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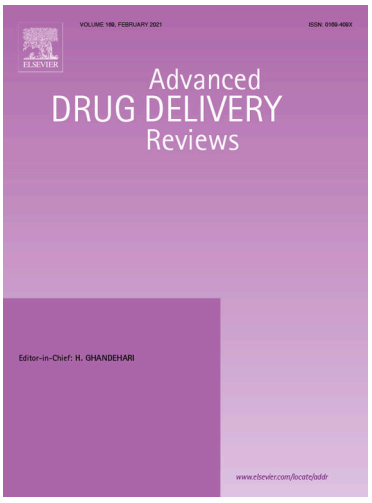

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Silica nanoparticles: A review of their safety and current strategies to overcome biological barriers

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1 ABSTRACT

Silica nanoparticles (SNP) have gained tremendous attention in the recent decades. They have been used in many different biomedical fields including diagnosis, biosensing and drug delivery. Medical uses of SNP for anti-cancer, anti-microbial and theranostic applications are especially prominent due to their exceptional performance to deliver many different small molecules and recently biologics (mRNA, siRNA, antigens, antibodies, proteins, and peptides) at targeted sites. The physical and chemical properties of SNP such as large specific surface area, tuneable particle size and porosity, excellent biodegradability and biocompatibility make them an ideal drug delivery and diagnostic platform. Based on the available data and the pre-clinical performance of SNP, recent interest has driven these innovative materials towards clinical application with many of the formulations already in Phase I and Phase II trials. Herein, the progress of SNP in biomedical field is reviewed, and their safety aspects are analysed. Importantly, we critically evaluate the key structural characteristics of SNP to overcome different biological barriers including the blood-brain barrier (BBB), skin, tumour barrier and mucosal barrier. Future directions, potential pathways, and target areas towards rapid clinical translation of SNP are also recommended.

2 KEYWORDS

Silica nanoparticles, Tumour, Blood-brain barrier, Mucosal barrier, safety, Biocompatibility, Nanocarriers, Mesopores, Retention time and membrane permeation

3 INTRODUCTION

Nanotechnology-based diagnostic, sensing and drug delivery platforms have gained significant attention in the last few decades [1,2]. When compared to conventional therapeutics, nanomaterials may offer many advantages, such as tailored drug delivery at the diseased site with reduced side effects and improved stability of formulations. The field of nanomedicine has progressed rapidly, and numerous nanoparticle-based delivery systems have been investigated preclinically and clinically [1,3].

An optimal nanocarrier would possess a desired cargo loading capacity, show excellent biocompatibility and stability (on shelf and during transport), exhibit particle size and shape uniformity, and allow for targeted (organ and/or cell) cargo release. In light of this, there has been growing interest in developing porous inorganic materials which have unique properties such as high specific surface area, tuneable (surface) chemistry, and enhanced stability across biological barriers, when compared to organic nanoparticles such as polymers and liposomes [4]. Many inorganic or hybrid drug carriers have been investigated, such as silica, silicon, quantum dots, and metal-organic frameworks (MOFs). Among these, research on silica-based nanomaterials has witnessed not only an exponential growth in the number of publications but also a progressive transition from basic research towards clinical translation (Figure 1) [5–13]. Silica-based nanoparticles, SNP, are very attractive due to their ease of synthesis, colloidal stability, tuneable particle size, ease of surface functionalization, biocompatibility, and potentially scalable synthesis [14,15]. Importantly, SNP have been reported to enhance the stability of diverse cargo without affecting their specific chemical and physical functions [16–20].

One of the earliest SNP synthesis protocols, known as the Stöber process, was pioneered by Werner Stöber in 1968 (Figure 1) [21]. Stöber particles are solid and monodisperse SNP with adjustable diameters in a wide range of 20-2000 nm. They are synthesized using sol-gel chemistry involving a silica precursor, such as tetraethylorthosilicate, in water and alcohol solutions containing ammonia [21]. In the last 50 years, many different types of SNP have been generated, having different particle sizes and shapes, as well as various pore structures [15,22–27]. Due to their versatile properties, SNP have now been used in a variety of applications such as food additives, excipients in pharmaceutical products and cosmetics, and as nutraceuticals [28]. For instance, the French cosmetic company L'Oréal uses SNP in many of their skincare products in order to improve skin texture and provide a matte finish [29]. Some sunscreens contain SNP, as they have a UV light scattering effect in addition to increasing dispersity of other UV blocking agents and thus improve the overall UV-attenuating efficacy of the formulations [30–33].

Porous silica as a drug delivery agent is not new. In 1983, the amorphous colloidal and porous silicas synthesis was published, by Klaus Unger in Germany [34]. Unger *et al.,* demonstrated that the porous silica can be used as a drug delivery and stabilizing agents. The early syntheses did not lead to the formation of nanoparticles, but rather non-uniform micron-sized particles. In the 1990's, a new class of high surface area mesoporous silica material emerged, where

surfactant assemblies served as supramolecular structure-directing agents, namely MCM-type ordered mesoporous silica. In 2001, Vallet-Regi *et al.,* introduced MCM-41 as a potential drug carrier for the first time using ibuprofen as a model drug. In 2003,Lin *et al* was pioneered the synthesis particle tunable size and surface functionality, and achieved the control drug release property of SNP . [35]. Mesoporous materials of this class are attractive due to their porosity features which create high specific surface areas and pore volumes which are useful for loading of high payloads. Moreover, well-calibrated and ordered pores also allows good control of adsorption/release rates enabling best fit between drug size and pore sizes/ openings [36,37]. Later developments along this direction allowed the synthesis of ordered mesoporous silicas with a narrow and adjustable pore size in the range 2-30 nm. [22,38,39] Importantly, the silica network is amorphous, which enhances the rate of biodegradation as compared to crystalline silica and silicates, which is also very important from a cytotoxicity point of view [40–43]. Crystalline silica has a highly ordered and repetitive atomic structure, whereas silicon and oxygen atoms are arranged into periodic, hexagonal 6-membered rings via siloxane bonds that bridge between two silicon centres. This regular structure makes it more stable and less susceptible to chemical reactions or breakdown by biological processes. In contrast, amorphous silica lacks this long-range order, with a disordered and irregular structure, which makes it more prone to chemical and biological degradation[43,44].

In 1999, a new hybrid class organic-inorganic silica-based material called periodic mesoporous organosilicas (PMOs) were developed independently by three research groups Ozin [45], Inagaki [46], and Stein [47]. Here, the solid wall of the porous material contained organic groups as part of the hybrid network, therefore the polarity of the pore walls differs from those of corresponding purely silica-based particles. Later, PMO-based nanoparticle systems, sometimes named as mesoporous organosilica nanoparticles (MON), were developed which have since become popular nanocarriers for drugs, genes and protein delivery as they may exhibit improved biodegradation and drug release profiles, as compared to the all-silica materials [48–50].

