Porous microspheres: Synthesis, characterisation and applications in pharmaceutical & medical fields

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ABSTRACT

Porous microspheres have interconnective external and internal pores leading to very low mass density and enormous specific surface area, enabling them to have excellent adsorption capabilities. Due to this uniqueness over traditional microsphere, they find extensive pharmaceutical applications. Porous microspheres are very promising for gastro retentive drug delivery, alveoli targeted drug delivery, high-speed chromatography, development of tissue regeneration scaffolds and as carrier of biopharmaceuticals. Pore structure and porosity are the main factors that govern their applications. There are different well-established methods for their synthesis, like seed swelling, solvent evaporation, polymerization, spray drying and phase separation. But most of the methods are time consuming and consists of numbers of complicated steps. The size, shape and pore structure of the particles depend on many experimental variables like temperature, pH, stirring speed, type and concentration of porogen, polymer and its concentration. Thus, synthesis of porous microparticle with predefined porosity is really challenging.

1. Introduction

Porous microspheres are identified as suitable materials in 1990s as carriers for drug delivery system. When compared with nonporous microspheres, they are unique regarding drug entrapment and release characteristics (Crofts and Park, 1995). Active substances are generally either adsorbed on the surface or dispersed throughout the matrix of microparticle. Capacity efficiency as well as release kinetics of drugs depends upon the porosity of the porous microspheres (Yu et al., 2014).

Traditional microspheres are either microcapsule or monolithic particles having diameter 1–1000 µm (Zdravkov et al., 2007). In microparticles, the cargo molecule is encapsulated at the core and the shell is made up of matrix material; whereas in monolithic particles the cargo molecule is dispersed within the matrix. The density of nonporous traditional microspheres depends upon the density of matrix material itself, whereas the specific surface area is determined by the size of the particles. Specific surface area increases with lowering of particle size. These limits the applications of traditional microspheres where large particles with low density and higher specific area are desirable e.g. pulmonary and gastro-retentive drug delivery. In case of porous microsphere, low density and large specific surface area are the inherent properties. These unique properties make them suitable as a carrier for pulmonary and gastro-retentive drug delivery. The external pores are present on the surface of the particles and internal pores are present within the core (Fig. 1). These pores are usually interconnected (Sing et al., 1985).

The number of pores, pore diameter and structure of pore mostly affect the properties of porous microspheres. According to IUPAC, porous materials can be classified as per their pore size (width) as Microporous materials (< 2 nm), Mesoporous materials (2–50 nm), Macroporous materials (> 50 nm) (Gao et al., 2010). Compared with small porous microspheres, large porous microspheres are...
characterized by large geometric diameters but low density and small aerodynamic diameters. They exhibit ideal lung deposition profiles (Cheng et al., 2009). Main criteria for the applications of the porous microspheres are based on the porosities, pore sizes, and surface area. These parameters determine the entrapment efficiency and release kinetics of entrapped molecules (Cai et al., 2013). Again, in the field of enzyme immobilization, the pore size of heterogeneous support is probably the most important parameter. It determines adsorption and release of biomolecules from porous microspheres. Generally, pore size 3–5 times than that of protein is considered ideal (Cheng et al., 2009). Hence, for appropriate and accurate application of the porous microspheres, pore structure should be precisely controlled (Nie et al., 2015). Porogens are most commonly used to control the pore size. The surface pore can be controlled by adjusting the temperature, pH and other factors related to method of synthesis (Cai et al., 2013).

2. Methods for synthesis of porous microspheres

The summary of different methods for synthesis of porous microspheres are shown in Table 1.

The synthesis of porous microspheres is typically accomplished by the following methods.

2.1. Solvent evaporation method

The schematic diagram is shown in Fig. 2. Polymers like PLGA is dissolved in non-polar organic solvents like di-chloro-methane to prepare oil phase solution. Nanoparticle of solids like hydroxyapatite, silicon dioxide is dispersed in the oil phase to prepare S/O suspension. This suspension emulsified into an aqueous phase (W1) containing emulsifier like polyvinyl alcohol (PVA) to obtain S/O/W1 emulsion. This S/O/W1 emulsion is poured into the second water phase (W2) containing PVA and salt like sodium chloride (NaCl) at M concentration. Due to salting out effect of NaCl, S/O/W1/W2 is formed. With continuous stirring organic medium starts to diffuse from the S/O/W1 droplet to W2 phase and polymer solidifies. With progress in the diffusion, S/O/W1 emulsion droplets becomes unstable due to increase in polymer concentration and relative volume of W1 phase in the droplet. So, nanoparticles in polymer droplet come out due of shrinkage of polymer chain and get adsorbed at the surface to stabilize the S/O/W1 emulsion. Upon complete diffusion of organic phase, the polymer gets dried into fine particles having pores on the surface. After removal of W1 phase the porous particles are separated and dried under vacuum (Takai et al., 2011).

2.2. Polymerization method

The schematic diagram is shown in Fig. 3. In this method polymers like methyl methacrylate or 2-hydroxyethyl methacrylate are dissolved in two different oil phases. The oil having higher hydrophobicity is emulsified using membrane emulsification technique to prepare uniform seed droplet. The other oil phase is homogenized in water to form the smaller secondary emulsion droplet. When these two emulsions are mixed under mild agitation, the secondary emulsion droplets diffuse into the aqueous phase and are adsorbed by the hydrophobic seed droplets to form uniform swollen droplets. Suspension polymerization of swollen droplets leads to the formation of uniform porous microsphere (Nie et al., 2015). A nucleation and growth mechanisms is proposed for the formation of the microspheres (Cheng et al., 2011; Shan et al., 2012).

