11
Porous Silicon Particles for Imaging and Therapy of Cancer

Rita E. Serda, Ciro Chiappini, Daniel Fine, Ennio Tasciotti and Mauro Ferrari

11.1 Introduction

The aim of the National Cancer Institute is to eliminate death and suffering from cancer by 2015, by harnessing the power of nanotechnology to provide new tools for diagnosing, imaging and treating cancer. Funded research projects include the design and fabrication of multifunctional nanodevices which promise to change the way we pinpoint cancer and deliver anticancer drugs. Major advances in our comprehension of how cancer develops come from the huge array of knowledge achieved by cancer genomics and proteomics. Unfortunately, to date only a very small fraction of the potential carried by these discoveries has been translated into clinical practice. Most of this gap can be accounted for by technological challenges inherent in designing systems able to function in the complex biological milieu [1, 2]. The ability to translate extremely developed and established technologies, such as silicon technology, into the biosciences offers an enormous advantage due to immediate access to a wide array of sophisticated tools.

Silicon technology is already established in terms of production, characterization and translation into nanotechnologies. An expansive set of miniaturized sensor and actuators have been developed that, in the biological context, could be used as building blocks for complex interacting systems [3–5]. Scalability, precision and reproducibility are characteristics of these processes that will be extremely valuable when translated into clinical applications. For these reasons, an enormous interest in the design and fabrication of silicon devices for biomedical application exists, and many research projects are now under way to characterize these systems within biological environments.

Since the 1980s, silicon-based sensors and microelectromechanical devices (MEMS) have been used ex vivo for tasks such as pressure sensing, blood chemistry analysis, flow cytometry and electrophoresis [6]. The term ‘BioMEMS’ was subsequently coined to describe the wide array of tools developed using silicon as a platform. Extremely sophisticated systems are now available, such as functionalized microcantilevers for molecule adsorption, recognition and quantification, as
Porous Silicon Particles for Imaging and Therapy of Cancer

well as ‘Lab-on-a-Chip’ devices which scale single or multiple laboratory processes into a compact chip-format [6].

Silicon-based implantable and transdermal devices have been proposed and realized. Arrays of implantable single-dose microreservoirs [7], or drug-releasing chips equipped with microneedles [8], have been prototyped and are currently undergoing animal testing, or are already in clinical trials. One of the major obstacles encountered in the successful implementation of implantable silicon devices has been their poor biocompatibility [9] and nonbiodegradability.

In 1995, the first study hinting at the biocompatibility and bioactivity of silicon-based devices was presented [10]. Scientists reported that silicon could be made biocompatible by making it porous, with the pore size directly affecting biodegradability. Porous silicon (pSi) and porous silica are obtained through sol–gel techniques [11] or from bulk silicon by means of electrochemical etch [12]. pSi was first observed some 50 years ago by Ulhir and Turner [13, 14] while experimenting with different techniques to electropolish silicon surfaces. The anodic electrochemical etching of silicon in an aqueous or organic solution of hydrofluoric acid (HF) creates pores the sizes of which vary depending on the experimental conditions.

In 1990, Canham discovered the photoluminescence properties of pSi, and shortly thereafter electroluminescence and chemoluminescence were observed [15], spurring an enormous interest in pSi as an optoelectronic material. However, almost 20 years of continual research in the field, the optical efficiency and long-term stability of pSi remain too low for any functional optoelectronic application [15].

In this chapter we present a complete overview of pSi bioapplications, from their discovery and synthesis to current biological testing and visionary ideas for bypassing multiple biological barriers for the effective delivery of drugs and imaging agents to the tumor site. First, we focus on the production of pSi, and then define the mechanisms used to characterize porosity. In the next section, we address biocompatibility and biodegradation, both of which are directly related to the degree of porosity. The loading and quantification of drugs (therapeutics and imaging agents), together with a description of nanovector design for optimal delivery, are then presented. Finally, the effect of particle size, shape and surface modification (including serum opsonization) on the phagocytosis of particles by cells is discussed, and an example of in vivo imaging with fused pSi and iron oxide nanoparticles is presented.

This chapter is unique in that it describes the fabrication, characterization and current biological applications of pSi, whereas previous reviews have focused either solely on the formation and modification of pSi, or on pSi microparticles for the detection of chemical and biological compounds. In 1997, Canham produced a book entitled, Properties of Porous Silicon, which contained a collection of the physical and chemical properties of pSi. In this chapter, we include details of tested and visionary ideas on nanosized and microsized delivery systems, both of which are today emerging as powerful tools for the systemic delivery of therapeutic molecules and imaging agents for different biomedical applications, from
cancer [16, 17] to cardiovascular diseases [18]. These delivery systems can be loaded with a multitude of drug molecules and contrast agents to provide simultaneous therapeutic and imaging capabilities.

11.2 Porous Silicon

Two top-down strategies have been developed for the production of pSi. The most widely used strategy is electrochemical etch, while the other approach, stain etch, is rarely used in practice due to its severe lack of flexibility [19]. The most widely employed top-down approach produces pSi through electrochemical etch of a bulk silicon wafer. The pSi can then be oxidized to take advantage of silane chemistry. Alternatively, its surface can be carbon-terminated to obtain a hydrophilic chemically stable interface [20].

Bottom-up strategies, which employ silicate precursors, such as tetraethoxysilane (TEOS), produce porous silicon dioxide. Silicon dioxide is a much more chemically stable interface than silicon, and precludes some forms of surface functionalization, such as carbonization, which is used to tune particle properties. Additionally, bottom-up approaches are limited to the production of either spherical or ellipsoid particles, unless used in conjunction with top-down lithography. Shape has been shown fundamentally to determine several properties of the particle that are relevant for drug delivery, such as flow dynamics, margination, degradation rate and cell uptake [21, 22]. For these reasons, top-down approaches to the production of pSi for biomedical applications have been historically favored.

Porous silicon dioxide is formed by the anodic dissolution of single-crystal silicon in an HF solution. A bulk silicon wafer is placed in an aqueous or organic solution containing less than 50% volume of HF. The cathode is immersed in the solution while the silicon wafer itself constitutes the anode. The porosification process is sustained as long as a current is applied. Figure 11.1 is a scanning electron microscopy (SEM) image of a cleaved pSi layer etched in a silicon wafer. The main characteristics of the porous layer—that is, porosity, pore size, length, orientation and morphology—are determined by the current intensity, the doping characteristics of the silicon wafer, the etch duration and the composition of the etchant solution. The most common solution used for the production of pSi is HF:H2O:ethanol. This solution is considered to be aqueous as ethanol does not take an active part in the porosification reaction, but is employed merely as a surfactant [12, 19].

The underlying porosification mechanism is still widely debated. All currently accepted theories require the presence of excess positive carriers (holes) at the silicon surface for etching to occur. In aqueous solution, two competing dissolution pathways are observed (Figure 11.2), both of which initially require the replacement of an H atom at the silicon surface with an F⁻ ion from solution. This step constitutes the electrical part of the etch, as the hole necessary to neutralize the SiF bond must be supplied by the silicon wafer. In the second dissolution step
Figure 11.1 A 45° cross-sectional scanning electron micrograph of a cleaved porous silicon layer etched in a highly doped p-type silicon wafer.

Figure 11.2 Competing silicon dissolution pathways during electrochemical etch leading to the formation of porous silicon. $h^+$ indicates positive charge carriers (holes) from bulk silicon.
of pathway I, the Si−SiF bond is broken by reacting with HF, which results in the removal of a silicon atom from the bulk. No carriers from the solid Si are required in this second step, which constitutes the chemical part of the etch process. In the competing pathway II, the backbone of Si−SiF is broken by reacting with H₂O; this results in Si−O−Si bonds that are unstable in HF. Thus, pathway I leads to the direct dissolution of silicon, while pathway II results in an indirect dissolution. Pathway II leads to a passivated surface in which dissolution depends heavily on the HF concentration. It is proposed that the ratio of the two pathways influences the final pore structure. Phenomenological analysis has shown that porosification occurs almost exclusively at the bottom of the currently formed pore, while the formation of lateral pores or an enlargement of the existing pore is a minor effect [12, 19].

Currently proposed porosification models disagree in delineating the factors that determine pore morphology, separation and orientation. Surface space charges, quantum confinement, surface curvature, local surface crystalline anisotropies, combinations of the former and many other effects have been proposed as mechanisms determining pore characteristics, but none is sufficiently convincing so as to achieve a consensus [12, 19].

As a result of the lack of a valid porosification model, general predictive guidelines to determine the final porous structure for a given set of etch parameters do not exist. Thus, several phenomenological rules, approximate guidelines and trial and error are employed to obtain structures with predefined morphological characteristics. Once defined, however, etching parameters used to obtain the desired configuration and pore characteristics are highly reproducible.

The silicon doping type and concentration influences pore size, porosity and pore structure. For highly doped \( D_c > 10^{15} \) p-type wafers, an increase in doping increases the pore size from 10 to 100 nm. In lightly doped p-type silicon \( (D_c < 10^{15}) \), the pore size increases with decreasing doping from 1 nm to the micrometer scale [19]. n-type wafers electrochemically etched in the dark instead tend to form larger pores. Heavily doped wafers \( (D_c > 10^{19}) \) produce pores in the 10 to 100 nm range, while lightly doped ones \( (D_c < 10^{19}) \) result in pore sizes ranging from 1 to 10 μm. The illumination of n-type silicon affects pore formation, both in structure and size, due to the significant increases in the amount of holes made available to the etch process. In general, n-type wafers tend to be less porous than p-type [12, 19]. The pore size distribution breadth is observed to increase with increasing pore size, and is independent of the details of pore formation [12].

The nature of the exposed surface is an extremely important factor contributing to the final pore structure. Pores tend to grow not only along the (100) crystalline direction but also towards the source of the holes, which is orthogonal to the surface. When the exposed surface is the (100) orientation there is no conflict, and in general straight pores orthogonal to the interface are observed, possibly with 90° branches depending on the wafer doping characteristics. However, if the interface is along another crystalline plane then, depending on the etch conditions, pores may orient either along the (100) direction, along the holes source, or somewhere in between, giving rise to a chaotic, branched porous structures [12, 19].
In the standard aqueous solution HF : H₂O : ethanol, the HF concentration determines a sub-range of pore sizes and porosity within the range set by the wafer doping. As a general rule, more diluted solutions tend to form larger-diameter pores [12, 19]. The etch current intensity also contributes to the pore size distribution; with all the other parameters kept equal, larger currents tend to form bigger pores. In order to obtain a porous layer with uniform pore size and porosity across the entire wafer, the current density must be kept constant across the surface. In contrast, it is possible to exploit anisotropic currents in order to form a continuous layer with a pore size gradient at the surface [19, 20]. Modulation of the current during the etch process allows the formation of pores with variable diameter along their growth axis. For every set of parameters there exists a limiting current \( J_1 \), above which electrochemical etch does not occur. For currents larger than \( J_1 \), but smaller than the threshold current \( J_2 \), a transitional regime is observed, while for currents larger than \( J_2 \), electropolishing dominates and entire layers of silicon are removed instead of pores being formed (Figure 11.3) [12, 19]. This property is often exploited in order to release porous layers from the wafer by removing entire layers at the interface between the porous silicon and the wafer.

