property of acetylene molecules to stick so strongly on the Si surface at room temperature that they remain on the surface although the temperature is increased. As the temperature is raised above 400°C, they dissociate, and the hydrogen atoms from the surface termination of the as-anodized PSi desorb and the carbon atoms bind to the silicon atoms resulting in the carbonized PSi surface. Two different surface terminations can be obtained. Using a treatment temperature below 700 °C, a continuous acetylene flow (mixed with nitrogen) can be used. The formed surface termination contains hydrocarbons, which have similar properties to the hydrosilylated PSi (Salonen et

![Figure 2. Scanning electron microscope pictures of differently doped p-type Si after electrochemical anodization with three different current densities. Note the numbers in the pictures, which refer to the porosity, growth rate, dissolution valence and pore diameter, respectively (Lehmann et al. 2000).](image)
al. 2004). If the treatment temperature is above 700 °C, continuous acetylene flow can not be used, but the acetylene flow has to be stopped just prior to the temperature treatment. The formed surface contains non-stoichiometric Si—C species, but it is completely hydrogen free and, thus, hydrophilic (Björkqvist et al. 2006). The thermally carbonized surface has been found to be very stable in chemically harsh environments and even in HF and KOH solutions (Salonen et al. 2001).

Besides electrochemical anodization, PSi particles can be produced also with stain etching without applying any electrical bias (Fauthauer et al. 1992). In the method, the (micro) particles made of silicon are immersed in aqueous HF and HNO₃ solution, and the stain films are produced on the surfaces of the particles. However, the control of the porosity, the layer thickness and the pore size is limited, and thoroughly porous homogenous particles are difficult to obtain.

### Drug loading of the particles

When a (therapeutic) solid material has been confined in a restricted nano-sized space (pores), its physicochemical properties differ dramatically from the properties of the corresponding bulk material. The space can be so narrow that any ordering of the atoms/molecules is prevented and, thus, no lattice energy is involved. If the material is crystalized, the surface of the material forms a major part of the confined material. In both cases, the interface of the material loaded in the pores and the pore walls play pronounced roles with regards to the interaction of the material with the surroundings, e.g., during drug dissolution. By alternating the pore size and the surface chemistry towards the molecule to be loaded in the pores, the properties of the drug delivery material can be controlled.

Typically, especially at a larger scale, the loading of PSi is performed by immersing the surface treated or functionalized microparticles into a loading solution (Salonen et al. 2008). An advantage of this method is that the loading can be performed at room temperature and the drug to be loaded is not exposed to harsh chemical conditions during the loading. In the cases of protein and peptide delivery, these features might be essential. After loading, the microparticles are filtered out from the solution and dried to obtain dry powder. There are several factors affecting the loading process (Fig. 3), e.g., solvent, pH of the solution, concentration of the solution, time scale and temperature of the loading process, the surface termination of the pore surfaces, and the drug molecule itself. As it is normally desired that all the drug molecules loaded in the mesoporous carrier are located inside the pores, it is essential to choose the loading parameters in such a way that no drug material is crystallized on the external surface of the particles. Figure 4 demonstrates that the correct choices of the solvent and the drug concentration of the loading solution can directly, without any extra washing of the sample, yield a high loading degree without any surface fraction of the drug. Choosing the wrong loading parameters, e.g. the solvent, can also cause chemical degradation of the drug during the loading process. Fourier transform infra-red (FTIR) spectroscopy can be utilized as a fast screening method for the degradation, but more reliable method is high performance liquid chromatography (HPLC) that has been utilized to quantify both the loading degree and chemical purity of the loaded porous particles. As the HPLC analyses are performed for the extracted samples, it is essential to use solvents which dissolve all the drug material from the porous carrier without causing any extra degradation. This is not always a trivial task and, thus, it is beneficial to use several parallel analytical methods like nitrogen adsorption, density measurement and thermal analysis (Salonen et al. 2005a and 2005b; Lehto et al. 2005).