Medical uses of SNP for delivery of anti-cancer, diagnostic and theranostic purposes are now becoming prominent [5]. SNP such as MSN and MON offer the ability to encapsulate and precisely control drug release at specific site which can be triggered by changes to pH, reactive oxygen species, temperature, enzyme and light [20,51,52]. For example, tumour tissues are known to have an acidic pH environment, whereas normal tissues typically maintain a neutral pH. In response to this biological distinction, researchers have engineered a dual-responsive

peptide, RGDFFFFC, designed to function as a 'gatekeeper' on the surface of MSN. Upon entry into the acidic tumours' microenvironment, the drug-loaded nanoparticles benefit from enhanced uptake by tumour cells, facilitated by the exposure of the peptide targeting ligand. Subsequently, the presence of the intracellular redox signal, glutathione, within the tumour cells plays a pivotal role in triggering rapid drug release [53]. In addition, the adjustable porous structure of MSN and MON enables co-delivery of various therapeutic agents to the desired sites to combat the development of disease, such as cancer, and its subsequent resistance while also reducing the drug amount needed, thereby limiting off-target side effects [52,54–57]. SNP are also a suitable carrier system to deliver antimicrobial agents, as they can be modified for targeted delivery to site of infection in order to avoid toxicity associated with systemic circulation [20,58–62]. In addition, unlike other nanocarriers such as gold, silver and polymeric nanoparticles, SNP such as MSN can have high drug loading capacity and improve the stability of active pharmaceutical ingredients by encapsulating the drug inside the pores, even including large antimicrobial peptides, which is important to both adequately treat the infection (especially biofilm) and prevent development of resistance [20,62–68]. Nonetheless, a limitation in drug release arises from the interactions occurring between drug molecules and the silica surface, potentially resulting in incomplete drug release. The magnitude of this interaction is contingent upon the silica surface's chemistry, which exhibits variations in response to pH levels. Moreover, it is influenced by the chemical composition and ionization states of the drug itself. This heightened adsorption, consequently, corresponds to a decreased extent of drug release [69]. Moreover, there has been high interest in using SNP for imaging and diagnostics, some formulations containing SNP have progressed to clinical trials in humans [5]. The traditionally-used gadolinium-based contrast agents used for magnetic resonance imaging (MRI) are highly toxic; the use of SNP as support for the Gd compounds can reduce the toxicity of these contrast agents by not only lowering the amount needed for high resolution MRI but by also limiting the leaching of Gd^{3+} ions into the circulation [70–73]. Recently, the photostability of fluorescence imaging agents, such as indocyanine green (ICG) or cyanine dyes, has also been enhanced by loading into SNP due to the entrapment and stabilisation of the dye in nanopores [10,18,74]. Moreover, Chen *et al.* reported a PEGylated mesoporous silica nanoparticle loaded with J-aggregates of NIR fluorophore IR-140, allowed nanoparticles to absorb and emit shortwave infrared light [75]. This development enabled high resolution imaging in vivo with 980 nm excitation and 1000−1700 nm acquisition, and the formulation was stable for weeks in buffer[75]. Due to the controlled mesopores, diverse surface modification, mesopores silica nanoparticles have become a popular image agent supporting

system including organic dyes, quantum dots and carbon dots, and their applications in bio-imaging have been extensively discussed elsewhere[76–80].

In addition to the advances related to the use of MSNs and MONs for drug delivery and bio-imaging, their use in overcoming biological barriers has recently been thoroughly investigated in various *in vitro* and *in vivo* models. Biological barriers such as skin, the blood-brain barrier (BBB), mucosal barriers in lungs and the intestinal tract are a part of the defense systems of the body that are involved in selective transport of substances. The nanoscale nature of SNP along with their specific properties such as size and shape, surface charge and functionality, stability and porosity generate complex physicochemical interactions with these biological barriers, in many instances leading to better transport of drugs across these hurdles. While numerous review articles cover SNP and their diverse applications in drug delivery [81–85] and theranostics [86,87], an up-to-date review focusing on linking the material characteristics to the ability to overcome biological barriers with special emphasis on their rational design was needed.