2.3. Seed swelling method

Ugelstad and co-workers first proposed seed swelling polymerization technique for the production of monodispersed, nanoporous polymeric microspheres. The particles are of larger size with well-defined nanopores. The synthesis method is shown in Fig. 4. Generally, seed particles are formed by polymerization reaction of monomer like styrene in presence of initiators like Sodium/potassium salt of peroxysulfate and porogen like Sodium Chloride. The polystyrene seeds are washed, dried and mixed with seed latex and the emulsion of oil like 1-Chlorodecane in water, stabilized with surfactants like SDS. Addition of acetone is also required under continuous stirring. After 10 to 12 hours, the emulsion droplets gradually disappear and the seed particles are grown larger. At this stage, the swollen polystyrene particles can be separated, washed and dried to get the porous microsphere. But to increase the particle size further, after complete evaporation of acetone, derivatives of acrylic acid like glycidyl methacrylate (GMA), ethylene glycol dimethacrylate (EGDMA) are added under continuous stirring at elevated temperature. 10–12 h is required to complete the polymerization reaction at this stage. The microparticles, thus obtained, are filtered and washed. Finally, the particles are dried to get the nanoporous microsphere (Ugelstad et al., 1979; He et al., 2012).

2.4. Sinter method

The schematic diagram is shown in Fig. 5. Fine power of solid like hydroxyapatite is mixed with aqueous solution of hydrophilic polymer like chitosan. This slurry is emulsified in oil phase. Under continuous stirring, cross linking agents like glutaraldehyde is added to harden the sphere. The settled particles are separated by filtration and washed with nonpolar solvents like acetone and petroleum ether to remove traces of oil. The particles are heated to burn off uncross linked polymer, followed by sintering at a temperature as high as 1100 °C. Finally the particles are thoroughly washed with water and dried (Paul and Sharma, 1999).

2.5. Synthesis method

The schematic diagram is shown in Fig. 6. Surfactant like CTAB is

![Fig. 1. The scanning electron microscopy image of microsphere. Adopted ion from reference Nie et al. (Nie et al., 2015). (A) Traditional microsphere with nonporous surface. (B) Porous microsphere.](image)
### Table 1

Summary of different methods for the synthesis of porous microspheres.

<table>
<thead>
<tr>
<th>Preparation method</th>
<th>Materials with categories</th>
<th>Result</th>
<th>Advantages</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Glycerol method</td>
<td>(polymer), Methacrylate (co-polymer), sodium chloride, sodium phosphate, calcium nitrate, ammonia solution</td>
<td>Average di. range = 176–471 µm</td>
<td>Easy to prepare</td>
<td>Diffusion of internal phase or oil phase affects pore size and porosity</td>
</tr>
<tr>
<td>(B) Exemplary sch. method</td>
<td>(polymer), PVA (co-polymer), sodium chloride, sodium phosphate, calcium nitrate, ammonia solution</td>
<td>Average di. range = 86–99.7%</td>
<td>Fast reaction speed, Low viscosity of medium, Controllable reaction temperature</td>
<td>Not suitable for heat sensitive substances, Microspheres agglomerate during sintering process.</td>
</tr>
<tr>
<td>Sinter method</td>
<td>Styrene (polymer), DVB (polymer), AMIN (amine), MB (methylene blue), PDDA (polymer)</td>
<td>Average di. range = 81 ± 24 µm</td>
<td>Monodispersed porous particle are obtained</td>
<td>Time consuming, Complicated</td>
</tr>
<tr>
<td>Synthesis method</td>
<td>TMOS (template), TMAH (alkaline agent or pH modifier), ethanol (co-solvent), DIW (co-solvent), BSA (protein), PVP (co-polymer), NaOH (alkaline agent or pH modifier), HCl (pore forming agent), urea (cross-linker), ( \text{NaCl} ) (salt), TMMS (template)</td>
<td>Average di. range = 81 ± 24 µm</td>
<td>Uniform pore channel distribution, Pore size can be used as variable by changing the amount of BSA</td>
<td>Easy to prepare</td>
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<tr>
<td>Preparation method</td>
<td>Materials with categories</td>
<td>Reaction condition</td>
<td>Result</td>
<td>Advantages</td>
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<tr>
<td>Phase separation method</td>
<td>(A) PLGA (polymer), PCL (solvent), dimethyl acetamide (solvent), dimethyl formamide (solvent), dimethyl carbonate (solvent), toluene (cross-linking agent), liquid nitrogen (immersion liquid or quenching fluid).</td>
<td>Stirring for 24 h at 25°C to evaporate the organic solvent. The microspheres were dried at 35°C for 3 days.</td>
<td>Diameter range = 150-300 µm</td>
<td>Sensitive to reaction condition, Poor monodispersity.</td>
</tr>
<tr>
<td></td>
<td>(B) PLA (polymer), Methylene chloride (solvent), n-hexane (non-solvent or hardening agent), PVA (co-polymer).</td>
<td>Stirring for 24 h at 25 °C to evaporate the organic solvent. The microspheres were dried at 35 °C for 3 days.</td>
<td>Number avg. dia. range = 176-471 µm</td>
<td></td>
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<td></td>
<td>(C) Ethyl acetocetate (chelating agent), octanol (non-solvent), zirconium propoxide (catalyst), alkylphenol ethoxylates (surfactant), span 80 (surfactant), SDS (emulsifying agent or surfactant), PVP (polymer or phase separation inducer).</td>
<td>Stirring for 1 h, stirring speed 1000 rpm, dried at 40 °C for 24 h, the resultant microspheres were heat treated at 500-900 °C for 2 h in air with heating rate of 3 °C/min.</td>
<td>Particle size range = 2-100 µm</td>
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<td></td>
<td>(D) Aluminium sec-butoxide (aluminum source), octanol (solvent), PVP (phase separation inducer), ethyl acetocetate (chelating agent), span 80 (surfactant), sodium dodecyl sulfate (emulsifying agent or surfactant), alkylphenolethoxylates (surfactant).</td>
<td>Stirring for 1 h, stirring speed 1000 rpm, dried at 40 °C for 24 h, the resultant gel microspheres were heat treated up to 1300 °C for 2 h with a heating rate of 5 °C min⁻¹.</td>
<td>Particle dia. range = 1-100 µm</td>
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<td></td>
<td>(A) Calcium nitrate tetrahydrate Ca(NO₃)₂·4H₂O (biodegradable polymer) and Ammonium hydrogen phosphate (NH₄)₂HPO₄ (fabricated materials)</td>
<td>Outlet temperature of the nozzle were adjusted to 170 °C and 100 °C, the product was dried at room temperature or calcined at 700 °C and 1000 °C for 1 h.</td>
<td>Particle size range = 2-20 µm</td>
<td>Suitable for industry, Easy to prepare.</td>
</tr>
<tr>
<td></td>
<td>(B) Fe (drug carriers), citric acid (crosslinking agent), lithium acetate (matrix material), PVP (polymer), NH₃ (precursors) and silica (coating materials).</td>
<td>Magnetic stirring at 80 °C, pH at 7.0-8.0, spray-dried at 108 °C, heated in a horizontal quartz tube oven under flowing argon gas at 700 °C for 10 h to obtain the final Li₂FeSiO₄, nitrogen adsorption/desorption at −196 °C.</td>
<td>BET surface area = 76 m²g⁻¹</td>
<td>Not many ways to form a porous structure and control the pore size. Not fit for heat sensitive substances.</td>
</tr>
</tbody>
</table>