Calculation of the loading degree by means of nitrogen adsorption is based on the difference in pore volumes of the sample before and after the drug loading. This gives the estimation of the volume of the drug in the pores, which can be turned into the mass fraction by using the density value of the drug. By measuring the density of the drug loaded sample and comparing the value to the corresponding values of the pure porous sample and the pure drug, the loading degree can also be calculated. Both nitrogen adsorption and density measurements give erroneous results if the drug blocks the pore openings preventing gas penetration into the pores. Also, both the methods need the density value of the drug loaded in the pores, which certainly differs from...
the bulk value. Use of thermogravimetry (TG) in the quantification of the total drug content in the sample is based on the fact that high enough temperature increase (especially at oxidative conditions) causes the organic compounds to decompose and desorpb from the sample. Comparison of the mass decrease with the corresponding values for the pure drug and the pure porous carrier gives the total mass fraction of the drug. If the decomposition product reacts with the surface of the carrier, erroneous results can be obtained. To distinguish the drug material adsorbed on the external surface (in crystalline form) from that located inside the pores,

Figure 3. A scheme describing the three principal components of the loading process affecting the obtained drug loading degree. The interactions of the components can also be regarded as different mutual affinities. The maximum loading degree is determined by the total pore volume of PSi.

Figure 4. The drug loading degrees of ibuprofen as a function of the ibuprofen concentration in the loading solution. The solvent used is chloroform, the particles are thermally carbonized PSi (TCPSi) and the loading time is 60 min. The total drug payload (solid squares) was obtained with thermogravimetric analysis (TG) and the surface fraction (open diamonds) with differential scanning calorimetry (DSC). The optimal loading can be obtained with the concentration of 400 mg/ml. The dashed line presents the theoretical content of ibuprofen in the pores, if it is assumed that the loading solution has totally filled the pores during the loading process and that the ibuprofen molecules remain in the pores upon drying.
differential scanning calorimetry (DSC) can be employed. If there is any crystalline material present in the sample, the melting endotherm can be observed at the same temperature as for the bulk material, and the accompanying energy is related to the amount of the crystalline material. By subtracting the amount of the crystalline material (DSC) from the total drug content (TG), an estimate for the drug material located inside the pores is obtained. If this material is in amorphous form, no melting endotherm(s) can be detected in the DSC thermograms, but if the material forms nanocrystals, a broad melting endotherm can be detected at depressed temperatures in accordance with the Gibbs-Thomson equation (Fig. 5). Typically these crystals are so small that they can not be detected with X-ray powder diffraction (XRPD).

**Enhancement of drug dissolution and permeability**

As suggested above, utilization of the mesoporous microparticles to increase the solubility and the dissolution rate is based on the fact that the formation of crystalline material is restricted by the confined space of the pores, which are only a few times larger than the drug molecule, thus retaining the drug in its amorphous/noncrystalline, disordered form (Salonen et al. 2005b). In their disordered state the compounds exhibit higher dissolution rates than their crystalline counterparts, especially when solubility is limited by high lattice energies (Yu 2001; Huang et al. 2004). The dissolution rates from the porous materials will also be improved by the high surface area (up to several hundreds of m²/g) characteristic for these carrier materials (see the previous chapters of this review). Further benefits for the improved dissolution are obtained through improved wetting properties of the particles (Salonen et al. 2005b). The mesoporous materials have also been shown to improve the permeability of large, hydrophilic drug molecules in combination with oral permeation enhancers (Foraker et al. 2003), and to provide sustained/controlled release (Andersson et al. 2004; Cavallaro et al. 2004; Vallet-Regi et al. 2004). In the case of controlled release carrier function, nano-sized mesoporous particles may serve as

\[\text{Figure 5. DSC thermograms for ibuprofen loaded in thermally oxidized PSi (TOPSi) with the average pore diameters of 11 nm (solid line) and 65 nm (dashed line). The melting endotherm for bulk ibuprofen (dotted line) is also added in the graph. Note that no crystalline ibuprofen on the external surface of TOPSi particles can be detected. The ordinate is scaled to the total mass of ibuprofen in the samples.}\]
aids to enhance the circulatory persistence of drugs and to target the drugs to specific cells (Aston et al. 2005).