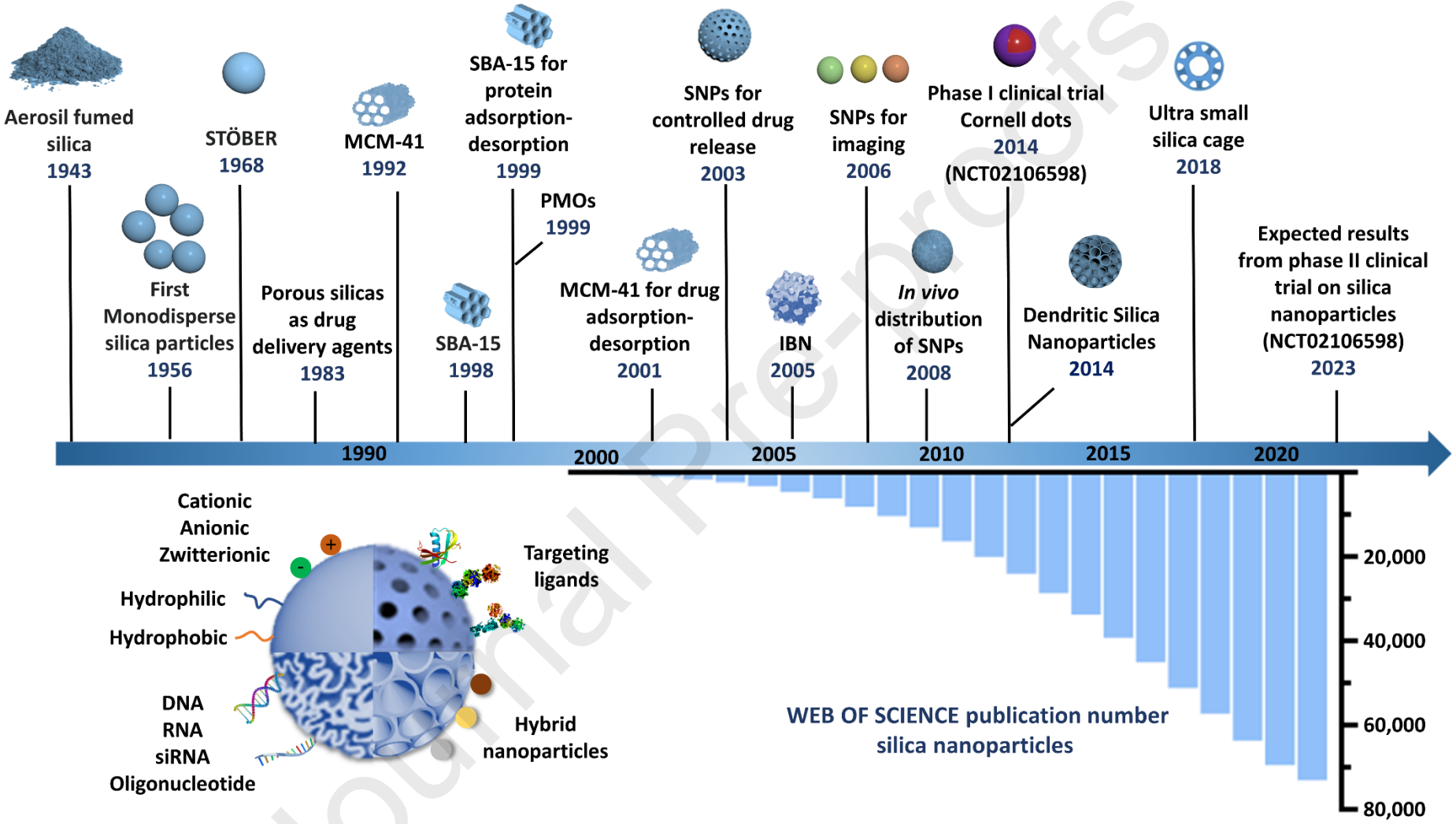

Figure 1. Schematic diagram representing the history and development of silica particles and silica nanoparticles (SNP) with different surface morphology and modifications. In the middle of 20th century, monodispersed silica nanoparticles were introduced, and in 1968 Stöber process was pioneered [21]. In 1983, the use of porous and surface modified silicas was realised for drug delivery [34]. In the early 1990's, new class of porous materials emerged called the ordered mesoporous silica including Mobil Composition of Matter (MCM), Santa Barbara Amorphous (SBA) and periodic mesoporous organosilica (PMO) types developed in 1992, 1998 and 1999, respectively [22,38]. In 1999, large-pore mesoporous silica (SBA-15) were first used to demonstrate their ability to adsorb and release of proteins from the porous structure [88]. In 2001, Vallet-Regi et al. introduced MCM-41 as a potential drug carrier for the first time, and the control drug release property of SNP was discovered in 2003 by Lin et al. [35,89]. In 2005, further advances in synthesis techniques allowed modification of porosity to generate large pore SNP such as IBN (named after The Institute of Bioengineering and Nanotechnology (IBN)) [39,90]. Later, more applications of SNP such as imaging were discovered in 2006 [16]. Further advances in material sciences lead to development of dendritic silica in 2014 [91] and ultra-small silica cages in 2018 [27]. In the recent decade, the first phase I clinical trial was launched in 2014 and the expected results from phase II clinical trial will be released in 2023. Before 2000, there were less than 200 publications about SNP. However, within two decades, more than 20,000 journal articles have been reported for SNP with multiple applications in diverse fields.

4 SAFETY OF SILICA NANOPARTICLES

4.1 METABOLISM, EXCRETION AND BIODISTRIBUTION OF SILICA PARTICLES

Many studies have been devoted to investigation of the mechanisms of SNP biodegradation and clearance both *in vitro* and *in vivo*[92–97]. The most critical particle characteristics that determine their behaviour in a biological system are surface chemistry, pore size/porosity, degree of silica condensation, and particle shape/size [92,93]. These factors have direct influence on the interaction with serum proteins, the cytotoxicity profile, the degradation kinetics, and clearance of SNP. Typically, most SNP undergo hydrolytic degradation, metabolism *via* the hepato-biliary route and are renally excreted (Figure 2).

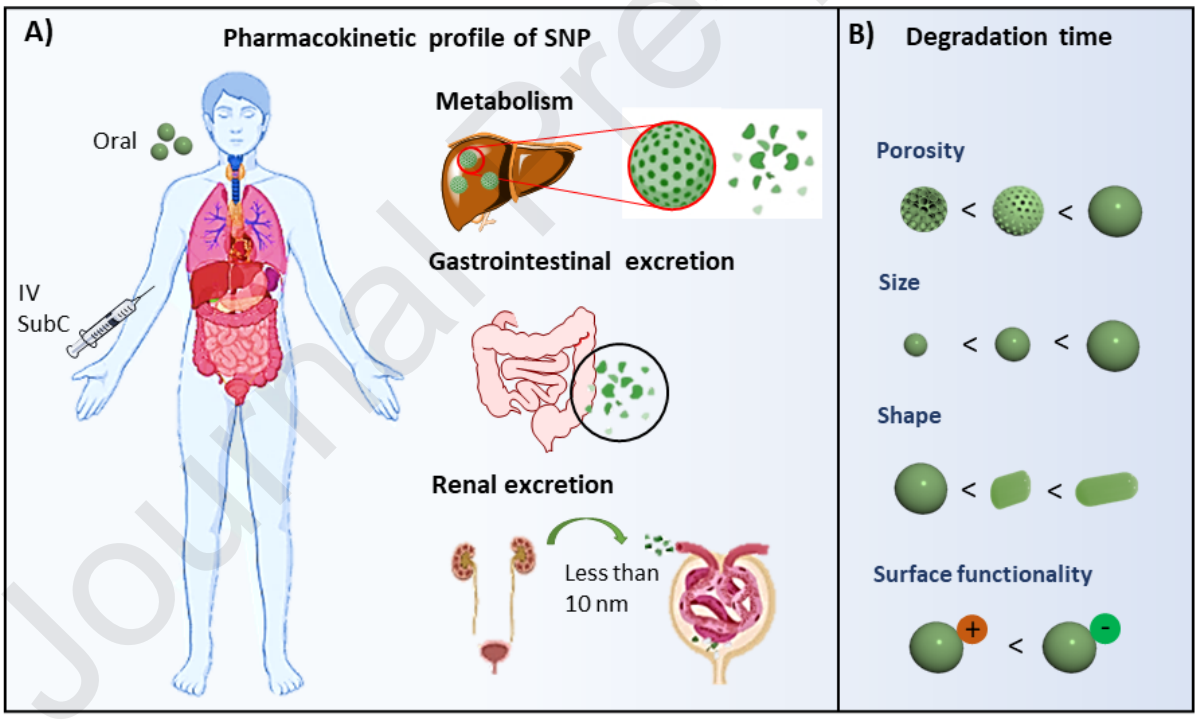

Figure 2. Schematic diagram of (A) Pharmacokinetic profile of SNP. I.V.: intravenous; SubC: subcutaneous. (B) Parameters that impact the degradation time of silica nanoparticles include its porosity, size, shape and surface chemistry. Figure created with Bio-render and Chemdraw.