PLA = Polylactide, PVA = Polyvinyl alcohol, ETOH = Ethyl alcohol, DVB = Divinyl benzene, AIBN = Azobisisobutyronitrile, DBP = = Dibutyl phthalate, ACN = Acetonitrile, THF = Tetrahydrofuran, PVP = Polyvinylpyrrolidone, PLGA = Poly(lactic-co-glycolic acid), PBS = Phosphate buffered saline, NaOH = Sodium hydroxide, PHB = Polyhydroxybutyrate, PCL = Polycaprolactone, BSA = Bovine serum albumin, TMOS = Tetramethyl orthosilicate, TMMS = Trimehox(methyl) silane, PGMA = Poly(glycidyl methacrylate), HCL = Hydrochloric acid, PGMA-SiO₂ = Poly(glycidyl methacrylate)-silica, SDS = Sodium dodecyl sulphate, Fe = Iron, NH₃ = Ammonia.
dissolved in water to make a micellar solution. Hydrophobic alkane like octane is added and solubilized in the hydrophobic inner core of the micelle which provide the medium of synthesis reaction. The spherical micelle act as the template for synthesis of spherical porous silica nanoclusters. Generally, Tetraorthosilicate is added as the source of silica and L-Lysine is used to catalyze the assembly of silica atoms. Polystyrene template is used to make the particle porous. This polystyrene template is formed in-situ with styrene monomer in presence of initiator like AIBA. After completion of reaction, the particles are separated, washed with water and ethanol. Finally, the porous particles are obtained after removal of template by heating at temperature as high as 500 °C (Bibby Mercier, 2002; Pang et al., 2010; Nandiyanto et al., 2009).

2.6. Phase separation method

The schematic diagram is shown in Fig. 7. Hydrophobic polymer like PLGA and drug to be encapsulated are dissolved in small amount
Dichloromethane. This solution is added dropwise in a dispersion medium like a mixture of mineral oil and DCM, under continuous sonication. The sonication bath should be kept at a temperature as low as 8–10 °C to control the rate of evaporation of DCM. After complete addition of polymer phase, the system is continuously stirred till the total removal of DCM. Finally, the microparticles are washed with nonpolar solvent like petroleum ether and dried to get free flowing powder (Mandal et al., 2001).

2.7. Spray drying method

The schematic diagram is shown in Fig. 8. Hydrophobic polymer like PLGA and phospholipid like DPPC are dissolved in methylene chloride to prepare the organic phase solution. An aqueous solution of porogen like ammonium bicarbonate is added to the organic solution and homogenized using a high shear mixer. This emulsion is spray dried using nitrogen as atomizing and drying gas (Straub et al., 2005).

2.8. Others method

With microfluidic technique, the polydispersity in particle size can be significantly reduced. The microfluidic devices used for the microsphere fabrication are generally consisted of a glass slide, polyethylene or teflon tubing, square glass capillaries, round glass capillaries and syringe needle tips. The outer aqueous phase may contain polymer like PVA, whereas a solution of polymer like PLA in DCM can be used as the inner oil phase. The volatile organic phase can be removed under reduced pressure. Traces of PVA must be removed.
removed by washing with deionized water. Finally, the particles are dried (Duncanson et al., 2012).

Molecularly imprinted polymeric microspheres are synthesized by polymerization of functional monomers around a print molecule. First, a complex of print molecule and functional monomer is formed by different noncovalent interactions (hydrogen bonding, ion-pair interaction etc.) or reversible covalent interactions. This complex is then incorporated into highly cross-linked macroporous polymer matrix by polymerization reaction. Upon removal of print molecules by extraction, microparticles with molecular imprinted surface is obtained. The pores on the surface have specific shape and functional group complementarity to the original print molecule (Lai et al., 2001; Mayes and Mosbach, 1996).