In our recent study (Salonen et al. 2005b), mesoporous silicon (PSi) microparticles were produced using thermal carbonization (TCPSi) or thermal oxidation (TOPSi) to obtain surfaces suitable for oral drug administration applications. Loadings of five model drugs (antipyrine, ibuprofen, griseofulvin, ranitidine and furosemide) into the microparticles and their subsequent dissolution/release behaviour were studied. The loading of the drugs into TCPSi and TOPSi microparticles showed that, in addition to the effects of stability of the particles in the presence of aqueous or organic solvents, the surface properties of the particles determined the compound affinity towards the mesoporous particles. Besides the surface properties, also the chemical nature of the drug and the loading solution were critical to the loading process. This was reflected in the obtained loading efficiencies, which varied between 9% and 45% with TCPSi particles (Salonen et al. 2005b). The release rates of the loaded drugs from the TCPSi microparticles were also found to depend on the characteristic dissolution behavior of the drug substance in question. When the dissolution rate of the free/
unloaded drug was high, the microparticles caused a delayed release. However, with poorly dissolving drugs, the loading into the mesoporous microparticles clearly improved and accelerated dissolution (Table 2). Moreover, pH dependency of the dissolution was reduced when the drug substance was loaded into the microparticles.

In Figure 6 some very recent results for the significantly improved dissolution rate of indomethacin after loading into mesoporous silicon (TCPSi) and silica (SBA-15) materials are presented. It is clearly shown that compared to pure indomethacin, for example at 60 min timepoint, 28 and 26 fold increase in drug dissolution/release takes place for the mesoporous TCPSi and SBA-15, respectively. Combined release and permeation enhancement behaviour was also recently shown for poorly soluble and poorly permeable furosemide loaded into thermally carbonized mesoporous silicon (TCPSi) microparticles (Kaukonen et al. 2007). Permeation was studied across Caco-2 monolayers at pH-values of 5.5, 6.8 and 7.4, from drug solutions and TCPSi particles. Furosemide loaded in the TCPSi exhibited improved dissolution from the microparticles with greatly diminished pH dependence. At pH 5.5 (the lowest furosemide solubility), the flux of TCPSi-loaded furosemide across the Caco-2 monolayers was over 5-fold higher compared to the pre-dissolved furosemide. The highest furosemide permeability was observed at pH 5.5 with the $P_{\text{app}}$ value from the TCPSi microparticles $18.0 \pm 1.3 \times 10^{-6}$ cm/s. Also at pH 6.8 and pH 7.4 higher flux- and permeability-values were observed when the drug was loaded in the TCPSi microparticles. During the Caco-2 permeability experiments, transepithelial electrical resistance (TEER) and mannitol permeability (membrane integrity marker) were monitored, and the results showed that the monolayer integrity was not compromised by the drug-loaded TCPSi microparticles. The improved permeation observed suggests that the high local concentrations provided by the enhanced dissolution properties of the TCPSi-loaded furosemide should be highly beneficial for the drug absorption (Kaukonen et al. 2007).

Surface properties form an essential aspect in the design of porous silicon particles to be used in oral drug delivery. Thermal carbonization (TCPSi) and thermal oxidation (TOPSi), showed that in addition to the effects regarding particle stability, also the surface properties affect significantly the compound affinity towards the particles and to drug release (Salonen et al. 2005b). This observation presents an important potential to tailor the surface properties accordingly to suite the compound in question. Professor Peter Swaan's group has studied the use of porous silicon particles in combination with permeability enhancers in order to deliver insulin across the intestinal Caco-2 cells (Foraker et al. 2003). A major disadvantage of permeation enhancers is the lack of specificity, which may open up a route for food-borne pathogens and toxins to migrate along with the therapeutic compounds. To minimize this risk, the group developed a system called OralMEDDS (Oral Micro-Engineered Delivery Devices) that consists of novel porous silicon particles that can be used as oral drug-delivery vehicles. Once prepared, the particles could be loaded with liquid drug formulation through simple capillary action. Interstitial air is removed by vacuum aspiration, and the formulation is dried using vacuum drying or freeze-drying. OralMEDDS particles are designed to target into the intestinal epithelial cells, adhere to the apical cell surface, and deliver the drug formulation containing a co-administered permeation enhancer that will open up the local tight junctions of the paracellular transport pathway (Foraker et al. 2003). Absorption of macromolecules (e.g. insulin) and hydrophilic drugs, which are unable to undergo transcellular transport across lipid membranes like the intestinal wall, is largely restricted to the paracellular route. Thus, the intestinal absorption of orally administered water-soluble drugs can be enhanced through the utilization of OralMEDDS particles; the drug transport efficiency could be augmented at least 10-fold when the drug formulations were delivered in porous silicon particles when compared to liquid formulations, and up to 100-fold when compared to formulations without the permeation enhancers. Further targeting and specificity could be obtained by attaching cytoadhesive lectins specifically bound to the intestinal mucosa, as previously demonstrated in vitro with similar microdevices by the research group of Desai (Ahmed et al. 2002).
MODELLING OF DRUG RELEASE KINETICS

Dissolution of drugs from silicon particles – theory of dissolution

To model the dissolution behaviour of the PSi particles, the system is depicted in Figure 7.