Great versatility is thus achieved through the electrochemical etch porosification approach. Fine-tuning the porosification parameters makes it possible to obtain pSi structures of extremely different characteristics, ranging from very low porosity (15–20%) to very high porosity (>90%), with pore sizes ranging from a few nanometers to tens of microns, and completely different pore structures and morphologies. As these materials have significantly different behaviors when employed as drug delivery systems, the remainder of this chapter will be devoted to microfabrication for clinical applications and biological validation.

The lack of reliable predictive rules for the effect of etch parameters on the final result may be seen as a drawback of the technique. However, phenomenological guidelines are usually sufficient to direct the exploration of the parameter space and significantly limit the number of trials required to obtain the desired results in terms of film porosity, pore size distribution and thickness.
Microfabrication

The production of pSi microparticles generally starts with the porosification of a silicon wafer in an apposite etch tank (Figure 11.4). The tank is composed of HF-resistant materials such as polytetrafluoroethylene or aluminum oxide. The wafer is placed in the tank with the frontside facing the etch solution and the rearmost side in contact with the anode of a power supply. If no backside illumination of the wafer is required, a metallic layer is deposited over the entire wafer backside to provide a better current uniformity; otherwise, an annular contact along the wafer edge is employed. As an alternative, a backside electrolytic solution can be used to obtain a better contact, but at the expense of flexibility [12, 19].

The cathode is composed of an HF-resistant material (e.g. platinum) which is immersed in the etch solution. If a uniform current density is required, a mesh cathode with surface area larger than the silicon wafer must be used and placed in the solution parallel to the wafer, and at a suitable distance so that the current flow at the interface between the wafer and the solution is spatially uniform. Alternatively, when a pore size gradient is pursued, a rod-like Pt cathode is immersed at a specific position within the solution [12, 19].

The current flow through this system induces porosification of the silicon wafer by the anodic electrochemical etch process previously described. Throughout the process, hydrogen is evolved at the anode while fluoride ions are bonded to silicon while HF is consumed; this in time causes a change in the solution concentration at the wafer interface. In order to prevent this phenomenon from interfering with the etch process, a pumping system that continuously provides fresh solution is usually present [19]. Once the porous layer has formed, a final electropolishing step at high current is performed which causes the layer to detach from the silicon wafer [12, 19]. The detached layer is then rinsed, transferred to a 2-propanol solution, and sonicated until it is broken down into microparticles within the desired
size range. The particles obtained when using this approach are characterized by their irregular shape and size polydispersion. Although a selection for size is usually obtained through filtering, selection for shape cannot be achieved [19, 23].

The size polydispersion, and the very small yield in the micron size range suitable for drug delivery applications, constitutes a significant drawback of this technique. Size uniformity is important for shared biodegradation rates, which depend on both size and porosity. Another drawback of this technique is the shape polydispersion, which affects both the cellular uptake of pSi particles and blood flow characteristics [24]. Microparticle uptake by the reticuloendothelial system (RES), as well as margination and adhesion dynamics in the bloodstream, are strongly correlated to both the shape and size of the particles [21, 25, 26].

Ferrari et al. developed a porosification process involving photolithography that allows extreme control over particle size and geometry [16]. In this process, an initial two-dimensional (2-D) shape is transferred through photolithography of a silicon nitride-coated wafer. Reactive ion etch (RIE) is then employed to transfer the pattern through the silicon nitride layer and confer an initial three-dimensional (3-D) structure to the particle, etching a trench within the silicon. The exact 3-D shape of the trench is determined by the 2-D shape of the pattern and the RIE conditions (Figure 11.5).

The patterned wafer then undergoes selective electrochemical etch. Here, the silicon nitride acts as an etch stop layer, and the wafer is differentially porosified only where the silicon is directly exposed to the etchant solution. A second etch step with current intensity in the transition region leads to the formation of a high-porosity layer at the interface between the porosified particle and the silicon wafer. The Si$_3$N$_4$ layer is removed by means of prolonged soaking in HF. Finally, the particles are detached from the silicon substrate by disrupting the high-porosity layer through sonication in 2-propanol. The final particle shape is determined

Figure 11.5 Scanning electron microscopy (SEM) images of hemispherical mesoporous silicon microparticles containing either large pores (a) or small pores (b). Reproduced from Ref. [16], courtesy of Nature Publishing Group.
by the initial trench shape and the effects of the electrochemical etch (Figure 11.5).
The electrochemical etch profile is determined by the local current intensity gra-
dients at the silicon interface. This technique is both extremely controllable and
reproducible, and allows the manufacture of particles with characteristic sizes in
the micron range and monodisperse in terms of both particle and pore size. The
particle shapes attainable are limited by the 3-D patterns achievable through
the combination of photolithographic techniques and the characteristic profile of
the electrochemical etch.

11.4 Characterization

Once the porous silicon microparticles have been fabricated, they must be carefully
characterized to insure that the desired particle specifications have been achieved
and that there is minimal batch-to-batch variation. Good Manufacturing Practice
(GMP) must be maintained to ensure replicability of microparticle fabrication
between lots and to exclude particle structural variability as a source of experimen-
tal observation in biological experiments. Given the complexity and size scales
involved with the internal structure of these microparticles, a battery of techniques
must be employed to quantify the important metrics, such as porosity, pore size,
density, interior volume, surface area, surface charge and surface modification.

11.4.1 Gravimetry

One of the most straightforward and accurate ways to measure the porosity and
density of pSi is through the gravimetric technique [27]. First, the silicon substrate
is weighed before any electrochemical etching is performed ($m_1$). The silicon sub-
strate is then electrochemically etched and then re-weighed again ($m_2$). Finally, the
pSi layer is removed using an etch solution of NaOH and the silicon substrate
weighed a third time ($m_3$). The porosity of the pSi is then determined according
to the following relationship:

$$p = \frac{m_1 - m_2}{m_1 - m_3}$$  \hspace{1cm} (11.1)

The thickness ($W$) of the pSi layer can also be determined from $m_1$ and $m_3$
according to the following relationship:

$$W = \frac{m_1 - m_3}{A \cdot d_o}$$  \hspace{1cm} (11.2)

where $A$ is the area of the silicon substrate exposed to the HF solution and $d_o$ is
the density of silicon [27].
11.4.2 Spectroscopic Ellipsometry

Although gravimetry is a straightforward and accurate technique [28], it is a destructive methodology for determining porosity. Spectroscopic ellipsometry is an optical technique (Figure 11.6), whereby the porosity of the pSi layer is determined by illumination of its surface with linearly polarized light, which then interacts with the porous material to emerge elliptically polarized upon reflection [29].

The reflected light is decomposed into its perpendicular (s-direction) and parallel (p-direction) components (with respect to the plane of incidence) and their amplitudes and phase shift are measured, as determined by the following relationship [29]:

\[
\rho \psi = R_p R_s \tan \Delta
\]

where \( R_p \) and \( R_s \) are the complex reflection coefficients, \( \tan(\psi) \) is the ratio of the reflected amplitudes, and \( \Delta \) is the phase shift [29]. Once the change in polarization has been determined, a complex dielectric constant (\( \varepsilon \)) can be calculated [30]:

\[
\varepsilon = \varepsilon_1 + i \varepsilon_2 = \sin^2 \varphi + \left[ \frac{1 - \rho}{1 + \rho} \right]^2 \sin^2 \varphi \tan^2 \varphi \tag{11.4}
\]

where \( \varepsilon_1 \) and \( \varepsilon_2 \) are the real and imaginary components of the complex dielectric constant and \( \varphi \) is the angle of incidence. Once the dielectric constant for the overall
porous silicon layer is obtained, an analytical model is used to determine the contribution of the dielectric constants of the crystalline silicon and the air-filled voids to the overall measured dielectric constant, which is dependent on the porosity of the film. One heavily used model for this purpose is the Effective-Medium Theory corrected for dipole–dipole interactions, which models the voids as ellipsoids inside the crystalline silicon matrix [31]. From this theory the porosity can be calculated as follows [31]:

\[
\frac{1}{3} C \alpha + \frac{(1 - C)(\varepsilon_m - \varepsilon)}{(\varepsilon_m + 2\varepsilon)} = 0
\]

for a 3-D system where \( \varepsilon_m \) is the dielectric constant of the surrounding crystalline silicon and \( C \) is the porosity. \( \alpha \) can be calculated through the following equation [31]:

\[
\alpha = \frac{1}{3} \sum_{i=1}^{3} \frac{(\varepsilon_v - \varepsilon)}{\varepsilon + L_i(\varepsilon_v - \varepsilon)}
\]

where the \( L_i \) indicates the depolarization triplets governed by the ratio of the axes of the ellipsoid shaped voids and \( \varepsilon_v \) is their dielectric constant [31]. Although this is a good approximation of the porosity, it is not perfect due to the fact that voids in the pSi layer cannot be completely approximated as ellipsoids leading to a discrepancy between the modeled and actual porosities [27].

11.4.3 X-Ray Diffraction

Another nondestructive technique for measuring the interaction of electromagnetic radiation with the pSi layer to determine its important characteristics is X-ray diffraction (XRD) [32]. This technique relies on pSi retaining the single crystal nature of the original silicon wafer even though it contains voids. Measurements are performed using monochromatic X-rays with a wavelength of 1.54 Å emitted from copper subjected to a high-energy electron beam. The X-rays are reflected off the surface of the pSi layer at varying angles of incidence, and collected by a detector at an equal angle of reflection [33]. The diffraction pattern thus reconstructed is related to the arrangements of interatomic planes in the crystal. The intensity peaks in the diffraction pattern are related to the wavelength of the X-rays (\( \lambda \)), the spacing between atomic planes (\( d \)), and angle of incidence according to the Bragg equation:

\[
n\lambda = 2d \sin \theta
\]
Porous Silicon Particles for Imaging and Therapy of Cancer

range around the equal angle of reflectance. The S Bragg peak corresponds to the sharp peak produced by the highly ordered single crystal silicon substrate. The P peak, which is lower in intensity and broader, is produced by the pSi layer. The lattice mismatch parameter between the lattice constant of the silicon and that of the pSi layer can be calculated by evaluating the angular separation of the two peaks and used in conjunction with the dynamical theory of XRD [35] to determine the porosity [32]. Furthermore, the pSi layer thickness can be ascertained from the frequency of the small intensity fringes at the edges of the rocking curves away from the main Bragg peaks S and P. X-ray diffraction, like spectroscopic ellipsometry, can be used to determine porosity nondestructively, and in the case of XRD the thickness of the pSi layers is usually in good agreement with measurements made using the gravimetric method [30, 32].