3. Characterization of porous microspheres

3.1. Particle size distribution

The volume average mean diameter, z-average diameter is determined with dynamic light scattering technique. The homogeneity of prepared particle is represented with polydispersity index (PDI) (Fan et al., 2014). A homogeneous sample is characterized with a single, sharp and narrow peak.

3.2. Morphology and particle surface topology

Morphology can be studied with Optical Microscopy (for larger particle), Atomic Force Microscopy (AFM) and Transmission Electron Microscopy (TEM). Surface topology is studied with Scanning electron microscopy (SEM) (Jabbari and Khakpour, 2000). The sample preparation method depends on the conductivity of sample to electricity. Non-conductive samples must first be sputter coated with an ultra-thin coating of an electrically-conducting material like gold or palladium before imaging. Conductive materials can be scanned directly (Lawrence and Jiang, 2017).

3.3. Measurement of pore size

Generally, SEM and TEM are used to determine the pore size of porous microsphere. Greater depth of field, higher resolution and magnification range are the main advantages of electron microscopy. We get direct and detailed structural information including the shape and size of individual pore. Porosity can be measured by image analysis based on SEM incorporating digital image processing technique. The steps of this digital porosity measurement technique are sample preparation, specimen scanning process, image enhancement, pixel classification, and pixel clustering (Kaestner et al., 2008; Jerram and Higgins, 2007). The accuracy of result depends on generation of a porosity threshold image where porosity voids are well separated from the rest of the objects in the image. In SEM analysis, the signals of the secondary electrons provide information on the surface topography, whereas the backscattered electrons (BSE) are used to get complementary information of the chemical composition of the sample surface. Since elements with higher atomic number backscatter electrons more strongly they appear brighter in an image. Thus, BSE are used to detect contrast between areas on the sample surface with different chemical compositions. The contrast in a backscattering electron image, C, can be calculated from following equation:

\[ C = \frac{n_1 - n_2}{\eta_1} \]

Thickness of section, threshold value, and pore circularity are the main factors that determine the calculated pore size distribution.

Fig. 6. Schematic diagram of synthesis of porous microsphere by Synthesis method.

Fig. 7. Schematic diagram of synthesis of porous microsphere by Phase separation method.
Inadequate preprocessing, high noise and improper measurement of threshold can lead to poor quality data. Sizing techniques, a two-point correlation technique, and fractal analysis can be used to analyze the individual pore size, shape and distribution (Lawrence and Jiang, 2017; Zhao and Darwin, 1992; Anovitz and Cole, 2015; Marinello et al., 2008).

### 3.4. Surface area of porous microspheres

Nitrogen adsorption/desorption analysis and Brunauer-Emmett-Teller (BET) absorption model are used to determine the specific surface area of porous microspheres (Cao et al., 2011).

### 3.5. Porosity of porous microspheres

Barrett-Joyner-Halenda (BHU) measurement is helpful for determination of porosity of porous microspheres. In this method, N2 adsorption-desorption is carried out at 77K. This method can simultaneously determine the pore size and pore volume (Si et al., 2011).

The average pore diameter, pore diameter distribution and pore volume of the large porous microspheres can be determined with the help of mercury intrusion porosimetry (Yoshizawa et al., 2004). Generally, the pore volume is measured from the volume of intruded mercury, and the pore diameter is calculated from the intruded pressure using the Washburn equation, assuming that the pore shape is a hollow-type cylinder (Yoshizawa et al., 2004).

### 4. Application of porous microspheres

#### 4.1. Tissue regeneration scaffolds

During the tissue regeneration, adhesion and proliferation of cells are usually required to carry molecules such as nutrients, cell growth factors to assist the proliferation of cells. The enormous specific surface area of porous microspheres provides the support for adhesion of cells, whereas the highly porous structure with well interconnected pores on inner and outer surface work as vessels facilitating in and out transport of nutrients, growth factors and oxygen. This is why three-dimensional porous microspheres are widely accepted as scaffolds in tissue regeneration (Kim et al., 2006). Particle size, pore structure, porosity and interconnectivity among the pores are most important characteristics of porous microsphere for use as scaffold of tissue regeneration. Again, these characteristics are regulated by the method of preparation and the physicochemical properties of polymers, like glass transition temperature, specific heat, crystallinity, viscosity and surface tension (Bellehumeur et al., 1996). The selection of polymer and choice of best suitable method depend on the characteristics of tissue (liver, cartilage, skin etc.) itself (Wang et al., 2009; Brown et al., 2008).

Thermally induced phase separation, microsphere sintering, electrospinning and hydro gelling methods are mostly applied for development of large size porous microspheres suitable for scaffold of tissue regeneration (Kim et al., 2006). Generally, the particles are implanted in the body by surgery. Their size and shape are so tailored that they fit into the dimension of a tissue defect. The alternative technique is to inject porous microspheres with tissue specific cells attached on the surface, to defected tissue. It is followed by photo-crosslinking or in-situ gelation to make cell-containing hydrogel. So, the polymer of choice must be biocompatible and biodegradable. Thus, PLGA is the most widely used polymer. Wang et al has prepared PLGA porous microspheres sintered scaffolds with controlled porosity. The biomimetic hierarchical structures of these porous particles were proved by enhanced viability and proliferation of synovium-derived mesenchymal stem cells upon incubation with them (Wang et al., 2009; Brown et al., 2008).