The rate of biodegradation is dependent on numerous factors. For the dissolution of SNP in biological environment, the breakdown of Si-O-Si bonds takes place through nucleophilic attack by OH^- ions in alkaline conditions [98]. The dissolution product is silicic acid or poly-silicic acid. Other than biological conditions such as pH, SNP degradation rate was shown to be dependent on porosity [91,98], size [98], shape [93,99] and surface charge [100] (Figure 2). However, for MSN, the main factors controlling the dissolution rates are specific surface area, and the degree of silica condensation [93,98,101]. Furthermore, the presence of additional organic functional group or even adsorbed proteins have an influence both on the dissolution rate and on the dissolution mechanism [97,101,102]. One commonly used strategy involves coating with hydrophilic polymers such as poly-ethylene-glycol (i.e., PEGylation) to limit serum protein adsorption on SNP and to improve colloidal stability and circulation time [103,104]. SNP that are conjugated with specific ligands may lose cell-specific targeting ability due to the shielding effect caused by the protein corona formation [105]. Protein corona are formed when nanoparticles bind with proteins in biological environment, which alters the physiochemical properties of nanoparticles and affects their subsequent interactions with biosystems [106,107]. In biological systems, nanoparticles (NPs) undergo significant transformations. Initially, a phenomenon referred to as the "soft corona" takes place as proteins are weakly adsorbed onto the NP surfaces through noncovalent interactions. Subsequently, over time, these weakly adsorbed proteins are displaced by more tightly bound molecules, leading to the formation of a "hard corona". It is important to note that the hard corona exhibits prolonged stability with a considerably longer lifetime, whereas the soft corona displays a more dynamic nature with significantly shorter lifetimes [107]. The formation of protein corona results in enhanced recognition of the particles by the innate immune system, ultimately facilitating their rapid clearance by phagocytic cells residing in organs including the lungs, liver, and spleen[108] .SNP shape is another critical factor that influences the biodistribution, metabolism and excretion from the body. It has been reported that spherical SNP are cleared at faster rate than rod-shaped SNP [109]. In a study by Li *et al.,* using *in vivo* murine model, it was reported that when orally administered at a dose of 40 mg/kg, the rod-shaped SNP had longer retention time due to their reduced rate of uptake by the reticulo-endothelial system (RES) [109]. Moreover, in comparison to the spherical SNP, the rod-shaped SNP tend to have slower rate of absorption in the gastrointestinal tract, degradation, and renal excretion [109]. Similar evidence was reported by Hao *et al*. who found that MSN with different aspect ratios (AR) exhibit different degradation in simulated media (intestinal and body fluids). The spherical nanoparticles (AR=1) indicated faster dissolution than the rod-shaped MSN (AR=2

and 4) in vitro [110]. This attributed to the higher surface area of spherical nanoparticles than the rod-shaped nanoparticles. Their fundings helped to explain the higher absorption rate in gastrointestinal tract, degradation, and renal excretion in previous studies [110].

Surface charge of SNP is an important parameter controlling the interaction with serum protein. Souris *et al.*, reported that cationic SNP were more likely to interact with serum proteins, and therefore rapidly excreted via the hepatobiliary route *in vivo* compared to anionic SNP [100]. Similarly, Clemments *et al.* reported that the surface charge of MSN determines the extent and variety of protein corona formation [104]. After exposure to 10% fetal bovine serum (FBS), cationic amino-functionalised MSN not only had the largest variety of proteins adsorbed but also the highest amount of proteins in the hard corona when compared to bare or carboxylic acid-functionalised MSN [104]. As most of the proteins found in FBS typically are anionic this could possibly explain the augmented protein corona formation with cationic MSN [104]. Marichal *et al.* found that the extent of protein adsorption was generally dependent on SNP size and smaller SNP (8.3 nm and 33.0 nm versus 78.0 nm) had less adsorbed proteins per surface unit [111]. Interestingly, Pochert *et al.* reported that in addition to factors such as surface charge and morphology of SNP, the loaded agent or therapy can also control its subsequent biodistribution [112]. Using *in vivo* murine models, the researchers reported that hollow MSN when loaded with contrast agent perfluoro-15-crown-5-ether (PFCE) had exclusive hepatic accumulation but the non-loaded hollow MSN were also found in other organs such as spleen or lung [112]. The difference in varying degree of organ accumulation in PFCE loaded versus unloaded hollow MSN was attributed to differences in protein corona formation [112]. Proteins such as apolipoproteins A-1 and A-2 were enriched on PFCE-loaded hollow MSN when compared to unloaded hollow MSN [112]. While many studies have focused on nanoparticle protein corona formation in serum and its subsequent influence on it biodistribution, this effect has also been observed in the digestive system [113–115]. It should be noted that it is a difficult task to accurately calculate the makeup of a protein corona formed on SNP due to its complicated and dynamic nature. Nevertheless, as the protein corona inevitably have an influence on the interactions between the NPs and the biological system, analysis of the protein corona composition under relevant conditions should be part of preclinical testing of SNP in order to allow the identification of predictors for their metabolism, excretion and biological performance [105].

Renal clearance of nanoparticles is desirable, as it not only excretes substances circulating in the blood stream rapidly but also avoids production of metabolites which potentially could be

toxic [116]. Depending on the route of administration and dose, silica species are either excreted through the gastrointestinal tract or are renally cleared. When compared to hepatobiliary clearance, the renal route is considered desirable, as it is rapid and thereby avoids long term retention and accumulation of SNP in tissues and organs. For renal clearance, SNP below the 10 nm diameter threshold are rapidly excreted in urine as evidenced by clinical trials of SNP in human (NCT03465618, NCT01266096 and NCT02106598) [5,10,11,117,118]. The renal clearance of SNP is dependent on both clearance across glomerular filtration system made up of the podocyte expansions with threshold of 10 nm and also bypassing reabsorption that occurs in proximal and distal renal tubules [119]. However, nanoparticles which has larger size (> 10 nm) cannot be easily cleared out by kidney as they cannot penetrate through the glomerular filtration barrier (GFB) [120]. Generally, SNP needs to break down to less than 10 nm to eliminate through urine clearance. However, by interacting with GFB, certain types of nanoparticles can cross the GFB and have been detected in urine using in vivo model [121,122]. Furthermore, the interaction of SNP with other circulating blood cells can also have an impact on their renal clearance rate [119]. Factors that influence SNP reabsorption from proximal and distal renal tubules are still poorly understood. However, it is known that glomerular filtration membrane is a charge-selective barrier [123,124]. The renal filtration system is made up of negatively charged membranes which both limits the filtration of anionic nanoparticles and also any reabsorption [123,124]. In contrast, nanoparticles that are neutral or positively charged are more likely to be reabsorbed back into blood circulation as compared to negatively charged nanoparticles [125,126].