Drug molecules in a solid or amorphous phase are inside the straight pores of the silicon particle, and water is assumed not to be able to penetrate into this phase. The solid drug material is consumed as its surface gradually dissolves and the dissolved drug molecules first diffuse from inside the pores towards the particle surface and then away from the particle. The drug/water interface is retreated as more drug is dissolved. The model shows how the erosion of the core proceeds as a function of time during the dissolution experiments (Crank 1956; Fogler 1999). Diffusion follows a spherical symmetry outside of the particle (area II) and linear diffusion inside the pores (area I), and there exists an adsorption/desorption balance at the solid - drug surface. The diffusion profile outside of the particle is spherical since the diffusion fields from the individual pores will overlap and create a spherical field even though each source as such is a single point source. While the model only takes into account the longest pore, i.e. the pore with length equal to the radius of the particle, $R_g$, the total dissolution rate can easily be calculated. Since all pores are linear, the drug material in them will erode at an equal rate until each pore is empty. This leads to a behaviour illustrated in Figure 7.

Figure 7. Illustration of the shrinking core dissolution model. Solid drug phase inside the linear pores is eroded (upper) and the material inside each pore in the whole PSi particle is consumed at an equal rate (lower).
where the remaining drug material in the particle occupies a segment of a sphere, which shrinks as more of the drug is dissolved. In fact, the length distribution for the pores is obtained from the curvature of the particle.

To model the dissolution, we start from the diffusion equations. Assuming pseudo-steady state diffusion and sink conditions \( C_b = 0 \) in areas I and II, the flux in both regions can be written as (Crank 1956; Fogler 1999)

\[
J^I = D_e \frac{c^s - c^{ps}}{R_0 - l} 
\]

\[
J^II = D \frac{R_0 (R_0 + h)}{h r^2} c^{ps} 
\]

where

\[
D_e = \phi D 
\]

At the boundary of Area I and Area II, the fluxes are equal and the concentration at the boundary can be solved from eqs. (1) and (2):

\[
c^{ps} = \frac{\eta c^s}{1 + \eta} 
\]

where

\[
\eta = \frac{D_e}{D} \frac{h R}{(R_0 - l)(R_0 + h)} 
\]

We can now write the molar flux at the solid drug surface as a function of the bulk concentration

\[
J^I_{r=R} = D_e \frac{c^s - c^{ps}}{R_0 - l} = k_c \left( \frac{c^s}{1 + \eta} \right) 
\]

where

\[
k_c = \frac{D_e}{R_0 - l} 
\]

At the surface the solubility is determined by zero-order desorption and first-order adsorption reactions. If they are in equilibrium, the surface concentration is equal to the equilibrium constant \( K \). Surface reaction rate can be written as

\[
r^s = k_d - k_a c^s 
\]

Surface reaction rate must be equal to the molar flux away from the surface. From Equations 6 and 8 we get for the surface concentration

\[
c^s = \frac{k_d}{k_a + k_c \frac{1}{1 + \eta}} 
\]

The surface is consumed at a rate proportional to the surface reaction rate:

\[
A r^s = - \frac{d l}{d t} (\rho A l) 
\]

\[
\frac{d l}{d t} = - \frac{r^s}{\rho} = - \frac{K}{\rho} \left( \frac{1}{1 + \frac{1}{k_a + k_c (1 + \eta)}} \right) 
\]

Final form for the rate that the surface is consumed is, thus

\[
\frac{d l}{d t} = - \frac{K}{\rho} \left( \frac{1}{1 + \frac{1}{k_a + k_c (1+\eta)}} \right) 
\]

The above equations are simplified in the case of solid drug particles, without a supporting matrix (Fogler 1999). Skipping the details, the result for the rate of erosion for a spherical particle is

\[
\frac{d R}{d t} = - \frac{K}{\rho} \left( \frac{1}{1 + \frac{R}{2D}} \right) 
\]