11.4.4 Nitrogen Adsorption

Spectroscopic ellipsometry and XRD measurements require large areas of continuous films. In order to directly measure the properties of pSi particles, it is possible to exploit the principle of gas adsorption [28], with the most commonly employed
gas for this analysis being nitrogen. This technique allows the total surface area of the porous material to be calculated, simply by knowing the adsorbate’s molar volume and adsorption cross-sectional area [36]. Furthermore, by knowing the surface tension of the gas species when it is in liquid form, and how the surface curvature adjusts the condensing properties of the gas from a flat surface, it is also possible to obtain a pore size distribution [37].

To perform these measurements, a known amount of gas is introduced into a chamber containing the porous sample. The system is then allowed to reach equilibrium, after which the deviation of the equilibrium pressure from the ideal pressure that would result if no adsorption was taking place, is measured [38, 39]. Under isothermal conditions, which can be achieved by immersing the sample in liquid nitrogen for example, the pressure difference can be related to a volume which, when divided by the mass of the sample, represents the specific volume of gas adsorbed (in cm³g⁻¹). The results of these measurements are known as isotherm curves (see Figure 11.8). Depending on the experimental apparatus used, the pressure can either be adjusted incrementally or, in some of the latest adaptations, adjusted continuously at a rate that guarantees the attainment of quasi-equilibrium conditions [40]. The pressure (as represented on the x-axis) is the relative pressure \( p/p_0 \) where \( p \) is the supplied pressure and \( p_0 \) is the saturation pressure.

Figure 11.8 Isotherms generated for microparticle samples with two different mean pore sizes. The green and dark red curves are the adsorption and desorption isotherms, respectively, for microparticles with a diameter of 3.2 μm and a mean pore size of 7.4 nm. The blue and orange curves are the adsorption and desorption isotherms, respectively, for microparticles with a diameter of 3.5 μm and a mean pore size of 24.2 nm.
of liquid nitrogen (the pressure at which liquid nitrogen boils at a given temperature). When the pressure in the chamber exceeds the saturation pressure, the gas condenses. The experiment is usually performed by sweeping the relative pressure in both directions to measure adsorption as well as desorption. As can be seen from Figure 11.8, hysteresis is usually observed; this is due to the particular structure of the pores (e.g. pores resembling straight cylinders versus pores with constrictions anywhere along their length) and/or the interaction between the adsorbate and the sample surface [40]. Although these interactions can be quite complex, the hysteresis for pSi tends to be quite consistent for samples fabricated under the same conditions, and so can serve as a qualitative check on the most likely pore configuration for a given sample [28]. A quick estimate of the total pore volume for pSi can be gleaned directly from the saturated area of the isotherm present at high values of relative pressure (this is not the case for all materials, as isotherms of different materials do not always saturate) [28]. Given that a calculation of the total volume of the pSi material can be made, the porosity can quickly be calculated (porosity = pore volume/total volume) and is usually in good agreement with data provided by the gravimetric method [28].

From the isotherms in Figure 11.8 a range of information can be obtained. The first important piece of information is the total surface area of the porous material, which can be calculated using the Brunauer–Emmett–Teller (BET) method. This derives a relationship between the volume and relative pressure, based on how the molecular layers of adsorbate build up on the surface of the porous material [36]. The BET equation is as follows:

$$\frac{p}{\nu(p_0 - p)} = \frac{1}{v_m c} \cdot \frac{c - 1}{v_m c} \cdot \frac{p}{p_0}$$  \hspace{1cm} (11.8)

where $p$ is the measured pressure, $p_0$ is the saturation pressure, $\nu$ is the adsorbed volume, $v_m$ is the adsorbed volume when the entire surface of the sample is covered with a unimolecular layer of adsorbate, and $c$ is a parameter obtained from the following relationship:

$$c = e^{-\frac{E_1 - E_L}{RT}}$$  \hspace{1cm} (11.9)

where $E_1$ is the heat of adsorption of the first layer of adsorbate, $E_L$ is the heat of liquefaction used for all subsequent layers of adsorbate, $R$ is the gas constant, and $T$ is the temperature [36]. In order to calculate the total surface area it is necessary to plot $p/(\nu(p_0 - p))$ versus $p/p_0$, using values of volume obtained from the isotherm over the range where the isotherm is approximately linear, which for silicon usually corresponds to values of $p/p_0$ from 0.05 to 0.35 (this range is material-dependent) [36]. Both $v_m$ and $c$ can be calculated from the line where $1/v_m c$ is the y-intercept and $(c - 1)/m$ is the slope. When $v_m$ has been determined, by multiplying the ratio of $v_m$ and the molar volume by Avogadro’s number and the adsorbate cross-sectional area, it is possible to determine the total surface area of the sample.
Outside the range of relative pressures listed above, either the entire surface area is not covered with adsorbate, leading to sublinear behavior, or condensation has begun inside the pores due to their extreme curvature at a pressure level lower than the onset of condensation on a flat surface, leading to super-linear behavior [37]. These two effects therefore exclude those regions of the isotherm from being used to determine total surface area through the BET model.

The super-linear region of the isotherm at higher values of relative pressure is used to determine the pore size distribution. As the super-linear behavior is due to the early onset of condensation in the pores, a mathematical relationship was developed by Barrett, Joyner and Helena (BJH) which equated the change in desorbed volume from one measurement point to the next to a release of condensate from pores of a particular radius, plus the thinning of the adsorbed layer in pores which were already empty [37]. The pore volume is then calculated according to the following equation [37]:

$$V_{pn} = R_n \left( \Delta V_n - \Delta t_n \sum_{j=1}^{n-1} c_j A_{pj} \right)$$  \hspace{1cm} (11.10)

where $V_{pn}$ is the average volume of the pores which are currently releasing their condensate (the range of pore sizes which must be averaged depends on the resolution of the measurement apparatus as it is related to the minimum relative pressure step), $\Delta V_n$ is the measured decremental desorbed volume change, $\Delta t_n$ is the decremental adsorbate layer thickness change, $R_n$ is the radial adjustment to convert the capillary volume (the volume of the actual pore minus the volume of the adsorbed sheath) to the actual pore volume, $A_{pj}$ is the actual area of each of the pores already emptied, and $c_j$ is the radial adjustment to convert the actual pore area to the capillary area. $R_n = r_{pn}^2/(r_k + \Delta t_n)^2$, and takes into account not only the capillary volume, with radius $r_k$, but also the decremental change in the adsorbate volume, with thickness $\Delta t_n$. Finally, $A_{pj} = 2V_{pj}/r_{pj}$, and is calculated for each pore which has already been emptied, multiplied by $c_j = (r_{pj} - t_n)/r_{pj}$, where $t_n$ is the total adsorbed layer thickness for the current relative pressure, and then summed together for all previous emptied pores [37]. This term constitutes the adsorbate volume released from all of the previously emptied pores, and must be removed from the measured decremental volume in order to accurately calculate $V_{pn}$. The value of the capillary radius, $r_k$, relates to the relative pressure by way of the Kelvin equation [41]:

$$r_k = \frac{-2\gamma V_m}{RT \ln(p/p_0)}$$  \hspace{1cm} (11.11)

where $V_m$ and $\gamma$ are the molar mass and surface tension of the adsorbate, $R$ is the gas constant, and $T$ is the temperature in Kelvin. The relationship between the total sheath thickness $t$ to the relative pressure—’the $t$-curve’—has been determined previously through a variety of techniques. One way is to determine the $t$-curve...
experimentally by measuring an isotherm for an unporosified sample of the same material, and dividing the isotherm by the BET-calculated unimolecular volume $V_m$ to extract the number of molecular layers present for a given relative pressure, and then multiply by the radius of molecular nitrogen [42, 43]. This can then be directly ported to the porous sample. If no exact reference material exists (i.e. the material does not exist in bulk form), then a similar material can be used. This reference substitute is selected based on its possessing a comparable $c$-value, as calculated by the BET model [43]. The other commonly used methodology for calculating the $t$-curve is to use a semi-empirical version of the Frenkel–Hill–Halsey equation:

$$ t = \frac{1}{\left( -\frac{RT}{\alpha} \ln \left( \frac{p}{p_0} \right) \right)^{1/s}} \quad (11.12) $$

where $s$ and $\alpha$ are constants, $\alpha$ being the Hamaker constant of the system (which is a measure of the van der Waals interactions between the adsorbate and the sample) [41, 44, 45]. In the literature, values of $s = 2$ and $s = 3$ have been used to generate $t$-curves, but for the case of porous silicon $s = 3$ has been shown to produce quite consistent results [29]. The various radii and thicknesses used in the model and listed above are depicted in Figure 11.9. When all of these components have been taken into account and the pore volume has been computed

![Figure 11.9](image-url)

**Figure 11.9** A schematic representation of the desorption mechanism described in the BJH pore size distribution model, indicating the origin of the physical parameters of the cylindrically modeled pores. $r_{kn}$, $r_{pn}$ and $r_{kn-1}$, $r_{pn-1}$ are the radii of the capillaries and pores which have released their condensate in the current relative pressure step $n$ and the previous relative pressure $(n-1)$ step. $t$ is the current thickness of the adsorbate, with $\Delta t_n$ and $\Delta t_{n-1}$ representing the current and previous change in adsorbate thickness. Adapted from Ref. [37].
for all pores with radii $r_p$, the values of $V_p$ are divided by $\Delta r_p$ (the change in the computed pore radius from one point to the adjacent point) to determine the differential volume for each pore size, given in units of $\text{cm}^3\text{g}^{-1}\text{Å}^{-1}$ [37]. The plot of the differential pore volume versus $r_p$ yields the pore size distribution representing the total area of the surface that each size of pore occupies. As an internal check for consistency, the summation of the surface area over all the radii from the pore size distribution should be within a few percent of the total surface area calculated from the BET model [37]. Figure 11.10 shows calculated pore size distributions for hemispherical microparticles similar to the microparticles depicted in Figure 11.5, but fabricated under two different electrochemical etch conditions [16]. One distribution constitutes microparticles with a diameter of $3.5\mu m$ and a mean pore size of $24.2\text{nm}$, while the other is comprised of microparticles with a diameter of $3.2\mu m$ and a mean pore size of $7.4\text{nm}$. These two distributions were calculated from the isotherms in Figure 11.8, and seem to agree quite well with the visual inspection of the microparticles as viewed using scanning electron microscopy.