Study of the biodistribution and clearance of SNP in a quantitative manner is crucial for evaluation of biological safety and authority approval before moving into clinical studies. Nevertheless, among the huge number of SNP with diverse structures reported to date, there is a lack of sufficient *in vivo* biodistribution or clearance data. Moreover, due to understanding of the factors that affect the biodistribution of MSNs is complicated due to the paucity of studies which have conducted head to head comparison using similar SNP's and often differ in the animal models used, type of SNP with varying dose and route of administration used in the biodistribution studies (Table 1) [92], a concrete structure-biodistribution relationship is yet to be investigated and understood. To this end, quantitative or semi-quantitative imaging techniques such as PET, single-photon emission computed tomography (SPECT) and fluorescence imaging can enable precise tracking of SNP biodistribution and elimination of SNP from the body [92,96]. These imaging techniques offer non-invasive visualisation and

quantification of the spatial and temporal distribution of SNP. Quantification of SNP accumulation in specific anatomical locations may also aid in understanding the systemic behaviour. By using complementary techniques in pharmacokinetic studies of the biodistribution and clearance of SNP in the body, comprehensive datasets, and understandings of how the structural parameter of SNP affect their distribution, metabolism and excretion of SNPs will be obtained, which are important for the clinical translation of SNP. A detailed discussion on this aspect goes beyond the scope of this work and biodistribution and clearance has been the topic of other excellent reviews [92,93].

Table 1. Summary of studies conducted in various in healthy animal models to establish the long-term safety of silica nanoparticles after multiple administrations from variety of different routes.

Type of silica nanoparticle	Size and modification of nanoparticle	Animal model	Route of administration	Duration of nanoparticle administration	Dosage	Purpose	Study outcomes	Reference
Amorphous silica	20 to 60 nm diameter	Wistar rats	Oral	92 days	0.1, 1.0, 10, or 100 mg/kg	Influence of silica nanoparticles on the immune function	NOAEL up to 100 mg/kg	[127]
Colloidal silica	20 nm and 100 nm diameter	Sprague-Dawley rats	Oral	90 days	2000, 1000 or 500 mg/kg,	Toxic effects of repeated oral administration	No toxic effect observed in the organs and tissues studied NOEL more than 2,000 mg/kg	[128]
Non-porous and mesoporous amorphous silica	100 and 300 nm methyl-coated diameter	Female Swiss mice	Oral	5 days	100 or 1000 mg/kg	Effects of repeated oral administration to induce local or systemic toxicity	No toxicological effects induced	[129]
Non-porous and mesoporous silica	Nonporous 50 nm diameter and 500 nm diameter Mesoporous 500 nm diameter	Immune-competent BALB/c mice	Intravenous	180 days	40 to 100 mg/kg	Sub-chronic toxicity after repeated injections	Nonporous 50 nm MTD of 103 ± 11 $mg.kg^{-1}$ for female and 100 ± 6 $mg.kg^{-1}$ for male mice. Nonporous 500 nm MTD of 303 ± 4 $mg.kg^{-1}$ for female and 300 ± 13 $mg.kg^{-1}$ for male Mesoporous 500 nm showed MTD 40 ± 2 $mg.kg^{-1}$ to 95 ± 2 $mg.kg^{-1}$ for male and female mice	[130]
Colloidal silica	20 nm diameter with L-arginine coating	Sprague-Dawley rats	Topical	90 days	2000 mg/kg	Toxic effects after repeated topical administration	No toxicity or organ damage reported	[131]
Mesoporous silica nanoparticles	short rod 185 nm length, and long rod length 720 nm	ICR mice	Intravenous	18 days	20 mg/kg	Toxic effects after repeated dose post injection	No significant *in vivo* toxicity. Short rods are cleared faster than long rods.	[99]

Abbreviations: No observed adverse-effects level (NOAEL), Maximum Tolerated Dose (MTD)

4.2 BIOCOMPATIBILITY AND SAFETY

Humans are frequently exposed to silicon since roughly 75% earth crust is made up of silicate materials [132]. In fact, foods such as vegetables and fish contain large amounts of silica, and silicon is considered an essential element in the diet [133], and silicates are an important component of bone, hair and skin [93,134,135]. However, silicosis, a long-term lung disease caused by inhaling large amounts of crystalline silica dust, is well documented [136,137]. In stark contrast, amorphous forms of silica particles have not shown to cause silicosis [136]. Amorphous silica is generally recognised as safe by numerous regulatory authorities such as Therapeutic Goods Administration Australia (TGA) [138], European Medicines Agency [139] and United States Food and Drug Administration [140]. Focusing more directly on mesoporous amorphous silica, it was recently shown in a human study involving 20 male adults that oral consumption of up to 9 grams/day of porous rod-shaped silica particles (1-3 x 0.4-0.5 micrometres with pore sizes 7-13 nanometre) for a duration of 21 days had no safety concerns nor severe side effects (NCT03667430) [141]. In another clinical trial, SiPore15™, a micron-sized silica particle, was given to 43 participants in doses of up to 3 grams/day for 12 weeks with no safety concerns observed (NCT03823027). These porous silica particles are currently undergoing clinical testing aimed at inducing weight loss in obese populations (NCT03667430 and NCT03823027) through adsorption of gastrointestinal lipase enzymes. The lipase enzymes are responsible for breakdown of fats, and adsorption of lipases can help reduce the absorption of fat into the blood circulation to ultimately reduce dietary fat intake [141].

When the particle size of SNP is reduced to nanoscale [142,143], their toxicity needs further investigation. The daily oral human consumption of SNP, including food additives such as E551, a solid 100 nm SNP, has been estimated to be between 0.28 and 4.53 mg/kg [144]. SNP are also used in some cosmetic products, such as lipsticks [28,145]. As a consequence, in the recent years, the human exposure to SNP has significantly increased [143,146].