Fraction of the dissolved drug from eqs. (12) and (13) is now simply

\[
f = 1 - \frac{3l^2}{2R_0} \left( \frac{R_0 - l}{3} \right) 
\]

\[
f = 1 - \frac{R^3}{R_0^3} 
\]
Using the equations above to model the dissolution of ibuprofen from PSi particles (Limnell et al. 2007), one can get fits such as the ones shown in Figure 8. Fitting parameter can be either the solubility, $K$, or the desorption rate constant, $k_d$. All other variables are known. The thickness of the diffusion layer is always assumed to be of the same size as the particle itself. The results reveal how the porosity and loading efficiency affect the drug release profiles. Counter-intuitively, low load rates will cause a faster release, since it will take less time for diffusion to transfer that much of the drug. For practical applications this is not of course the best approach, since it increases the amount of PSi particles needed to deliver a specific dose of the drug. Modelling parameters can also distinguish between different chemistries of different kinds of PSi particles. If the drug binds stronger into the pores and, thus, release is slower, the fitted $k_d$ or $K$ will be smaller, and vice versa. For comparison, the $k_d$ values obtained for different PSi materials are shown in Table 3. It seems that ibuprofen binds to the surface the strongest in the case of pure ibuprofen, i.e. without the silicon matrix. Binding is weaker with the PSi particles, which is logical since it is assumed that the drug is in an amorphous form inside the pores. TCPSi binds ibuprofen slightly stronger than TOPSi, and annealing seems to decrease the release rate.

### CELLULAR INTERACTIONS AND SAFETY/TOXICITY ISSUES

#### PSi biocompatibility

Biocompatibility is the ability of a material to interface with a natural substance without provoking unnatural response. The human body typically responds to contact with synthetic materials by initiating an immune response. This response can range from a mild inflammatory reaction to a severe allergic reaction. The biocompatibility of PSi materials depends on several factors, including the surface chemistry, particle size, and the presence of any toxic impurities. Studies have shown that PSi particles are generally considered to be biocompatible, with minimal to no immune response observed in in vitro tests. However, in vivo studies are necessary to fully evaluate their biocompatibility. This is especially important if PSi is intended for use in medical applications, such as drug delivery systems or tissue engineering scaffolds. In such cases, the safety and toxicity of PSi must be thoroughly investigated to ensure that it does not interfere with normal bodily functions or cause any adverse health effects. It is important to consider these factors when designing PSi-based medical devices, as the biocompatibility of the material can have a significant impact on its performance and long-term success.

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**Table 3. Fitted $k_d$-values for various PSi particles.**

<table>
<thead>
<tr>
<th>Pure ibuprofen</th>
<th>TCPSi</th>
<th>AnnTCPSi</th>
<th>TOPSi</th>
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<td>$k_d$ / mol s$^{-1}$ dm$^{-3}$</td>
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materials by depositing proteins and cells from body fluids at the surface of the materials. Materials that are 'tolerated' by the human body are then referred as 'bioinerts'. Application of PSi microparticles as drug delivery systems has to deal with the physiological environment when performing their functions during oral intake. Although silicon is known to be a biodegradable material, the use of silicon-based materials for medical applications is only possible if they are also biocompatible for the intended purpose. In this particular case, several factors need to be considered such as: (i) the different systemic pathway for removal and the toxicity of the degradation products; (ii) the mechanical integrity of the material during its degradation process; and (iii) the local interactions between the material and the surrounding tissues or cells. Furthermore, the degradation products of such biomaterials clearly need to have very low levels of toxicity and be readily removed from the body.

The possibility of toxic effects from PSi needs, therefore, to be considered. Silicon is essential in biological systems in small amounts as it affects both morphological development and metabolic processes. However, little is known about the biological processes that concern silicon at the molecular level. What is known is that silicon-induced toxicity may occur if a system is exposed to more silicon than is needed physiologically. Many studies have been carried out on the possible toxicity of implanted silicone, but there are few reports about the toxicity of silicon or silicon compositions. For mesoporous silicon materials there are just few biocompatibility tests published either, in particular concerning in vivo tests. Canham (1995) was the first to demonstrate that a thick layer of high porosity silicon was completely dissolved away within a day of in vitro exposure to a simulated body fluid due to possible adsorption of silicon. This was later confirmed by an in vivo study of both bulk and injected PSi at the subcutaneous site in a guinea pig model (Bowditch et al. 1999).