11.4.5 Sample Preparation for Electron Microscopy: Sectioning

Although the above-described techniques are quite effective for large areas and/or large ensembles of pSi microparticles, they require large sample sizes to be generated from either entire wafer surfaces or microparticles to be gathered from multiple wafers. It is therefore necessary to check a sampling of microparticles produced from each wafer by using electron microscopy in the form of scanning electron microscopy (SEM), transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) [16, 46–48].

SEM requires almost no sample preparation beyond fabrication of the microparticles because pSi is a semiconductor formed in solutions of HF and therefore
lacks any appreciable surface oxide layer that may prevent effective imaging. If an insulating oxide layer were to be formed on the surface, the SEM image would become distorted due to charging of the surface dielectric causing an electrostatic interaction with the incident electron beam [49]. Storing the particles in 2-propanol maintains this surface state, with at worst the formation of a thin native oxide layer which has a minimal effect on imaging. SEM is thus a quick but accurate technique to observe the overall microparticle shape and structural integrity, as well as allowing for the inspection of surface pore sizes, for a representative sampling from a single wafer. It is also possible to observe cross-sections of the microparticles using the SEM (Figure 11.5) by cleaving the wafers upon which the particles are fabricated before they are released [16].

Given the resolution achievable by SEM, several reasons lead to the use of high-resolution TEM (HRTEM) or STEM. One reason is to investigate the crystalline structure of the pSi layer [47], and indeed HRTEM achieves sub-Angstrom resolution by exploiting the change in phase that the electrons experience as they pass through and interact with the sample, essentially measuring the beam’s interference with itself [50]. STEM achieves the same result measuring electrons which are scattered from the sample at high angles, away from the optical axis of the beam [51].

A second reason to investigate pSi layers with high-resolution imaging is to assess the loading efficiency of primary-stage pharmaceuticals [52], secondary-stage delivery agents (such as liposomes [53]), or imaging agents such as iron oxide [54, 55]. In addition to HRTEM and STEM being able to observe loaded material at a higher resolution than SEM, STEM—by using high-angle scattering to create the image and employing a rastering beam with a 2 Å or less spot size containing electrons the kinetic energy of which is closely matched—can use electrons that are transmitted through the sample close to the optical axis for electron energy loss spectroscopy (EELS) [56]. EELS measures the change in the energy of transmitted electrons as they are inelastically scattered by the sample. Such scattering can arise from a number of sources, including phonon and plasmon excitations, but they can also occur through interband transitions in atoms which produce characteristic spectrographic signatures in the transmitted electron energies [57, 58]. These characteristic spectra can be used to identify and determine the relative abundances of different elements in the sample [57]. The effect range of EELS is capable of observing the interband transitions of lighter elements such as carbon [57], while the analysis of heavier atoms requires the use of electron dispersive X-ray spectroscopy (EDX) [59]. EDX relies on the radioactive emission from atoms excited from their ground state by the interaction with an electron from the STEM beam. When another electron from a higher energy level falls into the lower energy level that was just vacated, an X-ray is released [59]. The emission energies are characteristic of the specific energetic levels involved, and as they differ for different elements, they allow an estimation to be made of the chemical composition of the sample [59]. This technique has been employed to probe metal deposition on pSi [60]. Although SEM also has EDX capability, the penetration depth of the
SEM beam is in the low micron range [61], and so does not allow for sufficient z-resolution to differentiate thin samples from their substrates, thus precluding an accurate analysis of the sample composition. Therefore, the use of TEM and STEM, where the electron beam travels solely across the specimen, provides a more accurate characterization of the chemical composition of the sample. The 2Å spot size rastering beam of STEM also allows for minimum background noise and high-resolution x,y mapping of the sample surface.

11.4.5.1 Sample Preparation

Sample preparation for both HRTEM and STEM is significantly more complex than for SEM. The TEM sample must allow a certain degree of electron transmission in order for the electrons to be collected at the detector and form an image [62]. Conventional TEM sample preparation for hard materials such as semiconductors requires a combination of mechanical grinding, mechanical, ionic or focused ion-beam milling, or dry or wet etching [62, 63]. For pSi, the energy involved in these processes is known to lead to structural damage of the sample. However, by borrowing from a biological methodology for sample preparation, the authors’ group has developed an ultramicrotomy protocol to prepare samples for imaging [64, 65]. In the basic protocol, the sample—after being rendered into the desired state—is embedded in an epoxy resin which is cured and then shaved into nanometer-thick slices using a diamond-blade microtome [66]. In the initial protocol a formulation of Spurr resin was used [66]; this is a mixture of vinylcyclohexane dioxide (VCD), a flexibilizer made from the diglycidyl ether of polypropylene glycol (DER 786), a hardener consisting of nonenyl succinic anhydride (NSA), and an accelerator of dimethylaminoethanol (DMAE) [66]. The uncured resin is characterized by a very low viscosity that allows it to efficiently penetrate the nanosized features of the sample. The main drawback is the hydrophobic nature of the resin, which prevents the embedding of hydrated samples [66], and it is therefore necessary to dehydrate the sample first by using ethanol and then acetone. Although this method is effective for pristine porosified silicon, problems can occur when the microparticle has been loaded, as the acetone may dissolve out any loaded material that is not firmly bound in the sample. One means of alleviating this problem is to use a water-soluble embedding material such as agarose [67]. Agarose contains a crosslinkable polysaccharide that can be used in aqueous solution, but unfortunately it is quite soft, which makes it a poor choice for sectioning relatively hard silicon microparticles. It can, however, maintain the relative positions of the components within the aqueous media once it has been polymerized. The agarose can then be crushed and embedded in a firmer epoxy, such as Spurr.

When the sample has been embedded it is necessary to shape the block face down to a small area (<0.7 mm on each side). The sample is then sectioned using an ultramicrotome (e.g. the Leica-UCT) [64, 65]. The ultramicrotome uses a mechanical or thermal advance to move the epoxy block towards a stationary glass or diamond knife (in this case, a diamond knife with a 35° blade) while
simultaneously sweeping the sample vertically (perpendicular to the blade edge) [64–66]. A glass knife is used initially to smooth the block face and to supply thick sections for imaging by optical microscopy (this indicates the best location from where to take the ultrathin sections with the diamond knife) [66]. As the sample approaches the blade edge, the sweep rate of the arm slows dramatically so that a thin slice is removed. Due to the porous nature of the silicon microparticles, the block can be sectioned without damaging the blade (this would not be possible with bulk silicon). The tool is capable of stepping the arm anywhere, from many microns down to 20–30 nm [66]. The diamond blade is mounted in a chassis which can accommodate a liquid reservoir directly behind the blade [64]. When the block face has been sliced, the hydrophobic sections float on top of the reservoir in a ribbon, and may be collected by pressing a copper grid, treated by immersion in hydrochloric acid, onto the liquid’s meniscus where the sections are located [66]. The grids can then be loaded into the TEM for observation. The process of embedding and microtoming provides an effective means of preparing porous silicon TEM samples and, in a more general sense, of creating thin cross-sections of microparticles in order to examine their structure and/or the effectiveness of loading protocols. SEM can also be used to examine sections to check for loading efficiency; whether the material being loaded is sufficiently large and has good contrast, such as gold nanoparticles. Images of sectioned microparticles taken using SEM, TEM, STEM and HRTEM are shown in Figure 11.11. The loading of quantum dots in porous silica beads using fluorescent images has also been demonstrated [69].

As mentioned above, the hydrophobic nature of the epoxy and the embedding procedure may cause any loaded material to dissolve out of the pSi during the process. As an alternative to using a water-soluble embedding material, it is possible to use flash-freezing, cryosectioning and cryo-TEM to prepare and subsequently image the material [70]. If an aqueous solution can be frozen quickly enough, the water surrounding it can be vitrified, as opposed to frozen into a crystalline form that causes sample damage [70]. The usual protocol consists of plunging a small droplet of the sample containing an aqueous solution into a liquid, such as ethane, at liquid nitrogen temperature [70]. Immersion directly into liquid nitrogen is ineffective due to the Leidenfrost effect, whereby a liquid in contact with another material at a temperature much higher than its boiling point will be insulated for a certain period of time by a thin vapor film that is present due to the flash boiling of the cooler liquid at the interface [71]. This vapor film will insulate the bulk of the liquid, thus slowing down the transfer of heat along the temperature gradient [71]. The water in the aqueous solution containing the sample then has sufficient time to crystallize and damage the sample [70]. When the sample has been frozen, both ultramicrotoming and TEM can be performed under cryostatic conditions [70]. In this way, a ‘snapshot’ of the material interaction between the silicon microparticle and the material to be loaded can be achieved. This is an especially useful technique for visualizing the loading of soft material, such as liposomes, as the more standard embedding techniques are too destructive for these organic structures [72].
Having described some of the standard techniques used to characterize microparticles from a materials standpoint, it is also important to understand their behavior from a biological standpoint. This requires another set of tools and techniques, some of which will now be discussed in the context of using pSi particles as vectors for the targeted delivery of drug molecules and contrast agents.

11.5 Nanovectors for the Delivery of Therapeutics

11.5.1 Biocompatibility and Biodegradation

Silicon is the most abundant element on Earth after oxygen [73]. When in solution, silicon is not ionized but rather is present as orthosilicic acid, Si(OH)$_4$, the dissolution product of silica in water. Silicon is used for skeletal strengthening by sea sponges, and by organisms for bone development [74]. Experimentally, silicon implanted into humans has been shown to contribute to bone growth by stimulating the formation of hydroxyapatite [74]. In addition to bone, silicon is required for cartilage and connective tissue formation [75, 76].

![Figure 11.11](image-url) (a) SEM; (b) TEM; (c) STEM; and (d) HRTEM images of sectioned pSi microparticles. In (d) the crystal lattice of the pSi can be observed.
Crystallized silicon is very nonreactive and requires extremely high temperatures to become reactive. It is also known to be a nonbiocompatible material with very poor hemocompatibility [9]. However, in 1995, Canham [10] demonstrated the bioactivity of pSi layers in simulated body fluids (SBFs). Here, the term ‘bioactive’ refers to silicon as a biomaterial, which is defined as a nonviable material intended to interact with biological systems when used in a medical device. As noted by Canham, the transition of silicon to a bioactive state via the introduction of pores is consistent with the fact that all other natural biological materials are porous [77]. In Canham’s study, 1 μm-thick pSi layers were incubated in various SBFs for periods ranging from 6 h to 6 weeks. While the highly porous Si (porosity >70%) dissolved in all SBFs tested, the silicon with medium porosity (<70%) was slowly biodegradable. Similar to solid silicon, very low-porosity silicon was shown to be bioinert. Thus, porosity is directly related to bioactivity.