Since the introduction of SNP in the biomedical field, numerous cell lines have been tested *in vitro* to demonstrate the safety of amorphous SNP, including epithelial [147], endothelial [148,149], fibroblast [147,150] and immune cells such as dendritic cells [151,152], macrophages [151,153,154] and T-cells [151,155]. The safety of SNP has also been investigated in animal and human. Indeed, data from both animal (Table 1) and human studies (Table 2) demonstrate good safety. SNP safety profile has been evaluated in humans with evidence available from 13 clinical trials (Table 2) (Figure 3) [6–12,141]. Six trials have already been completed with results demonstrating excellent biocompatibility of SNP (Table 2). A further six trials are ongoing, with one study which has progressed into phase II (NCT02106598). A summary of case studies of SNP in clinical trials has recently been published by our group [5].

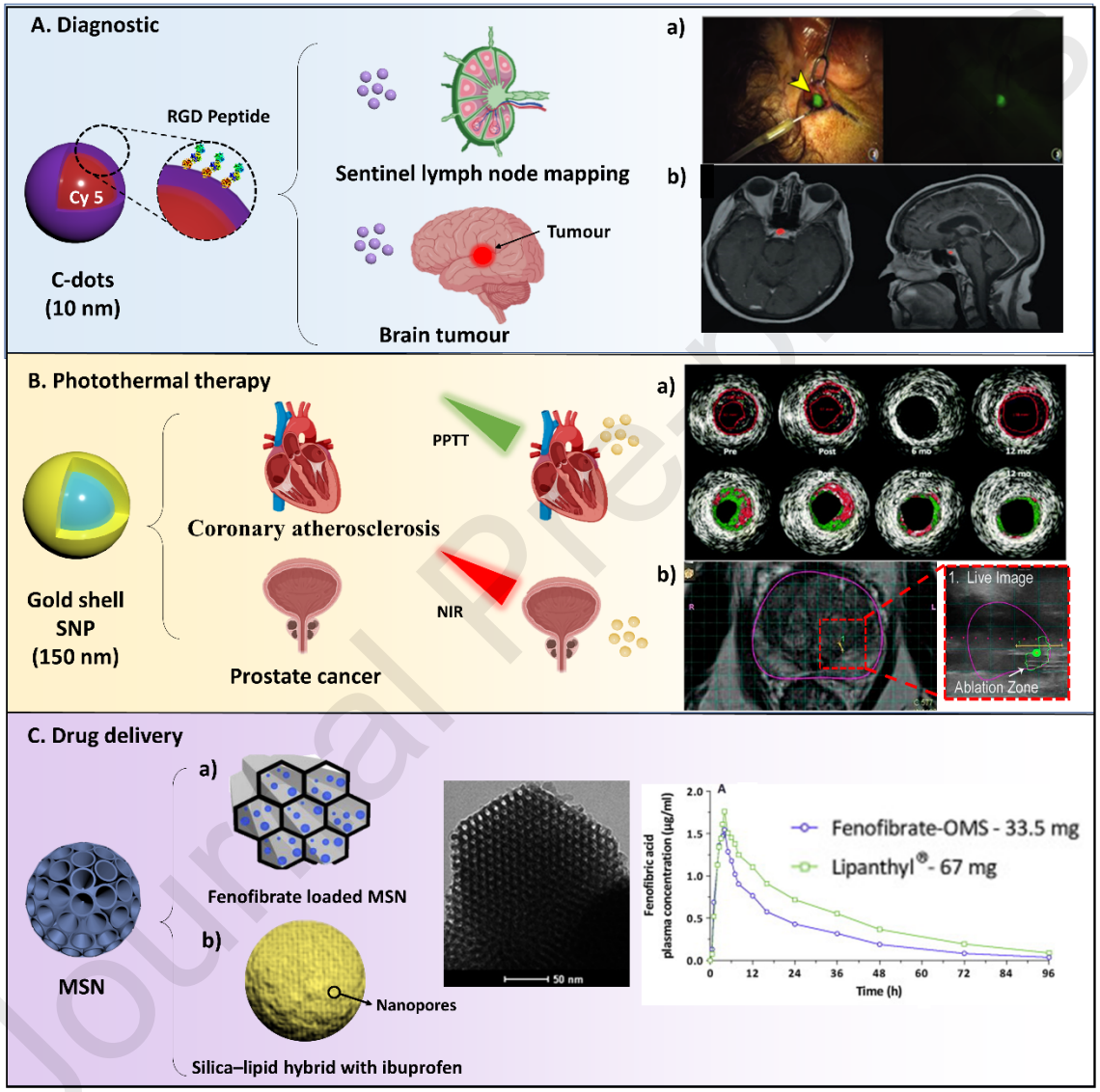

Figure 3. Human studies using different types of silica nanoparticles (SNP) for application in diagnostic, tumour ablation and drug delivery. (A). Cornell dot – 10 nm SNP with Cy5.5 fluorescence dye and tumour homing Arg-Gly-Asp-Tyr peptide [79]. *(A, a). Cornell dots could identify the sentinel lymph nodes in patients with head and neck melanoma, which overcame current probe limitations image, reprinted from Zanoni DK et al. 2021 JAMA Network Open.* [10]. *(A, b). Renally excreted Cornell dots were used to image with pituitary tumour, image reprinted with permission from Phillips et al. 2014, American Association for the Advancement of Science* [11] *(B). Silica nanoparticles with gold shell. (B, a). Plasmonic photothermal therapy and the delivery of nanoparticles could significantly reduce coronary atherosclerosis, image reprinted with permission from Kharlamov et al. 2015 Royal Society of Chemistry Publishing* [6] PPTT: plasmonic photothermal therapy. *(B,b). The treatment of prostate cancer using gold-silica nanoparticle based photothermal cancer therapy, image reprinted with permission from Rastinehad et al. 2019 National Academy of Sciences.*[8]. *(C). Clinical studies in human for delivery of poorly soluble drugs like fenofibrate and ibuprofen using SNP. (C, a). Mesoporous silica enhanced bioavailability of hydrophobic fenofibrate in human study, image reprinted with permission from Bukara et al. 2016, Elsevier* [12]. *(C, b). Lipid and silica nanoparticle based formulation proved safe and effective for oral absorption of poorly water-soluble compounds* [9]. *Created with BioRender.com*

Many studies highlight that SNP have minimal toxicity *in vivo* despite chronic administration, as summarized in Table 1. For instance, Ryu *et. al.*, conducted a large study of 100 Sprague Dawley rats with chronic exposure of SNP. SNP with a diameter of 20 nm SNP and with doses up to 2000 mg kg^{-1} were applied on the hairless back skin of rats fixed with gauze [131]. The treatment lasted for 6 hours, and it was repeated daily for 90 days. Long-term expose did not cause any toxicity nor any change in internal organs, which indicated the safety of silica nanoparticles upon dermal administration [131]. In another study, Kim *et. al.* orally administrated colloidal SNP of 20 and 100 nm in Sprague-Dawley rats over a period of 90 days, and found that doses of up to 2000 mg kg^{-1} showed no signs of toxicity despite chronic exposures [127].