One feature of the PSi materials is that their biocompatibility can be modified according to the PSi porosity and pore size, as already mentioned before. For example, highly porous Si (p > 70%) dissolves in all the simulated body fluids except in the simulated gastric fluid, whereas PSi with medium porosity (p < 70%) is bioactive and slowly biodegradable. The very low porous Si and macroporous Si are both quite bioinert materials similar to the non-porous Si. An important issue for biomedical applications is the toxicity of the dissolved Si. In the human body, PSi degrades mainly into monomeric silicic acid (Si(OH)₄), which is the most natural form of the Si in the environment. The average daily dietary intake of silicon in the Western world is about 20–50 mg/day (Jugdaohsingh et al. 2004) and Si is an essential nutrient, which the human body needs. Human tests have revealed that the silicic acid concentration in the bloodstream rises only very briefly above typical values of about 1 mg/l (Popplewell et al. 1998). Urine excretion of silicic acid is also very efficient and expels all the ingested silicon. Furthermore, degradation products of mesoporous silicon found in blood of normal healthy individuals were typically 10 μM (Dobbie and Smith 1986; Bissi et al. 2005). In vivo tissue compatibility of as-anodised mesoporous silicon discs has been demonstrated by Rosengren et al. (2000). In this both porous and non-porous silicon implants originated a foreign body reaction similar to the corresponding titanium surfaces.

Another important aspect is the physico-chemical properties of the degradation products of silicon in the body, in particular their pKa. The degradation product of porous silicon is orthosilicic acid (pKa = 9.5), which is the bioavailable form of dietary silicon that is readily excreted through the kidneys (Refitt et al. 1999). However, when in body fluids (pH 7.4), only a small fraction of the silicic acid is deprotonated. Thus, this would have little effect, for example, on protein delivery, since the pH changes would not be relevant to induce loss of their biological activity. In addition, silicon is possibly important in human physiology by protecting against the toxic effects of aluminium. The in vitro dissolution studies of PSI confirm the fact that the silicic acid concentrations remain quite low and can be controlled with the porosity of PSi (Anderson et al. 2003). In vivo tests of tissue compatibility of PSi have provided evidence for PSi promoting calcification (Bowditch et al. 1999). Beyond the simulated environments and in vivo tests, PSi has also been found to support living cultures of mammalian tissues (Sapelkin et al. 2006; Low et al. 2006). Interestingly, in these studies it was
shown that PSi is able to act as a reducing agent and, hence, when redox-based assays are used together with PSi microparticles, care should be taken in order to correctly evaluate the possible toxic effects of PSi particles on cells.

**Cellular interactions and safety/cytotoxicity issues**

Although the fabrication of the aforementioned PSi particles is very well documented and known (see the previous sections), literature data on both in vitro and in vivo quantification and analysis of the cellular toxicity of these mesoporous materials is still premature. Before the intended use of PSi materials in drug delivery, it is first very important to use adequate methods to study the toxicity/cellular viability after a contact with the PSi particles. Below we address the in vitro safety and/or toxicity issues of PSi microparticles on cells.

Subsequent to the abovementioned studies concerning the cytotoxicity effects of PSi microparticles, particularly in vitro tests, we have recently shown that many of the traditional widely used toxicity assays, such as MTT and LDH assays, are inappropriate to correctly evaluate the Caco-2 toxicity of PSi microparticles (Laaksonen et al. 2007). As can be seen in figure 9, the MTT assay shows that the viability of the cells was increased when the PSi concentration was increased. However, even at relatively low PSi concentrations (0.1 mg/ml), the measured viability values caused by the artificial formazan production were higher than 100%. This is a clear evidence that the MTT cell viability assay interacted with the PSi particles (TCPSi, TOPSi, As-anod.).

![Figure 9. Caco-2 viability values after 5 h of incubation with PSi microparticles for size fractions of 1–25 (a) and 53–75 (b) µm. The MTT assay was performed using Caco-2 cells at 37 °C in the presence of PSi at concentrations of 1 and 0.1 mg/ml (n = 3 ± SEM) (modified from Laaksonen et al. 2007).](image-url)
and as-anodized). The results indicated that the observed viability is even higher than what would be predicted from the reduction of MTT by the PSi particles alone. Therefore, the common toxicity indicator, MTT, fails to accurately predict the toxicity of formulations containing PSi particles as a result of the spontaneous redox reactions where the MTT is reduced and the PSi particle surfaces are oxidized simultaneously, which is in good accordance with previous reports (Low et al. 2006). Consequently, the viability results are overestimated when the cytotoxicity of PSi particles is analyzed using the MTT assay alone. To avoid such effect, other toxicity indicators/tests should be used with any drug formulation testing involving the PSi particles.