#### 4.2. High speed chromatography

Due to huge specific surface area, porous microspheres are applicable for adsorption and desorption purposes like high speed chromatography where high column efficiency is required to separate protein molecules or phytochemicals from complex mixtures. The presence of macropores reduce the flow-resistance and provide the easy passage for the mobile phase to pass through. Whereas the micropores provide the enormous surface area for binding of solute molecules and connect the macro pores. This, together with specific interaction of target molecules with active sites on porous particles, enable high speed, efficient separation at low pressure. Zhiguo et al. compared the chromatographic performance of silica based C18 medium and polystyrene-type uniform porous microsphere to separate icariin from a complex mixture of crude extract. Using porous microsphere as packing material, they were successful to purify icariin up to 90% purity at an operating pressure of less than 0.05 MPa with a recovery of 99.9% in a single run (Wu et al., 2003).

Narrow particle size within the range of 5–10 µm, good mechanical strength and chemical stability in a wide range of pH are the basic requirements for use of porous microparticles as stationary phase.

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**Fig. 8.** Schematic diagram of synthesis of porous microsphere by Spray drying method.
Silica, polystyrene and acrylic acid polymers are mainly used (Haihong et al., 2005). In most of the cases, suspension co-polymerization is reported to be the method of choice. The type and amount of porogen used determine the pore size distribution. Sun et al. used this technique to develop rigid spherical biporous poly-(glycidyl methacrylate-co-ethylene dimethacrylate) microsphere having specific surface area as high as 91.3 m²/g (Haihong et al., 2005). They tested this polymeric particle for high speed protein chromatography. Broad particle size distribution is the inherent problem of this method. The larger particles cause lower column efficiency whereas fine particles increase column pressure drop leading to shorter column lifetime. These problems can be solved with multi-step swelling and polymerization method that produces monodisperse porous polymeric particle. But pore structure can better be controlled with suspension polymerization method. So, seed swelling methods are developed where first large seed particles are synthesized by an emulsifier free emulsion polymerization technique, which are then used for multistep swelling and polymerization method (Haihong et al., 2005).

Liapis and McCoy (1992) has developed theoretical model describing the dynamic adsorption-desorption process of porous microsphere when used as absorbent in column chromatography. They studied the dynamic behavior of column systems for different particle sizes, column lengths, column fluid superficial velocities, intraparticle fluid velocities and different values of the effective pore diffusivity and of the total number of active sites per volume of adsorbent.

Fig. 9. Application of porous microparticles as tissue regeneration scaffold. The enormous specific surface area of porous microspheres provides the support for adhesion of cells, whereas the highly porous structure with well interconnected pores on inner and outer surface work as vessels facilitating in and out transport of nutrients, growth factors and oxygen. This is why three-dimensional porous microspheres are widely accepted as scaffolds for bone and cartilage regeneration (A), nerve regeneration (B) and wound healing (C). When the particles are loaded with tissue specific growth factors, endogenous or transplanted progenitor cells are easily differentiated into appropriate cell types. Generally, the particles are implanted in the body by surgery. Their size and shape are so tailored that they fit into the dimension of a tissue defect. The alternative technique is to inject porous microspheres with tissue specific cells attached on the surface, to defected tissue. It is followed by photo-crosslinking or in-situ gelation to make cell-containing hydrogel. Especially, in case of treatment of bone injury, the microparticles are loaded with antibodies like gentamicin sulphate for prophylactically protecting the patient from infections.

4.3. Carriers for biopharmaceuticals and drugs

Porous microspheres are widely studied as porous scaffold for controlled and sustained release of small drug molecules and biopharmaceuticals like therapeutic proteins, peptides, genes, siRNA into the cellular microenvironment (Jang and Shea, 2003). The cargo molecules are generally loaded in the matrix material (Fang et al., 1996; Bonadio et al., 1999; Shea et al., 1999; Nof and Shea, 2002; Steinbacher and Landry, 2014; Edelstein et al., 2007; Reischl and Zimmer, 2009; Bridge et al., 2003; Persengiev et al., 2004; Slecz et al., 2003; Svensson et al., 2012; Nishimura et al., 1986; Donohue and Rosenberg, 1983; Morikawa et al., 1987; DeLoach et al., 1988; Jung et al., 2016), whereas the surface of the particle is made functionalized by conjugation with ligand molecules, specific antibodies, and immune stimulators Fig. 10) (Jang and Mok, 2016; Baki et al., 2017). Due to enormous specific surface area of porous microsphere, the loading capacity is higher in comparison to nonporous particles. Physical adsorption, hydrophobic interaction, H-bond formation, ionic interaction and even formation of covalent linkage are mechanisms behind surface conjugation. Biodegradable polymers like PLGA, Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (Wu and Zreiqat, 2010); alginate/chitosan (Liu et al., 1997); ceramics like hydroxyapatite (Yu et al., 2014); inorganic salts like Calcium Carbonate (Volodkin et al., 2004) are widely studied. Porous microsphere itself acts a scaffold for tissue regeneration; when they are loaded with tissue specific growth factors, endogenous or transplanted progenitor cells are easily differentiated into appropriate...
cell types (Jang and Shea, 2003; Blesch et al., 2002; Bottaro et al., 2002; Varkey et al., 2004). Controlled delivery of VEGF in combination with PDGF with porous microsphere caused rapid growth of vascular network (Jang and Shea, 2003; Richardson et al., 2001). A better alternative is to load the polymer scaffold with DNA encoding for therapeutic or tissue inductive proteins. As cells invade through the interconnective pores of the scaffold, they uptake DNA that is either released from or entrapped within the matrix. The transfected cells act as bioreactor for local synthesis of tissue specific growth factor (Jang and Shea, 2003; Bonadio et al., 1998). Collagen and PLGA are well studied matrix material for controlled delivery of non-viral DNA like plasmid (Jang and Shea, 2003; Fang et al., 1996; Bonadio et al., 1999; Shea et al., 1999; Nof and Shea, 2002). They have the capability to hold DNA till the DNA is internalized by the infiltrated fibroblasts. Thus, the rate of clearance of delivered DNA from tissue is significantly reduced leading to increased rate of gene transfer.