The effect of long term-exposure of SNP in humans is still unknown. However, results from phase I and II clinical trials give support for the biocompatibility and safety of SNP in humans (Table 2) [5]. It is noteworthy that chemical composition of the SNP used in most of the long-term animal studies have used pure SiO_2, while most particles applied for human studies had surface-functionalized SNP which may influence their safety (Table 1 and 2). As new silica-based nanomaterials are developed, their long term toxicity may need to be tested using high-throughput assays in the latest organ- or human-on-chip models [156–158]. Eventually, this can be substantiated by evidence from clinical studies that elucidate the effects of chronic exposure of SNP in humans. In light of this, it is essential to understand the mechanisms of SNP metabolism and excretion, and to investigate the influence of SNP on various biological barriers.

Studying the safety of new materials such as SNP should involve a comprehensive approach that needs to go beyond standard methods such as blood tests and histology. Firstly, the SNP need to be adequately characterised for their physicochemical properties. Size, shape, surface charge, surface composition and morphology can all influence SNP's subsequent interaction with biological barrier and safety profiles [92,159,160]. In this case, it would be pertinent to adhere to minimum reporting standards for nanomaterials [159]. Secondly, it is crucial to ensure in both animal and human studies to report SNP's effect on hemocompatibility, reproductive toxicity, induction of oxidative stress, genotoxicity, carcinogenicity, mutagenicity, organ-specific toxicity, and immunogenicity. For instance, there is a possibility of SNP triggering immunogenic reactions that is dependent on their compositions, which could be evaluated with tests such as monitoring antibody production, cytokine profiling, and assessing immune cell activation. However, the initiation of an immune response by SNP carries both advantages and disadvantages. The utilization of distinct surface-functionalized silica nanoparticles presents a promising avenue for the application of allergen-specific immunotherapy. Through tailored surface characterizations, functionalized SNPs have the potential to stimulate heightened micropinocytosis uptake [161]. In cancer treatment, proper surface modification could trigger antitumor immune responses and enhance immunogenic cell death of tumour cells [162]. Moreover, organ-specific toxicity is highly dependent on the specific route of SNP administration. For instance, inhaled SNP are more likely to induce pulmonary inflammation compared to orally administered [163].

Organ-specific toxicity can be evaluated using imaging modalities, such as MRI or computed tomography (CT), which can show anatomical images of specific organs affected by SNP exposure [164,165]. Utilisation of imaging techniques to study safety of SNP could be very useful. SNP can be easily radiolabelled with ^{64}Cu, ^{45}Ti or ^{89}Zr providing long half-life for long term biodistribution and toxicity studies with positron emission tomography (PET) scanning [92]. By utilising imaging techniques, one can easily obtain a real-time, non-invasive, and quantitative information on the behaviour of SNPs within the human body. This information complements other safety assessment methods, providing a more comprehensive understanding of SNP toxicity, biodistribution, and potential risks post-administration. However, the quality and reliability of studies can vary significantly. The inherent variability in the quality and methodology, and rigor employed across various studies can lead to

inconsistent findings when assessing the safety of SNP. Therefore, it is pertinent to report and utilise a combination of various methods, to gain a comprehensive understanding of SNP safety profile.

Table 2. Summary of studies conducted in human subjects using silica nanoparticles

Study start date	Type of Silica nanoparticle	Size and modification of nanoparticle	Number of participants	Route of administration	Disease/ condition	Purpose	Study outcomes	Phase	Status	Trial registration number or reference
April 2007	Silica-gold iron-bearing NP	60/15–70/40 nm core/shell silica–gold NP	180	Intracoronary infusion	• Stable Angina • Heart Failure • Atherosclerosis • Multivessel Coronary Artery Disease	Plasmonic photothermal therapy of flow-limiting atherosclerotic lesions with silica-gold nanoparticles: a first-in-man study	• Total atheroma volume • Major adverse cardiovascular events free survival	Not Applicable	Completed	NCT01270139
April 2008	Silica nanoparticles with gold shell (Aurolase™)	Silica core and a gold shell. Spherical 150 nm	11	Intravenous injection	• Head and Neck Cancer	Photothermal ablation of recurrent or refractory tumour	• Adverse events	Not Applicable	Completed	NCT00848042
December 2010	Gold nanoparticles with silica-iron oxide shells	60/15–70/40 nm core/shell silica–gold NP	62	Intracoronary infusion	• Coronary Artery Disease • Atherosclerosis	Plasmonic photothermal and stem cell therapy of atherosclerosis versus stenting	• Total atheroma volume	Phase 1	*Terminated	NCT01436123
January 2011	Silica nanoparticles	10 nm 124I-labeled cRGDY	10	Intravenous injection	• Melanoma • Malignant Brain Tumours	PET Imaging of Patients with melanoma and malignant brain tumours	• Characterise biodistribution, pharmacokinetics, and metabolic stability of nanoparticles	Not Applicable	Active, not recruiting	NCT01266096