Other studies have also analyzed the degradation of pSi layers in SBFs [68, 78]. Both, inductively coupled plasma (ICP) and mass spectroscopy (MS) measurements of solutions containing porosified wafers were used to determine the amount of dissolved pSi from 64%, 83% and 88% porosity layers over 24 h at different pH levels. Triplicate experiments were performed on five different buffered solutions with pH values of 2, 4, 6, 7 and 8, after which ICP-MS was used to determine the amount of silicon in solution at different time points [68]. At pH 7, medium-porosity (62%) pSi showed almost no dissolution at 6 h, and very little at 24 h. Both, high (83%) and very high (88%) -porosity films showed an exponential increase in silicon dissolution over time, with a maximum of 61.3 ± 7.6% for high-porosity films and 45.5 ± 3.3% for very high-porosity films [68] (Figure 11.12). Dissolution at 6 and 24 h under different pH conditions showed a positive correla-

![Figure 11.12](image-url)
tion between dissolution and increasing pH (Figure 11.12). The low- and high-
porosity layers dissolved completely at pH 8 at 24 h, whereas at pH ≤4, little or no
dissolution was observed [68].

Ferrari’s research team has confirmed the impact of pore size on degradation
kinetics [79]. 3-Aminopropyltriethoxysilane (APTES) -modified silicon microparti-
cles, which are hemispherical in shape and have a diameter of 3.0–3.2μm, con-
tained either small (5–10 nm) or large (30–50 nm) pores. The microparticles were
incubated in phosphate-buffered saline (PBS) with shaking at 37°C. Degradation
was measured by inductively coupled plasma atomic emission spectrometry (ICP-
AES) analysis of the released silicon. While small pores particles remained intact
at 4 and 6 h, large-pore particles released 15% and 35% of their silicon content,
respectively. After 32 h, small-pore particles were 25% degraded, and large-pore
particles 85% degraded. In agreement with previous reports, the degradation
kinetics was heavily dependent on the pore size, with small-particle degrad-
ning much more slowly than their large-pore counterparts.

The hydrosilylation of pSi layers with 1-dodecyne yields a dodecenyl-terminated
surface which is completely stable for weeks in both simulated blood plasma and
alkaline solutions [78]. Another surface modification which has been shown to
slow degradation is to coat the pSi with a hydrophilic ‘stealth’ polymer, such as
poly(ethylene glycol) (PEG). In a study conducted by Godin et al. [79], the pSi was
surface modified with one of seven PEGs of which the molecular weight (MW)
ranged from 245 to 5000, in either PBS or 100% serum at 37°C with constant
shaking. The pSi was seen to degrade faster in serum than in PBS, while the MW
of the PEG correlated negatively with the degradation rate. Conjugation of the
shortest PEG (PEG-245) to the particle surface did not affect the degradation kinet-
ics in serum compared to the parent APTES pSi, but did delay pSi degradation in
PBS. pSi particles with longer PEG chains degraded within 18–24 h in serum, and
within 48 h in PBS. The most dramatic effect on degradation rate was observed
for particles coated with PEG-3400 or PEG-5000, both of which extended the life
of the particles to more than three days under these harsh conditions.

The ‘stealthing’ of delivery vehicles with PEG is also used to prolong the blood
circulation time by reducing the surface charge and creating steric hindrance,
both of which interfere with the binding and uptake of particles by cells of the
mononuclear phagocyte system, such as macrophages. One mechanism by which
PEG reduces the uptake of pSi by phagocytic cells is through reduced serum
opsonization, which mediates recognition by phagocytic cells. The main draw-
backs of this approach include a reduced ability to attach targeting ligands to the
particle surface, the involvement of distally located ligands which may accelerate
removal, and the possible elicitation of an immune response [80]. Additional
drawbacks of using PEG to limit phagocytosis include the shielding of pores
during the loading process and hindered drug release in vivo at the pathological
site. In order to enhance the therapeutic efficacy of ‘stealthed’ particles, tech-
niques aimed at causing the polymer coat to be ‘shed’ at the lesion site are being
developed [81].
For the extracellular release of drugs, a delayed degradation of the silicon particle with polymers, followed by shedding after arrival at the target site, would lead to a directed drug release [81]. The extracellular shedding of the polymer coat at the pathological site also restores interactions with target cell membranes and facilitates uptake if desired. Shedding approaches include using linkers with predetermined cleavage points between the polymer chain and anchoring moieties. Stimuli eliciting such release include proteases, pH changes and reducing agents. For example, the extracellular pH of solid tumors is significantly more acidic than that of normal tissues, leading to a selective release at the tumor site [82]. Alternatively, internalized particles encounter consecutive drops in pH as they transition from early to late endosomes, and then to lysosomes. Intracellular delivery could also benefit from linkers sensitive to protonation for the release of therapeutics. One example of a linker which is sensitive to hydrolysis of the linker bond is diorthoester [83]; at low pH, this bond forms a stable dialkoxy carbonium intermediate and leads to dissociation of the polymer. Other acid-labile linkers include vinyl ether, hydrazone, beta-thiopropionate and phosphoramidate [81]. With regards to reducing agents, a switch from an oxidizing to a reducing environment occurs as particles transition from the extracellular to the cytosolic environment. The reduction of disulfide bonds, such as a dithio-3-hexanol linker, for example, could release polymers, carrier caps or covalently attached drug molecules. The third cleavage approach—proteolysis-induced shedding—relies on enzymes present at high concentrations at the pathological site. For example, lysozymes and cathepsins are present at inflammatory sites, including cancer lesions [81].

One important issue concerning biocompatibility is the toxicity of the dissolved silicon. Fortunately, pSi degrades predominantly into monomeric silicic acid (Si(OH)$_4$) which, as stated previously, is the natural form of silicon in water. The average daily dietary intake of silicon, an essential nutrient, in the Western world is approximately 20–50 mg per day [84]. The mean daily silicon intake in men is 30–33 mg, significantly higher than for women (24–25 mg); for both men and women, the mean daily intake is decreased with age. As an example of dietary silicon content, an average beer contains 19.2 mg l$^{-1}$ silicon [85]; due to a 55% absorption rate, serum and urinary silicon levels are considerably increased following the consumption of 600 ml of beer. Fortunately, the dietary intake of silicic acid was balanced by its urinary excretion, which is highly efficient. The dietary importance of silicon was demonstrated definitively by excluding silicon from the diet of rats; this led to a growth reduction of 30–35% and to the development of bone deformations [86].

Consistent with variations in dietary silicon intake, serum silicon levels in adults vary with both gender and age. In a study of 1325 healthy volunteers [87], median serum concentrations were higher in women aged 30–44 years (11.1 μmol l$^{-1}$), as compared to women aged less than 30 years, and decreased in both men and women with increasing age (7.7 and 8.0 μmol l$^{-1}$, respectively). The decrease in silicon levels in relation to age was faster in women. For men, steady-state silicon levels (9.5 μmol l$^{-1}$) were maintained between 18 and 59 years.
Sapelkin et al. [88] cultured rat hippocampal neurons (B50 cell line) with stain-etched pSi and showed that cells were capable of adhering to the silicon surface. Portions of the crystalline silicon surface were treated, creating either square regions with pore sizes varying from 50–100 nm, or 30 μm- and 100 μm-wide stripes, separated by untreated surfaces. The cells showed a clear preference for adherence to porous regions, with growth ending abruptly at boundaries between the crystalline and porous regions. In the case of porous lines etched onto the silicon, the cells aligned along the channels. Thus, the surface topology clearly influenced cell proliferation. Khung et al. [89] further showed that SK-N-SH neuroblastoma density and morphology were dramatically influenced by pSi surface topography. The cells were sensitive to nanoscale surface topography, responding to features of less than 20 nm. Neuroblastoma cells were grown on pSi gradients, with pore sizes ranging from 5 to 3000 nm. Unlike B50 cells, the SK-N-SH cells appeared healthy and had well-spread processes when grown on either unetched flat silicon or pSi with 5 to 20 nm pore sizes. For large pore sizes, the cell morphology mirrored the structure of the pores, but for pore sizes <1000 nm (but >20 nm) the increased cell clustering and shortening of neuritic processes continued with decreasing pore size. In addition to increased clustering, the cell density was decreased with decreasing pore size (from 1000 to 20 nm), but increased on pore sizes below 20 nm; this suggested that the nanoscale surface topography had been ‘sensed’ by the growing cells.

Bayliss cultured Chinese hamster ovary (CHO) cells and rat hippocampal neurons (B50) on pSi layers in tissue culture media for four days [90]. The cell viability in terms of respiration and membrane integrity was measured using MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] and neutral red (NR) assays. Cell viability was significantly higher for B50 neurons cultured on pSi than on poly-silicon or bulk silicon, as well as control glass coverslips, supporting the nontoxicity of pSi under these conditions [90]. However, CHO cells were less viable on pSi than on crystalline silicon and glass, which suggested that coating with matrix proteins such as collagen might be important for specific populations.

In another study, primary mammalian hepatocytes isolated from a three-month-old female Lewis rat were cultured on either untreated pSi, fetal bovine serum-treated pSi or collagen-coated pSi [91]. After a 24 h incubation period, measurements of cell adhesion showed the collagen-coated pSi to be by far the optimal substrate, but after five days the cell viability in the presence of pSi was similar to that in the presence of polystyrene. The production of albumin and urea, which were respectively considered as markers of hepatocyte synthetic function and of intact nitrogen metabolic pathways, was monitored in the cell culture for 14 days and shown to be comparable to values observed in the presence of polystyrene. Taken together, these data suggest that pSi does not exhibit any significant cytotoxic effects towards primary mammalian cell lines.

Although studies to determine in-vivo biocompatibility, biodegradation and biodistribution studies are currently under way, no results have yet been presented. As mentioned above, in vitro dissolution studies have shown that silicic acid
concentrations can be controlled by adjusting the porosity of pSi [92]. The major obstacle to successful in-vivo studies is the dietary dose of silicon in animals. Therapeutic doses of drug-carrying pSi are two to three orders of magnitude lower than the dietary intake, and therefore administered silicon is difficult to detect accurately in biodistribution assays. Whilst radioactive silicon may help to overcome this detection issue, the half-life of $^{31}$Si (the only isotope that can be obtained through the irradiation of Si) is 157.3 min, and clearly inadequate for long-term studies. The production of single-crystal silicon wafers enriched with more stable Si radioactive isotopes is, as yet, unheard of. Studies of the biodistribution path of other elements (i.e. fluorescent molecules), either loaded into or covalently attached to the pSi particle, represent an indirect alternative that is currently being used to measure the biodistribution of silicon.