siRNA is widely accepted as good therapeutics for treatment of cancer (Steinbacher and Landry, 2014; Edelstein et al., 2007). But there are many barriers that limits their clinical use. Unprotected RNA is rapidly degraded in the body by endogenous ribonucleases (RNases), and the negatively charged RNA strands cannot pass through cell membranes (Steinbacher and Landry, 2014; Reischl and Zimmer, 2009). Moreover, the systemic administration of siRNA can cause immune responses and off-target gene effects (Steinbacher and Landry, 2014; Bridge et al., 2003; Persengiev et al., 2004; Sledz et al., 2003). So, smart delivery vehicles are need to be developed for overcoming these barriers and improving the biodistribution and pharmacokinetics. Steinbacher et al. developed nanoporous large silica particles known as acid-prepared mesoporous spheres (APMS) as transfecting agent. They used diethylametriamine to modify the pore so that siRNA was adsorbed inside the particles rather than on the surface. Thus, it was protected and did not affect the cellular uptake of the particles. These particles do not cause disruption of normal cell functions and are not accumulated within body thus they are free of toxicities associated with use of nanoparticles. They functionalized the surface of the particles with tetraethylene glycol to facilitate cellular uptake. Moreover, these particles are not membrane-bound upon internalization, thus endosomal escape is avoided.

Nanoporous silicon particles are used as Multistage Nano-Vectors (MSV) to deliver imaging and therapeutic agents like protein, RNA, lipid and nanoparticles to the site of action. They exhibit superior margination, firm cellular adhesion, and internalization properties. Even, they can be tailor-made to deliver the payloads to different subcellular structures. This is possible as the unique geometry and pore morphology can be mathematically optimized (Svensson et al., 2012).

Further, the surface of the nanoporous silicon particles can be so controlled that they reach simultaneously or sequentially to diverse cell types and different intracellular locations and release the cargo molecules selective to the target location. This makes contact independent cell signaling possible.

Since, the delivery cargo can be a combination of imaging and therapeutic agents, they are good theranostics. Thus, they are suitable for real-time monitoring of drug delivery or therapeutic efficacy.

Controlled delivery of IL-2 is an important strategy to active the immune system of cancer patients against the tumors cells (Liu et al., 1997; Nishimura et al., 1986; Donohue and Rosenberg, 1983; Morikawa et al., 1987; DeLoach et al., 1988). Liu et al. (1997) developed IL-2 loaded porous microsphere by gelation of polyanionic sodium alginate, Calcium chloride and polycationic chitosan, followed by lyophilization. These spherical particles of chitosan/alginate polyelectrolyte complex were of 20–100 µm size. 100% of encapsulated active IL-2 was released slowly in the release medium, fetal calf serum, in 5 days.

The biocompatible and biodegradable porous microsphere of calcium carbonate is suitable for delivery of proteins (Volodkin et al., 2004). Simply rapid mixing of equal volume of calcium chloride and sodium carbonate resulted in colloidal crystallization of porous calcium carbonate from supersaturated solution. The specific surface area of porous particles of average size 4.75 µm was 8.8 ± 0.3 m²/g. The target protein molecules were physically adsorbed on the surface of porous particles followed by coating of oppositely charged poly-electrolyte like sodium poly-(styrene sulfonate), poly(allylamine hydrochloride) by means of electrostatic layer-by-layer assembly. The core was subsequently dissolved with EDTA to form protein containing polyelectrolyte microcapsule.

Micromolding is well studied as a robust technique for fabrication of porous hydrogel microspheres. Yi et al followed this micromolding based approach for production of monodisperse microspheres with controlled macroporous structures composed of biopolymeric-synthetic hybrid polymer (i.e., chitosan-polyacrylamide) hydrogels (Jung et al., 2016). Due to the presence of primary amine groups of glucosamine monomer units of chitosan, these microspheres are very suitable for conjugation of specific proteins by amine-reactive conjugation chemistry. The protein conjugation kinetics and capacity depend on the pore size. Long chain PEG is the most suitable porogen to tune the pore size and synthesize macroporous particles.

Porous microspheres are well studied also as a carrier of drug molecules. The bone defect treatment in trauma and orthopedic surgeries involve use of different biomaterials as implant. Those materials
combined with potent antibiotics like gentamicin sulphate is utilized to support bone healing as well as prophylactically protecting the patient from infections. But, the limited blood supply in the bone, and the toxicity of antibiotics reduce the effectiveness of systemic antibiotic therapy. So, one good option is to develop bone regeneration scaffold loaded with the antibiotics. Zhou et al developed ice-templated spray drying technique to fabricate gentamicin sulphate loaded porous hydroxyapatite microspheres with the mean particle size of 42–50 µm (Yu et al., 2014). Poly-vinyl alcohol was used to control the pore structure and porosity of the particles (Liu et al., 2016).