In order to overcome the problems with the MTT and LDH assays, we have measured cytotoxicity of PSi microparticles using two other test assays: luminescence-based assay and flow cytometer. Caco-2 cells were used here to mimic the intestinal epithelial cell barrier during incubation times similar to typical transit times for humans (which correspond to the first 1 to 10 hours). Since the particle size of biomaterials may possibly induce toxicity, especially when the particle size is reduced to a nanosize-range, we have tested several PSi size fractions ranging between 1.2 and 75 µm to evaluate the effect of the particle size on the cellular toxicity. Moreover, the biopharmaceutical characterization of the particles is addressed. For that purpose, an in vitro pharmacodynamics study of the PSi microparticles on Caco-2 cells were analysed, by determining the effect of both the PSi concentration and size thresholds.

Figure 10a shows the cell viability results obtained for Caco-2 cells after 3 h incubation with PSi microparticles (TCPSi, TOPSi, and THCPSi) using a luminescence assay. In this assay the number of viable cells in culture is quantified based on the amount of ATP produced by metabolically active cells. In these experiments different size fractions of the particles were tested at different concentrations. The results show that the cell viability is rather high for all the studied particles for concentration values of ≤ 4 mg/ml and size fractions ≥ 38 µm. For these particular size fractions and concentration values, the results are rather independent of the surface chemistry of the particles, since similar behaviour was observed for TCPSi, TOPSi, and THCPSi. Thus, no compromise of the cellular toxicity was observed in this case. On the other hand, in the case of TCPSi, when the size fraction of the PSi microparticles was decreased (< 38 µm), the Caco-2 cell viability decreased rather significantly for high amounts of PSi particles (2 and 4 mg/ml). This effect was even more pronounced at the smaller size fractions of TCPSi (1.2–25 µm) indicating that the smaller size fractions of TCPSi are somewhat toxic to the Caco-2 cells. In the case of TOPSi, the cell viability also decreased for small size fractions, but here the effect was less pronounced than that observed for TCPSi. For smaller size fractions of the PSi microparticles, it seems rather evident that the Caco-2 cell viability is strongly dependent on the surface chemical treatment of the particles as well as on the PSi concentration.

We have further measured the Caco-2 cell viability using flow cytometer after the cells were treated with PSi (Fig. 10b). The same concentrations were used as in Figure 10a, with the size fractions of 1.2–25 and 53–75 µm for TCPSi and TOPSi, and 38–75 µm for THCPSi. For this purpose, Caco-2 cells were stained with propidium iodide dye in order to distinguish the living and death cells. The results obtained in Figure 10b are in very good accordance with the results in Figure 10a, except for the smallest size fraction (1.2–25 µm) of TCPSi with a possible toxic effect in the Caco-2 cells. Overall, the mesoporous silicon microparticles were well tolerated at ≤ 4 mg/ml in Caco-2 cell cultures for size fractions > 25 µm. In this case, even at the highest particle concentrations used (4 mg/ml), both the luminescent assay and the flow cytometer gave consistently high cell viabilities, which demonstrate that the mesoporous silicon microparticles were not toxic to the cultured intestinal epithelial cells. The surface chemical treatments and the particle size of PSi microparticles both seem to affect the toxicity. Also, when dealing with PSi mesoporous microparticles, the use of an appropriate method to quantify cellular viability is crucial, as we have shown here.
Figure 10. (a) Cell viability values for Caco-2 cells determined by a luminescent assay after 3 h incubation at 37 °C with the PSi microparticles (TCPSi, TOPSi, and THCPsi) of different size fractions (1–25, 25–38, 38–53, and 53–75 µm) at concentrations of 0.2–4 mg/ml. (b) Number of living Caco-2 cells (%) determined from flow cytometer measurements after 3 h incubation at 37 °C; PSi microparticles with respect to the untreated cells (control). Data are expressed as mean ± SEM of at least 3 independent measurements.
CONCLUSIONS

Our recent results on the feasibility (fabrication, characterisation, loading and release, incl. modelling, and cellular compatibility and safety) have clearly confirmed the fact that the meso-/nanoporous silicon may indeed offer a distinct advantage in dissolution enhancement and precisely controlled (oral) drug delivery applications. Moreover, the results of the newly developed toxicity assays also suggest that the microparticles could be utilised in (oral) drug delivery formulations based on the ordered mesoporous silicon materials. Clear incentive is presented for future animal and toxicity testing of the materials and drug delivery formulations based on ordered mesoporous silicon microparticles. For a more comprehensive review about the biomedical applications of the mesoporous silicon materials, the reader is directed to a recent review article in the Journal of Pharmaceutical Sciences (Salonen et al. 2008).