Reports on the use of $^{32}$P-doped pSi (Brachysil®) for brachytherapy in non-resectable hepatocellular carcinoma have so far accounted for the first—and only—published study of an in-human use of pSi particles [93]. A collection of phosphate-doped 20$\mu$m pSi particles were neutron-irradiated to obtain a significant increase in the level of radioactive $^{32}$P. Previous animal model studies have established that nonirradiated particles were biologically inert; irradiated $^{32}$P pSi implantation into pig livers also failed to show any systemic toxicity, with minimal leakage. For human studies, all eight subjects considered for a clinical trial were characterized by the Eastern Cooperative Oncology Group performance status 0–2, Okuda Stage I and II, life expectancy greater than 12 weeks, as well as adequate hematologic, renal and hepatic functions. Any patients with encephalopathy, prior radiotherapy, significant history of cardiac disease or a serious, active infection were excluded. A maximum of three tumors per patient was identified and monitored, after which patients were scheduled to receive intratumoral implantation of Brachysil under radiologic imaging guidance and local anesthesia. None of the patients had any detectable radioactivity levels in their blood immediately after implantation, which showed that the device had remained in situ, without systemic dissemination. The 24-week follow-up showed no adverse events related to the therapeutic formulation, and the authors concluded that, ‘The lack of any serious adverse events, and the absence of systemic leakage, indicate that this device is relatively safe for use in clinical settings’. All target tumors showed a size reduction at 12 weeks after implantation, with 100% regression in two patients. Further tumor size reductions were observed in four patients at 24 weeks. The final assessment, at 24 weeks, showed two complete responses, two partial responses, three stable diseases, and one progressive disease.

As with all therapeutic applications, aseptic techniques are necessary for production. It has been shown, using in vitro cell cultures, that both bacteria and fungi will readily colonize pSi, thus establishing a need for sterilization prior to their use in clinical applications [77]. Although some preliminary studies on pSi sterilization, including autoclaving, have been presented, few reported data are available. Current clinical applications, which are limited to BioSilicon carriers, use irradiated products and a sterile formulant [93].
11.5.2
Drug Loading and Quantification of Drug Load

A variety of techniques have been used to load drugs into pSi mesopores, including the immersion of particles into a loading solution containing an appropriate solvent for the target drug [94–96]. Another method is to load dry pSi particles or chips with a drug solution by capillary action; this is also known as the incipient wetness method [97–99]. Drying pSi particles has been achieved by freeze or vacuum drying [96, 100] as well as thermal drying [52]. While these loading techniques may appear simple, factors such as pH dependency, time and temperature, as well as interactions between the drug and the particles, the drug and the solvent, and the solvent and the particles, must all be taken into account. Retention of the drug occurs by a nontrivial combination of electrostatic attraction, van der Waals forces, H-bonding and hydrophobic interactions, depending on the pSi surface chemistry and the drug choice. pSi can be made hydrophobic for the loading of hydrophobic drugs, such as dexamethasone; conversely, the loading of hydrophilic drugs may be aided by an appropriate surfactant [99]. Once loaded, a balance between sufficient washing and drug retention must be achieved whilst at the same time releasing the surface fraction.

A more irreversible loading technique involves entrapment by volume expansion [55]. For example, an iron oxide nanoparticle payload was loaded into pSi and then locked into place by oxidation of the pSi. When silicon is oxidized to SiO₂, a volume expansion occurs which shrinks the pores and traps the payload. This procedure involves loading nanoparticles immersed in a basic aqueous solution and then drying the sample in an oven (both of these steps may contribute to oxidation). Another loading technique is covalent attachment of the payload, in which the hydrosilylation layer on oxidized silicon provides a means for using bioconjugation chemistry. As an example, in order to attach a payload to pSi, it might be possible to react the oxidized pSi with a carboxyalkene to create functional carboxyl units on the pSi surface. The final result of loading is pSi particles containing a payload, the release of which is dependent upon degradation of the matrix. An advantage of the more stable loading techniques is that the loaded pSi can be more vigorously washed, thus facilitating release of the surface fraction.

A unique attribute of pSi is the ability to optically report on the loading of a molecule within the porous nanostructure. The optical interference spectrum from multilayers has been used in sensor applications, where changes in the optical reflectivity spectrum indicate the capture of a chemical or biological species [101–106]. However, these properties can also be used to monitor the release of a drug loaded into pSi. The Fourier transform of the optical reflectivity spectrum provides a simple means of monitoring the drug loading and release [107] since, when the loading has been completed, both the quantity and chemical purity of the drug can be determined using high-performance liquid chromatography (HPLC) analysis. For this, two different methods can be used. In the first method,
any depletion in the drug concentration of the loading solution can be determined during the loading procedure. In the second method, the drug concentration is determined after complete extraction of the drug residing in the PSi particles [97]. In addition, as noted in Section 11.4.3, high-resolution imaging (e.g. STEM and EELS) can be used to visualize and quantitate the sample loading.

In a detailed in vitro study, Salonen and coworkers studied the loading of five model drugs into pSi particles, and their subsequent release [52]. Here, antipyrene, ibuprofen, griseofulvin, ranitidine and furosemide were chosen to represent a wide range of solubilities, as well as different acid–base characteristics and lipophilicity. The pSi surface was treated with a two-step thermal carbonization process in order to obtain a chemically stable hydrophilic Si–C interface. The drugs were then loaded in different solutions and conditions due to their different solubility characteristics. The loaded particles were filtered and dried at high temperature (65–105 °C) for 1 h. The characterization of the drug loading was performed using techniques which included thermogravimetry, differential scanning calorimetry, helium pycnometry, N₂ desorption, X-ray diffraction and HPLC. Such use of multiple techniques allowed the investigators not only to obtain more reliable results but also to localize the drug in the pores, or on the surface of the microparticle. Drug release was studied at different pH values (5.5, 6.8, 7.4) and quantified through HPLC. The five drugs each showed different loading capabilities and release profiles:

- **Antipyrene:** The average load of antipyrene, a highly soluble drug, was 53.4% (w/w) after 1.5 h in aqueous solution, but 14.5% of the loaded drug was in crystalline form on the surface. A rapid early release, which slowed after 5 min, was due to the dissociation of surface antipyrene. A pH-independent dissolution of 80% of the loaded drug occurred at 75 min, while unloaded drug required 45 min for 80% dissolution.

- **Ibuprofen:** The average ibuprofen load, in ethanol after 1 h, was 30.4%, with a minimal amount of crystallized drug on the surface. The lower loading efficiency and negligible presence on the surface were expected due to the lower solubility of ibuprofen. Although ibuprofen solubility is pH-dependent, dissolution of the loaded drug was less pH-dependent than unloaded ibuprofen, and the release profile of loaded ibuprofen was similar to that of loaded antipyrene.

- **Griseofulvin:** The griseofulvin loading efficiency was 12.4–16.5%, with minimal traces on the surface; this was an extremely good result considering the poor solubility of the molecule. Griseofulvin solubility is not pH-dependent while loaded, however, griseofulvin release was pH-dependent, which suggested that the enhanced release at high pH might be due to effects of the pH on surface interaction between the pSi and the drug, or to an enlargement of the pores at high pH due to surface degradation.

- **Furosemide:** Furosemide, which also is poorly soluble in aqueous media, had an average load of 41.3%, with no drug crystallized on the surface. Its release at pH 5.5 was significantly improved by loading into porous silicon. At pH 6.8 and 7.4,
furosemide release was still slightly increased by loading, as was the case for the other poorly soluble molecules.

- Ranitidine: Surprisingly, despite the high aqueous solubility of ranitidine, the average load was only 13.2%, with no drug crystallized on the surface. As in the case of antipyrine, loading of the drug into porous silicon particle slowed the release rate of this highly soluble molecule.

The results of this study indicated that the chemical nature of the drug and the loading solution were critical for efficient loading. Clearly, the surface properties constitute an essential aspect in the design of porous silicon particles for drug delivery, and there is great potential for tailoring the surface properties of silicon to suit the compound being loaded. The release rate of the pSi-loaded drug was dependent on the solubility of the drug molecule. For highly soluble molecules, the pSi loading caused a slightly delayed release with respect to the unloaded drugs, whereas for poorly soluble drugs their dissolution was significantly improved by loading. In addition, loading in general reduced any pH-dependence of dissolution. Another important result of this study was the direct observation and quantification of actual loading of the drug inside the silicon matrix pores. The advantages of having the drug on the surface of the silicon particle are extremely limited, both in terms of sustained release enhancement and of protection from physiological biobarriers. It is necessary, therefore, to achieve a significant pore loading in order to fully exploit any advantages of pSi as a drug carrier.

PSi loading and release of a model protein have also been studied [108]. Here, aqueous solutions containing papain at different concentrations (1–10%, w/w) were incubated with pSi particles that had been presoaked in methanol to enhance loading. The particles were then washed, and a portion of the loaded particles examined using Fourier transform infrared (FTIR) analysis to assess the loading level. The FTIR analysis was used to characterize the chemical state of the pSi loaded with papain by monitoring any absorbance that was correlated to specific functional groups. The remaining particles were transferred to PBS, at 37°C, and at multiple time points the PBS was collected for the quantitation of released papain. FTIR analysis showed a decrease in the Si–H signal which indicated a strong physical binding or chemisorption between the Si–H groups and specific functional groups in the papain. Absorbance at 2100 cm⁻¹ due to Si–H stretching decreased with increasing papain solution concentration, which suggested an increased loading level. A significant initial burst release was observed that indicated the presence of surface-bound papain. Increasing the loading level of papain decreased the initial release percentage, suggesting that with increasing protein concentration a greater proportion of papain was being loaded into the pores. After the initial burst, a linear relationship between papain release and time was observed, suggesting a zero-order release. The results of this study support pore internalization of the protein through FTIR analysis, as well as the presence of a possible loading interaction mechanism and how this reflects on the release profile. Also, the selected surface termination of the silicon, and its interaction
with the loaded moiety, was also indicated as an extremely important factor affecting loading.

In the future, additional knowledge regarding the loading process itself will undoubtedly be needed. Typically, some drugs seem to be easily loaded into pSi, while the loading of other drugs is quite complicated, especially when the behavior is not dictated solely by the surface chemistry of the pore walls. In summary, the use of alternative solvents, an optimized pH value and an appropriate temperature may enhance loading efficiency, especially when considering hydrophobic drugs and the loading of nanoparticles such as liposomes.