4.4. Pulmonary drug delivery

The efficient treatment of respiratory inflammation, pulmonary infections, cystic fibrosis, lung cancer and other lung diseases require local and controlled release of drug molecules. Again, due to good vascularization, large area for drug absorption (approx. 75 m²), low thickness of alveolar epithelium (0.1–0.5 µm), immense capacity of solute exchange and avoidance of hepatic first pass metabolism, bio-pharmaceuticals can be delivered through pulmonary route for systemic absorption (Uchenna Agu et al., 2001; Lee et al., 2007; Edwards et al., 1997). But there are a number of barriers to pulmonary absorption of proteins and peptides: respiratory mucus lining the pulmonary epithelium, pulmonary surfactants secreted by epithelial type II cells, mucociliary clearance, alveolar lining layer, alveolar epithelium, basement membrane, pulmonary enzymes, and macrophages. Most of the peptides and proteins, upon reaching to alveoli, are either degraded by proteases or scavenged by alveolar macrophages. The pulmonary macrophages also release short-lived peroxidases, inflammatory and immunomodulatory mediators that cause degradation of proteins and peptides (Uchenna Agu et al., 2001; Lee et al., 2007; Edwards et al., 1997). So, there is the requirement of such a delivery system that can overcome all these barriers and increase the bioavailability of administered biopharmaceuticals. Not only that, it is quite desirable that the delivery system should deliver the drug at a controlled rate for prolonged period of time. Drug loaded particles can be administered with Dry Powder Inhalers (DPI). In case of nonporous particles, the efficient pulmonary delivery requires that the mass median aerodynamic diameter (MMAD) should be within the range of 1–5 µm. Larger particles are deposited in the oropharynx, whereas smaller particles (< 1 µm) are exhaled during normal tidal breathing.

Human lung can easily sweep the larger particles from upper respiratory tract due to the presence of ciliated epithelia. Again, alveolar macrophages are capable of phagocytosing the fine particles as soon as they deposit to lower respiratory tract and alveoli. Porous microspheres may have mass density less than 0.4 g/cm³. So, porous particles of MMAD within the range of 1–5 µm have larger geometric diameter than the corresponding nonporous particles. As a consequence, they can avoid phagocytic clearance and are aerosolized more efficiently from DPI (Uchenna Agu et al., 2001; Lee et al., 2007; Edwards et al., 1997).

There are complex processes for clearance of deposited particles from respiratory tract through three routes of clearance; i.e. to the blood, to the lymphatic circulation and to the gastrointestinal tract (Stuart, 1984). Upon deposition of particles on respiratory epithelium, the number of macrophages is increased. They phagocitize the insoluble particles and are translocated to air way region of lungs, lined with ciliated epithelium. As the ciliated cells propel mucus towards the oral-pharynx, the deposited particles and particles-bearing macrophages are cleared into the gastrointestinal tract. This process plays predominant role in early clearance (within first few hours) from alveolar region. But the size, shape, physicochemical properties, surface reactivity and the amount & site of deposition of the deposited particles determine the clearance pattern. The remaining insoluble particles within alveolar region can be transported via the lymphatic drainage system to regional lymph nodes that may act as secondary reservoir and principal site for whole body retention of the particles. But this pathway is mainly influenced by the relative toxicity of the inhaled particles. Since, porous microspheres for pulmonary drug delivery are made up with biocompatible, nontoxic materials, they are not supposed to follow this route (Stuart, 1984).

The inhaled porous microparticles, upon deposition on respiratory epithelium may get dissolve in alveolar lining fluids and enter into blood. Then it is cleared of body through metabolism in liver. If the deposited particles are not phagocytized, then the model proposed by Mercer (Mercer, 1967), provide an adequate description of overall clearance rates for many soluble materials and some relatively insoluble particles that are deposited within the respiratory tract. This dissolution model is based upon physicochemical characteristics of particle size and in vitro solubility.

Edwards et al. (Edwards et al., 1997) has shown that inhalation of insulin loaded large porous poly[lactic acid-co-glycolic acid] particles resulted in elevated systemic levels of insulin and suppressed systemic glucose levels for 96 h, whereas nonporous particles had this effect for only 4 h.

Al et al. (Bot et al., 2000) has formulated human immunogobulin loaded lipid-based hollow-porous microparticles for local and systemic delivery via the respiratory mucosa. This PulmoSphere when administered into the trachea of Balb/c mice, resulted in generation of higher titers of specific IgG antibodies in serum as well as bronchoalveolar lavage fluid.

Lee et al. (Kwon et al., 2007) used W/O/W multiple emulsion as a template for synthesis of protein loaded porous microparticle for pulmonary delivery. They used BSA as model protein. The synthesized low density porous particles having MMAD of 3 µm were accumulated in the deep lung epithelium. It was the Sucrose ethyl acetate, added during secondary emulsification, that controlled the release of BSA up to 7 days.

Yeo et al. (Yang et al., 2009) used ammonium bicarbonate as porogen to develop highly porous large PLGA microparticles having MMAD of 6.2 µm. They established the capability of this particle to deliver model therapeutic macromolecules like lysozyme and small molecular weight drugs like doxorubicin-hydrochloride for over 4 days.

Meenach et al. (2012) used biodegradable polymer acetylated dextran to prepare Camptotheacin loaded porous microsphere for the treatment of lung cancer (Fig. 11). The molecular weight of Ac-DEX can be controlled to tune the degradation rate of particle. Thus, the rate of drug release can be controlled for the duration of few hours to month. This biocompatible and biodegradable porous particle could be a promising delivery system for treatment of lung cancer.

Hickey et al. (Conterras et al., 2015) were able to prepare porous rifampicin particles, simply, by spray drying a aqueous solution of ri-fampicin and L-leucine. They compared the pharmacokinetic parameters after pulmonary administration of this porous rifampicin to guinea pigs with that of administration of rifampicin as intravenous solution and oral suspension. When one half of oral dose was administered as porous rifampicin through pulmonary route, similar systemic concentrations were obtained. Again, there was 3–4 fold higher rifampicin concentration in bronchoalveolar lavage for prolonged period of time, even when systemic concentration was below the detection limit.