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List of symbols used in the modelling equations

- \( A \) Surface area of a single pore (m\(^2\))
- \( c^s \) Concentration at the solid drug surface (mol m\(^{-3}\))
- \( c^{ps} \) Concentration at the silicon particle surface (mol m\(^{-3}\))
- \( c^b \) Bulk concentration (mol m\(^{-3}\))
- \( D \) Diffusion coefficient (m\(^2\) s\(^{-1}\))
- \( D_e \) Effective diffusion coefficient inside the particle (m\(^2\) s\(^{-1}\))
- \( f \) Fraction of drug dissolved from the particle
- \( h \) Thickness of the diffusion layer (m)
- \( J \) Molar flux (mol s\(^{-1}\)m\(^{-2}\))
- \( k_a \) Rate constant for adsorption (m s\(^{-1}\))
- \( k_{d} \) Rate constant for desorption (mol s\(^{-1}\)m\(^{-2}\))
- \( K \) Equilibrium constant / solubility of the drug (mol m\(^{-3}\))
- \( l \) Length of the drug phase inside the pores (m)
- \( r_s \) Rate of reaction at the surface (mol s\(^{-1}\)m\(^{-2}\))
- \( R \) Radius at the solid drug surface (m)
- \( R_0 \) Radius of the silicon particle (m)
- \( \rho \) Molar density of the drug in the solid phase (mol m\(^{-3}\))
- \( \phi \) Porosity
TIIVISTELMÄ

Tässä katsausartikkelissa esitellään meso-/nanohuokoiset piimateriaalit (PSi) uutena mahdollisuutena suu'n kautta annettavien lääkevalmisteiden formulointimehitykseen. Mesohuokoisten piipartikkelien valmistusparametrit ja karakterisointimenetelmät sekä farmaseuttisen kehityksen avainkysymykset, lääkeainelataus, lääkeaineiden liukoisuuden ja lääkevapautumen paranminen sekä biohygienen-sopivuus (solutason elinkyky/toksisuus), muodostavat katsauksen pääsisällön. Mesohuokoisia materiaaleja (huokosten läpimitta 2–50 nm) valmistetaan tyypillisesti etsimällä mahdollisimman puhtaita piikiekkoja tai -partikkeleita kemiallisesti vahvassa HF-liuoksessa. Tämän jälkeen mikronoidut PSi-materiaalit tulee pintakäsittelä esimerkiksi hapen tai hiilen avulla korkeissa lämpötiloissa, sillä etsauksen jälkeinen vetytermi on anodisoituna pinta on epästabiili ja eristäntesiin tapahtuu helposti säilyttävän aikana. Syövytysprosessi määrittelee sen, minkälaisia huokosrakenteita piihin syntyvän, kun taas pintamuokkauksen avulla voidaan säädetä sitä, miten materiaalit reagoivat ympäröivien molekyyljen kanssa, esimerkiksi lääkeaineliuoksessa tai soluympäröistössä. Suun kautta tapahtuva etsaamisen jälkeen pintojen funktionalisointi merkitsy liittyä mahdollisimman suureen lääkelatauksen saavuttamiseen sekä siihen, miten nopeasti tai hitaasti lääkeaine liukenee ja vapautuu mesohuokosista elimistössä. Huokoksen piin pintakemia on avainroolissa myös partikkelien ja solujen vuorovaikutusten ja mahdollisen solutoksisuuden suhteen. Kaiken kaikkiaan tutkimusryhmämme viimeaikaiset tulokset (valmistus ja karakterisointi, lääkelataus ja -vapauttaminen (ml. ko. ilmiöiden kineettinen mallitus) sekä elävien solujen kanssa tapahtuva yhteensopivuus- ja toksisuuskarakterisointi) osoittavat selvästi, että meso-/nanohuokoiset piimateriaalit tarjoavat erittäin suuria hyötyjä ja kehitysmahdollisuuksia (oraalisten) lääkevalmisteiden formulointoon ja kehittämiseen lääkevalmisteiksi.

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