11.5.3 Nanovectors for the Delivery of Therapeutics

Nanovectors are drug delivery vehicles engineered with details and features in the nanosize range. These nanovectors are designed to optimize delivery to pathological sites and to provide a sustained, controlled release of drug therapies. Alternatively (or simultaneously), nanovectors can deliver concentrated payloads of contrast agents that can be used to image the pathological site and/or monitor drug delivery [109]. The localization, controlled release and monitoring of drug delivery within the body represent key challenges in the design of effective, targeted drug therapies [110–116].

In its simplest embodiment, a nanovector comprises the particle and the biologically active principle it carries (Figure 11.13a). Experience with particulates, such as liposomes, has shown that nanovectors of dimensions between 10 and 1000 nm are cleared very rapidly from the bloodstream by means of uptake by phagocytic cells of the RES [117]. In order to decrease the clearance time, and thereby allow

![Figure 11.13](image-url)
longer circulation time and improve localization at the desired target location, vectors can be surface-decorated with PEG or other shielding moieties, as described previously [118]. A nanoparticle comprising a drug and a stealth layer is defined as a second-generation nanovector (Figure 11.13b). The liposomal drug formulations that are currently available in the clinic belong to this class of nanovectors. First- and second-generation nanovectors accumulate at the tumor site by escaping the vascular compartment through fenestrations’ these are openings that characterize the tumor-associated, neovascular endothelium and render the blood vessels hyperpermeable compared to the normal vasculature. This passive localization mechanism is known as enhanced permeation and retention (EPR).

During the past two decades, several different strategies have been proposed to add biological targeting capabilities to nanovectors [119]. The dominant strategy involves the conjugation of targeting moieties to the surface of the nanovector, including antibodies [120], ligands [121], aptamers [122] and small peptides [123]. Targeting has been directed towards cancer cell-surface markers and to molecules expressed in the tumor microenvironment, most notably on the tumor-associated vascular endothelium.

After loading with drugs, the pores in the nanoparticles can be closed by constructing appropriate cap structures (Figure 11.14). The ability to control the release of anticancer drugs provides mesoporous silicon nanoparticles with advantages over other drug delivery systems, such as liposomal particles or albumin-based nanoparticles [124]. For example, environmentally responsive nanovectors are designed to release their therapeutic payload upon encountering external conditions that are associated with cancers. For instance, pH-sensitive polymers exist that become destabilized in the acidic environments of tumor lesions [125, 126], leading to a localized release of the drug cargo (Figure 11.15a). Tumor-associated enzymes, such as matrix metalloproteinases, may also be employed for preferential release at lesions that present a markedly invasive phenotype [128] (Figure 11.15b).

11.5.4 Towards a Multi-Stage Drug Delivery System

In order to develop the optimal drug delivery system it is important to consider the localization and negotiation of biological barriers. Following injection, therapeutic agents encounter a multiplicity of biological barriers that adversely impact their ability to reach the intended target at the desired concentrations [129]. Barriers of epithelial and endothelial nature, such as blood vessel walls and the blood–brain barrier, are among the most common examples of biobarrriers to therapeutic action (Figure 11.16). These barriers are based on tight-junctions, which either prevent or limit the paracellular transport of agents. Endothelial/epithelial barriers are themselves multiplex and sequential in nature.

An entirely different biobARRIER against the penetration of therapeutic agents, especially if they are formulated as large particulates, is the increased, adverse osmotic oncotic pressure developed by cancer lesions during their growth (Figure
Recent studies have shown that the hemodynamics within cancer lesions constitutes another type of barrier to particle localization [21]. Delivery vehicles of different shapes and sizes can offer a dramatic increase in therapeutic index, by optimizing their properties of margination, extravasation, firm adhesion to the vascular endothelium and control of phagocytic uptake.

In response to the need to bypass multiple biological barriers, third-generation nanovectors are being introduced which perform multiple functions in a sequential fashion. These nanovectors contain all the characteristics of the previous two generations—that is, they contain a therapeutic payload and have targeting and shielding moieties on their surfaces—but they are also able to perform a time sequence of functions which involves multiparticle coordination (see Figure 11.13c). An example of a third-generation nanovector is the ‘nanoshuttle’, which is a cluster of bacteriophage-entrapped metal nanoparticles [123]. The multistage systems which recently were demonstrated represent another example of
Figure 11.15  (a) pH-responsive particles (blue) retain their payload while in the normal circulation. At the site of the tumor lesion, the characteristic enlarged fenestrations (leaky vessels) favor extravasation of the particles into the tumor tissues. After diffusion and penetration into the tumor mass, the conditions of the tumor microenvironment (acidic pH) trigger the release of the therapeutic payload; (b) Another release mechanism is enzymatic cleavage or dissociation of the particle, for example, via overexpression of matrix metalloproteinases in highly invasive tumors.

Figure 11.16  Major obstacles exist that prevent the majority of the injected drug from reaching the intended target. An efficient drug delivery system must be able to negotiate each of these obstacles in order to localize the therapeutic action at the target site. The nature of these biological barriers is sequential, and a delivery vector requires multiple layers of function in order to overcome each of these barriers. Examples of biological barriers are: reticuloendothelial system (RES) uptake, enzymatic degradation, hemorheology, endothelial barriers and the tumor-associated osmotic and interstitial pressures. Adapted from Ref. [154].
third-generation nanovectors [16]. As illustrated in Figure 11.17, these comprise a first-stage module that houses groups of different nanoparticles (Figure 11.17a); the particles then dock on the target vessel walls (Figure 11.17b) where they release the second-stage nanoparticles (Figure 11.17c). The second-stage nanoparticles can be released with different kinetics and rates in accordance with their structure, size, shape and chemical composition [16]. Complete drug release is accomplished
by the degradation of first-stage particles into biologically benign components (Figure 11.17d). The multistage strategy, summarized in Figure 11.17 combines the ability to perform sequential functions, offers opportunities to negotiate multiple, serially presented biological barriers, and therefore opens new frontiers in drug delivery [16].

11.6 Cellular Uptake of pSi Particles

As stated previously, pSi particles are targets for internalization by cells of the mononuclear phagocyte system, and ‘stealthing’ with PEG delays their uptake. For third-generation delivery systems, the first level of targeting for intravascularly administered particulates is the vascular endothelium. In vitro, vascular endothelial cells are able to internalize micron-sized pSi particles by phagocytosis and macropinocytosis (R.E. Serda et al., unpublished results). This is more complicated in vivo, where serum opsonization coats the microparticles and alters their ability to adhere to the vascular wall. In this section we describe cellular uptake of pSi nanoparticles and microparticles, and examine the characteristics of pSi microparticles which alter this phenomenon.

11.6.1 Tumor Microenvironment

In order to target cancer lesions, an understanding of the microenvironment is essential for the design of the delivery vector. For instance, a clear association exists between chronic inflammation and malignant transformation. As an example, inflammatory bowel disease and Helicobacter pylori are associated with high rates of colon and gastric cancer, respectively [130]. Also, the relative risk of prostate cancer is increased in men with a history of sexually transmitted disease and prostatitis [131]. In fact, 15% of all cancers are associated with microbially induced inflammation [132]. In breast carcinogenesis, affinity mature B cells are found in tumor-associated stroma and secondary lymphoid tissues, and they are enriched in sentinel lymph nodes [133]. The proportions of B cells in sentinel and axillary lymph nodes of breast cancer patients correlate with increases in disease stage and total tumor burden [134, 135]. The high incidence of chronic inflammation in cancer explains why the long-term use of non-steroidal anti-inflammatory drugs is correlated with a decreased risk of various types of cancer, including colorectal and esophageal adenocarcinoma [136, 137].

Inflammatory mediators released by resident neutrophils and mast cells in solid tumors attract migrating monocytes, which differentiate into macrophages. Local chemokines and cytokines activate macrophages, which become phagocytic and release additional inflammatory mediators. When mice deficient in macrophages were crossed with mice predisposed to mammary cancer, the rate of tumor pro-
Progression decreased and metastasis was inhibited [138]. This was most likely due to the positive role of tumor associated macrophages in stimulation of tumor growth and progression [139]. An example of inflammatory cytokine secretion by macrophages is the release of tumor necrosis factor-α (TNF-α), which increases the surface expression of adhesion molecules on endothelial cells, including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and endothelium leukocyte adhesion molecule-1 (ELAM-1) [140]. Enhanced numbers of adhesion molecules enhance the capture of leukocytes [141, 142], and may also increase the adhesion and capture of pSi particles (i.e. drug delivery vehicles).

The luminal surfaces of blood vessels contain a monolayer of endothelial cells that govern vascular tone and create a blood–tissue interface/barrier. As most drugs lack an affinity for endothelial cells, the endothelium is an important therapeutic target for delivery vectors. Endothelial cells are diverse in nature, and an understanding of their surface determinants guides the selection of targeting ligands that decorate the nanovector. The optimized treatment for cardiovascular and cancer pathologies benefits from a knowledge of endothelial receptor expression induced under inflammatory conditions. As stated previously, targeting ligands include antibodies, peptides and thioaptamers; these ligands target receptors for growth factors, cell adhesion molecules and transport proteins. Currently, ‘Zip codes’ are being identified which describe the molecular topography of surface determinants for defined vascular areas [143]. That is, addresses are being defined based on endothelial surface determinants expressed at pathological lesion sites. Ligands which recognize these addresses bind to the respective surface receptors, and can either internalize adherent delivery vehicles into cells through endocytic pathways, or provide prolonged residence on the surface, allowing time-dependent drug release.

11.6.2 Effect of Microparticle Shape on Margination

Theoretical models [21, 25], recently corroborated by experimental results [26], have shown that nonspherical objects have superior margination and endothelial adhesion properties compared to spherical objects of the same characteristic size. These findings give nonspherical particles significant advantages over their spherical counterparts. Hemispherical pSi particles have been fabricated as drug delivery vehicles (Figure 11.18) [16]. Enhanced margination improves the ability of particles to sense the endothelium and detect biological and biophysical aberrations, such as the overexpression of specific antigens or the presence of openings and fenestrations in the endothelium. Better margination abilities also enhance the particle’s ability to leave the larger for the smaller vessels at branching points, resulting in a more complete exploration of the vascular system. As stated above, an enhanced adhesion of microparticles to the endothelium at the pathological site either permits the extracellular release of a payload or facilitates cellular uptake.
11.6.3 Effect of Microparticle Size on Cellular Uptake

As demonstrated by Chung et al. [144], human mesenchymal stem cell internalization of pSi nanoparticles (108–115 nm) can be regulated by altering the degree of positive surface charge on the nanoparticles. For example, increasing the positive charge on pSi nanoparticles, from a zeta potential of 15.6 to 19.5, caused a significant increase in nanoparticle uptake in serum-free conditions. However, a change in zeta potential from −4.90 to 15.6 did not significantly alter the number of nanoparticles internalized.