Tafaghodi et al. (Alipour et al., 2015) has developed paclitaxel loaded large porous PLGA microsphere for treatment of lung cancer. The mass median aerodynamic diameter (MMAD) was 5.74 ± 0.09 µm. The lung targeting efficiency of this particle was 11.9-fold higher than intravenous (i.v) administration. Endotraechal administration of this particles in rats exhibited paclitaxel plasma concentration in the therapeutic range lasting for 4-fold longer period of time than i.v injection.

4.5. Gastric drug delivery

Though oral route is the most preferred route for delivery of drugs,
it is very difficult to develop oral controlled release product to provide a sustained level of plasma drug concentration for prolonged period of time. Sustained plasma drug concentration is achievable only if a fixed and predictable amount of drug can be made available to the site of absorption throughout the desired period of time. The factors that mainly affect the rate of drug absorption from g.i.t (Boxenbaum, 1982) are i. site of drug release; as the pH (affecting the solubility and permeability of drug molecule) and area available for drug absorption are variable throughout the gut ii. gastrointestinal motility and transit time, particularly gastric emptying time iii. presence of food in gut that affects the gastric motility, pH, level of enzymes and the availability carriers free for drug absorption. So, to achieve sustained level of plasma drug concentration, it is not enough that the delivery system releases the drug at predetermined rate, preferably at zero order kinetics. The prolonged residence of the delivery device within gastrointestinal tract, especially in the upper part of gastric region is of prime importance. These types of delivery systems are known as Gastro Retentive Drug Delivery System (GRDDS). Again, this gastric retention is most suitable for those drug molecules that (i) act locally/absorbed in the stomach, (ii) are poorly soluble at an alkaline pH, (iii) have a narrow window of absorption, and (iv) are unstable in the intestinal or colonic environment (Singh and Kim, 2000).

Among different approaches to achieve prolonged gastric retention of drug delivery system (Moës, 1993; Deshpande et al., 1996), floating drug delivery system is of great importance (Desai and Bolton, 1993; Oth et al., 1992; Whitehead et al., 1998). Due to very low mass density, porous microspheres remain buoyant in gastric content (~1.04 g/cm³) resulting in prolonged gastric residence time (Fig. 12).

Srivastava et al. (2005) developed cimetidine-loaded floating microspheres of hydroxypropyl methylcellulose and ethyl cellulose. The prepared microspheres exhibited prolonged drug release (~8h) and remained buoyant for > 10h. Streubel et al. (2002) used polypropylene foam powder as porous carrier for the development of verapamil HCl-loaded floating microparticles. More than 83% of the particles kept floating for at least 8h. By increasing the percentage of polymer (Eudragit RS), burst release of drug was prevented and entrapment efficiency as high as 93.4% was achieved. Jain et al. (2006) used gamma scintigraphy to study the gastro-retentive behavior of calcium silicate based porous microsphere, radiolabeled with 99mTc, for delivery of repaglinide, orlistat. The particles had gastric retention time of more than 6hr and enhanced bioavailability than the marketed product, in rabbit. Bhaskar et al. (Pandya et al., 2011) developed glipizide loaded floating microspheres by the emulsion solvent diffusion technique, using calcium silicate as porous carrier and Eudragit ® S as polymer. When tested in simulated gastric fluid, more than 80% of the particle kept floating for at least 10h. The amount of calcium silicate was the key factor to control the drug release. The extent of drug release was inversely proportionate to the content of calcium silicate.

4.6. Other applications

Other than pharmaceutical, porous microspheres have potential application in developing high performance lithium ion battery anode materials. Guo et al. used one step surfactant free, hydrothermal reaction method for the self-assembly of tin dioxide (SnO₂) porous microspheres. In comparison to non-assembled SnO₂ octahedral nanoparticles and irregular SnO₂ nanoparticle, it had the higher current density of 500 mA g⁻¹ and highly stable capacity about 690 mA g⁻¹ after 50 cycles. Actually, the porous nanostructures favor the diffusion of electrolyte, rendering better Li⁺ ion transfer. It also provides the buffer space for the volume change during the alloying and dealloying reaction between Sn and Li, thus delay the pulverization and improve the cyclability (Wang et al., 2011).

Recently Liu et al. fabricated 3D giant graphene sheets-carbon nanotubes-porous SnO₂ octahedrons aerogels as high capacity anode material for Li-ion batteries (Liu et al., 2016). GCNT-SnO₂ aerogel film with vertically aligned pores, had excellent electrochemical performance with the largest specific capacity of 1190 mAh/g, as well as long-term cycling stability up to 1000 times.

5. Conclusion

Porous microspheres have low density and very large surface area. Excellent absorption capability makes them unique over traditional microspheres. They are extensively used as carriers for biopharmaceuticals and drugs. Porous microspheres have wide application in
developing tissue regeneration scaffolds. Due to enormous specific surface area they are very suitable as stationary phase of high speed chromatography. Low density and proper diameter of microspheres promise for alveoli targeted drug delivery. Since porous microsphere inherently float in liquid, they are extensively used for gastro retentive drug delivery system. Though there are number of well-established methods like seed swelling, solvent evaporation, polymerization, spray drying, phase separation etc. are available for their synthesis, most of the methods are time consuming, consists of numbers of complicated steps and have poor yield. The size, shape and pore structure of the particles depend on many experimental variables like temperature, pH, stirring speed, type and concentration of porogen, polymer and its concentration. Thus, synthesis of porous microparticle with predefined porosity is really challenging.

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References


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