When using human umbilical endothelial cells (HUVECs) as a model of the vascular endothelium, hemispherical pSi microparticles were shown to be inter-

Figure 11.18 (a, b) Scanning electron micrographs showing phagocytosis of pSi hemispherical microparticles by endothelial cells (HUVECs). HUVECs were incubated with pSi microparticles for either 15 min (a) or 60 min (b) at 37°C in serum-free media; (c) Confocal micrographs showing HUVECs with internalized pSi microparticles (green) after incubation for 60 min at 37°C.
nalized in a process characterized by the formation of pseudopodia—that is, the extension of rope-like protrusions from the cell surface (Figure 11.18a). Within 1 h, the HUVECs had completely internalized multiple pSi particles (Figure 11.18 b and c). Two sizes of microparticles, with diameters of 1.6 and 3.2 μm, were both shown to be internalized via an actin-dependent process. While the uptake of larger particles was consistent with classical phagocytosis, with the hallmark image of an actin cup holding a microparticle, the smaller particles were internalized by both phagocytosis and macropinocytosis. Macropinocytosis, similar to phagocytosis, is actin-dependent, but results in the bulk uptake of both fluid and solid cargo [145]. Support for macropinocytosis as a mechanism utilized by endothelial cells is derived from a study conducted by Hartig et al. [146], in which nonspecifically bound nanoparticulate complexes were taken up by microvascular endothelial cells via macropinocytosis. The role of macropinocytosis in pSi microparticle uptake of 1.6μm particles is supported by an enhanced uptake of fluorescein isothiocyanate (FITC) dextran from the cell media (Figure 11.19).

Based on TEM analysis, HUVECs incubated with 1.6μm oxidized pSi microparticles for 2 h at 37°C show microparticles located in the perinuclear region of the cell (Figure 11.20a) indicative of microtubule-based transport [147]. A clear membrane can be seen surrounding an internalized 1.6μm pSi particle. The larger 3.2μm pSi particles, on the other hand, are more disperse and the majority of the microparticles show no evidence of membrane enclosure (Figure 11.20b). When pSi microparticles were labeled with DyLight 488 NHS ester (Pierce) to give them green fluorescent properties, a large number of the 1.6μm particles colocalized with acidic vesicles (labeled with Lysotracker Red; Invitrogen) at 2 h. However, only a few of the 3.2μm particles colocalized with acidic vesicles, supporting a cytoplasmic localization for the majority of the larger particles (R.E. Serda et al. unpublished results).
Slowing et al. [148] reported that mesoporous silica nanoparticles are internalized by human cervical cancer cells (HeLa) within 1 h in D-10 growth media. For pSi particles ranging from 150 to 300 nm, smaller particles were taken up more efficiently. To examine the endosomal escape of pSi nanoparticles, cells were stained with a red fluorescent endosomal marker (FM 4-64). The more negatively charged silica nanoparticles were able to escape from endosomes within 6 h, while the more positively charged particles remained trapped within endosomes. This effect was attributed to the ‘proton sponge effect’, in which the negative charge on the particles could buffer the acidic environment and disrupt endosomal function.

A comparison of cellular uptake of mesoporous silica nanoparticles and microparticles was performed using dendritic cells [149]. Two sizes of particles, namely 270 nm (AMS-6) and 2.5 μm (AMS-8), were incubated with dendritic cells in
Porous Silicon Particles for Imaging and Therapy of Cancer

culture medium for 24–48 h. For both sizes, the majority of cells had internalized the particles within 60 min. Based on TEM analysis, AMS-6 nanoparticles were encapsulated into vesicular compartments, while the larger microparticles appeared to lack membrane enclosure. Clearly, for drug therapy the intracellular release of delivery vehicles and their therapeutic payload is highly desirable; hence, a more detailed study relating particle size, charge and temporal endosomal release of particles is required.

The degree of apoptosis and necrosis in dendritic cells with internalized pSi particles was measured using the Annexin V-fluorescein and inclusion/exclusion of propidium iodide. Only at the highest concentration (50 μg ml\(^{-1}\)) was any decrease in cell viability seen at 24 h (viabilities: control 89%, AMS-6 81%, AMS-8 69%). However, the larger AMS-8 particles induced the expression of activation (costimulatory) markers at 24 h, suggesting that pSi microparticles might induce specific immune regulatory signals in dendritic cells. The nature of the immune signals is currently unknown, and whether they elicit proinflammatory or anti-inflammatory responses is yet to be determined.

11.6.4 Effect of Surface Modification on pSi Particle Uptake

Since attractive and resistive forces govern the binding and uptake of microparticles, the effect of surface chemistry on the uptake of pSi microparticles by HUVECs was investigated. Three classes of microparticle were examined: (i) negatively charged oxidized microparticles; (ii) positively charged APTES-modified microparticles; and (iii) methoxy poly(ethylene glycol) (mPEG)-5000-modified microparticles. Both, oxidized and APTES-modified pSi microparticles, were internalized under serum-free conditions by actin-dependent phagocytosis/macropinocytosis. Therefore, the binding of pSi microparticles to the cell membrane is not mediated solely by electrostatic interactions. As seen by others, PEGylated pSi microparticles are less able to be internalized.

In a separate study, the effect of surface charge, as well as labeling pSi nanoparticles with a cancer-specific targeting ligand, was examined by attaching various functional groups to the surface of pSi nanoparticles [23]. Nonfunctionalized, negatively charged silica nanoparticles were taken up by HeLa cells via nonspecific adsorptive endocytosis in serum-free media. On the other hand, the uptake of N-folate-3-aminopropyl pSi particles was sensitive to the addition of folic acid to the culture media, thus supporting a role for folic acid receptor-mediated endocytosis. Similar to studies with HUVECs in serum-free media, all particles were internalized, although the mechanism varied. Fluorescein (−34 mV) and folate (+13 mV) grafted particles were internalized by a clathrin-dependent mechanism, 3-aminopropyl (−5 mV) and guanidinopropyl (−3 mV) particle uptake was caveolae-dependent, and 3-[N-(2-guanidinoethyl)guanidine]propyl (+0.5 mV) uptake was via an unidentified mechanism.

With regards to other types of nanoparticles, a recent in vivo study showed that cationic liposomes have a propensity for localizing in tumor vessels [150]. Increas-
ing the cationic charge on PEGylated liposomes lowered uptake by the spleen and increased uptake by the liver. While the overall localization of liposomes in tumor cells in a severe combined immunodeficiency (SCID) mouse model did not increase, there was an increase in the accumulation of liposomes in tumor-associated blood vessels. Using a dorsal window chamber to view tumors, liposomes containing 10 and 50 mol% of cationic lipids were associated with 14 and 27% of the vascular area, respectively. These findings suggest that the physicochemical properties of drug carriers (e.g. liposomes and pSi particles) should be optimized to exploit the physiological features of the tumor and thereby enhance vascular targeting.

11.6.5 Serum Opsonization Inhibits Uptake of Oxidized pSi Microparticles

The results of several in vivo studies have suggested that the pattern of opsonins adsorbed to the surface of particulates determines the population of phagocytic cells responsible for their clearance [151]. For example, plasma protein adsorption by poly(D,L-lactic acid) nanoparticles enhances uptake by monocytes, while decreasing binding to lymphocytes [152]. In a recent study, regardless of the initial surface functionalization (oxidized, APTES or mPEG), all serum-opsonized pSi microparticles had a negative surface charge based on zeta potential measurements. The internalization of opsonized oxidized microparticles by HUVECs was inhibited (ca. 70%) compared to uptake under serum-free conditions. However, serum opsonization failed to have any impact on the internalization of APTES-modified microparticles. Serum opsonization also inhibited the uptake of negatively charged 1 μm polystyrene microparticles by HUVECs. Similar to the in vivo data described above, these data also support preferred vascular uptake of cationic particles in the presence of serum.

11.7 Cancer Imaging

Si-han Wu (2008) [153] demonstrated imaging capabilities for mesoporous silicon particles by fusing FITC-conjugated mesoporous silicon nanoparticles with Fe₃O₄·SiO₂ nanoparticles (1 MSN:1 Fe₃O₄·SiO₂). Their product, known as ‘Mag-Dye·MSNs’, possessed magnetic resonance imaging (MRI)-enhancing, luminescent and porous properties. The iron content of the Mag-Dye·MSNs was 1 wt%, with a T₂ relaxivity (r₂) of 153 μM⁻¹s⁻¹. Within 1 h of incubation with 40 μg ml⁻¹ Mag-Dye·MSNs in serum-free media, more than 90% of NIH 3T3 fibroblasts and rat bone marrow stromal cells were labeled. Confocal images showed that the fused nanoparticles were internalized and localized around the nucleus.

In the same study, Wu et al. [153] showed contrast agent in the liver of mice by MRI as soon as 5 min after the administration of Mag-Dye·MSNs. Immunohistochemical staining and Perl’s Prussian blue staining of liver sections demonstrated
a limited uptake by macrophages. In addition, the perfusion of mice at 30 min after injection of Mag-Dye·MSNs showed reduced fluorescent foci, indicating that the Mag-Dye·MSNs may still be located in the blood vessels at this early time point. One unsettling finding was that MR contrast in the liver lasted for up to 90 days, although whether the Mag-Dye·MSNs were still intact or only the contrast agent remained was not addressed. This study emphasizes the need for further in vivo studies of biocompatibility and biodegradation, which are currently under way.

11.8 Conclusions

A thorough knowledge of silicon’s fundamental physico-chemical properties, its relative ease of production, limited costs, high scalability and solid—as well as extensively developed—manufacturing technology, makes pSi drug delivery vehicles good candidates for the mass production required for clinically available applications. The high flexibility in system design can accommodate a multitude of delivery mechanisms and payloads, which represents a significant advantage compared to many currently explored alternatives.

Ferrari’s vision of multistage carriers, where each stage performs part of the journey from the site of administration to the cancer cell, negotiating one or more biological barriers, and adding a degree of targeting selectivity in each step, is a promising approach. The biological selectivity comprising cell target recognition by surface molecules, as well as mathematics-based rational design of the size, shape and physical properties of the vector particles, enhance the probability of recognition of the target cell—a synergy between molecular biology, physics, engineering and mathematics.

References

References


References


75 Schwarz, K. (1973) A bound form of silicon in glycosaminoglycans and polyuronides. Proceedings of the National
11 Porous Silicon Particles for Imaging and Therapy of Cancer

Academy of Sciences of the United States of America, 70, 1608–12.


HPLC evaluation for controlled gentamicin drug delivery. *Journal of Controlled Release*, 97, 125–32.


Wernicke, M. (1975) Quantitative morphologic assessment of immunoreactivity in regional lymph


Keywords

silicon; porous; microparticles; nanoparticles; drug; delivery