April 2014	Silica nanoparticles	10 nm cRGDY-PEG-Cy5.5-C-dot	105	Injection intra-tumour	• Head and Neck Melanoma • Breast Cancer, • Colorectal Cancer	Targeted silica nanoparticles for real-time image-guided intraoperative mapping of nodal metastases	• Possibility of conducting pre-operative sentinel lymph node mapping	Phase 1/2	Recruiting	NCT02106598
February 2016	Silica nanoparticles with gold shell (Auroshell)	150 nm spherical silica core and a gold shell.	45	Intravenous injection	• Neoplasms of the Prostate	Neoplasms of the Prostate	• Efficacy of focal ablation • Side effects	Not Applicable	Completed	NCT02680535
March 2018	Silica nanoparticles	10 nm 89Zr-cRGDY-Cy5 C-dot	10	Intravenous injection	• Malignant primary brain tumour or known metastatic cancer with brain lesion	PET scans for detecting brain tumours.	• Distribution of nanoparticles in high-grade gliomas	Phase 1	Recruiting	NCT03465618
November 2019	Silica nanoparticles	6.2nm Sphere NH_2 functionalised PEG-Cy5.5-C' dots	10	Intravenous injection	• Prostate Cancer	Use of nanoparticles to guide the surgical treatment of prostate cancer	• Side effects	Phase 1	Recruiting	NCT04167969
January 2019	Silica nanoparticles	Aerosil 300 and Syloid 224.	12	Oral	• Hyper cholesterolemia	Use of nanoparticles to improve pharmacokinetics profile of simvastatin	• Bioavailability	Phase 1	Completed	ACTRN12618001929291
January 2020	Silica nanoparticle with gold shell (Auroshell)	150 nm spherical silica core and a gold shell	60	Intravenous injection	• Neoplasms of the Prostate	Nanoparticle mediated focal therapy for ablation	• Efficacy of focal ablation • Side effects	Not Applicable	Recruiting	NCT04240639
Unknown	Silica nanoparticles	282 nm silica–lipid hybrid made of Aerosil 380 fumed silica (diameter 7 nm)	16	Oral	• Healthy male adults	Bioavailability and tolerability studies of a silica–lipid hybrid loaded with ibuprofen	• Effectiveness and tolerability	Not Applicable	Completed	[9]
Unknown	Mesoporous silica nanoparticles	500 nm hexagonal nanoparticle	12	Oral	• Healthy Caucasian adults	Mesoporous silica to enhance the bioavailability of fenofibrate	• Bioavailability	Not Applicable	Completed	[12]

	with 5.8 nm pore size

*NCT01436123 terminated due to political pressure by the Federal Security Service of the Russian Federation.

5 INFLUENCE OF SILICA NANOPARTICLES ON BIOLOGICAL BARRIERS

5.1 BLOOD-BRAIN BARRIER

The blood-brain barrier (BBB) is one of the most restrictive and complicated biological barrier to permeate across [54,166–168]. BBB is composed of brain endothelial cells, surrounded by a network of pericytes and astrocytes. The brain endothelial cells are connected to each other via tight and adherent junctions [167]. This distinctive architecture of BBB is responsible for controlling brain homeostasis and movement of substances into brain parenchyma [167]. The complex cellular architecture of the BBB with presence of tight junctions and lack of any fenestrations represents a significant challenge to transport therapeutics across BBB. This leads to a restrictive criterion for passive permeation across BBB limited to small size (< 500 Da) and/or lipophilic molecules [169]. Other substances that can permeate BBB require active transport via specific transporters [169]. The BBB, while necessary for preventing neurotoxins entering in the brain, also creates challenges for diagnostics and therapies to access the brain tissues.

Many researchers have shown that SNP are an emerging platform for transporting therapeutics and their unique properties are particularly important at the interface of the BBB (Figure 4) [54,73,170–172]. SNP can interact with the BBB via several mechanisms, including (a) inducing transient relaxation of tight junctions and acting as a permeation enhancer; (b) transcytosis of SNP from brain endothelial cells; (c) endocytosis of SNP from luminal side of brain endothelial cell followed by exocytosis into abluminal side [173–176]. In addition to these routes, SNP surface can easily be modified to enable receptor-mediated transport across the BBB. For instance, *in vitro* and *in vivo* testing of large pore SNP conjugated with lactoferrin have demonstrated an improved utility of chemotherapy agent such as temozolomide and doxorubicin, by utilising overexpressed lactoferrin receptors present on the BBB [172,177]. Other researchers have shown that SNP can be easily decorated with BBB-homing targeting moieties such as folic acid [178,179], transferrin [180–182], and lactoferrin [172,177,183] as these receptors are overexpressed on the BBB.

As the BBB is a very restrictive barrier, the size of nanoparticles plays an important role in permeation [168]. For example, Cornell dots, a 6-10 nm fluorescently tagged SNP, have shown excellent penetration across the BBB with tumour homing properties for the purpose of tumour mapping in clinical trial [10,11]. Mo *et. al.,* found that when compared to larger diameter MSN (80 nm), smaller-diameter MSN (20 and 40 nm) showed higher accumulation in *in vitro* BBB models [170]. In another study, this effect was also observed *in vivo* in a BALB/C murine model, where larger PEGylated MSN (160 nm) demonstrated high non-specific binding while smaller PEGylated MSN (50 nm) displayed specific targeting to transferrin receptors in the BBB when conjugated with an Ri7 antibody [73]. As a consequence, there has been a focus in generating smaller sized SNP for central nervous system (CNS) and delivery to other cancers in the brain [184].

Other parameters such as surface chemistry of SNP also play vital role in predicting the SNP interaction with the BBB. Using transgenic larval zebrafish Tg(zfli1:EGFP) *in vivo* model, Chen *et. al.,* established that a high anionic surface charge on MSN is crucial for penetration into the brain [185]. It was found that MSN which were more anionic (−20 mV and −40 mV versus +18 mV and +42 mV) and smaller (50 nm versus 200 nm) had the highest accumulation in zebrafish brain [185]. Notably, upon analysis of the protein corona surrounding these mesoporous silica nanoparticles (MSNs), it was observed that the MSNs with highly anionic phosphonate functionalization (-40 mV zeta potential) and small hydrodynamic diameter (50 nm) showed the greatest abundance of proteins associated with blood-brain barrier (BBB) penetration, including basigin, afamin, and apolipoprotein E. This observation was found to be a contributing factor to the enhanced penetration of the BBB [185]. These findings therefore provide an alternative strategy of modulating SNP surface chemistry to achieve BBB targeting, instead of conjugating large BBB-targeting proteins such as lactoferrin, albumin, transferrin onto SNP which can increase the overall size of nanoparticle. In addition, the protein corona can render SNP targeting ability to BBB useless even if targeting moiety is properly conjugated, unless its effect are not properly elicited on its corresponding receptors [186]. Salvati *et. al.,* used transferrin-conjugated, PEGylated SNP and found that the protein corona formation on SNP prevents transferrin from binding to the target receptors [186]. Thus, while SNP offer ease of conjugating with targeting moieties, it may not necessarily translate in BBB-specific targeting because of SNP interactions in complex biological milieu upon administration.

The blood circulation time of SNP also needs to be sufficient to allow optimum BBB penetration. In the field of nanomedicine, it remains a significant challenge to ensure accumulation of nanoparticles into the brain parenchyma, because of their short plasma half-life and ensuing clearance by the RES [8]. The short plasma half-life of many nanoparticles is responsible for reducing the exposure time for nanoparticles to interact with BBB and therefore limits their penetration into the brain. As discussed earlier, PEGylation has been shown to prolong plasma circulation time by minimising SNP aggregation and limiting protein corona formation [104,187]. Indeed, in a study using Wistar rats as an *in vivo* model, Ku *et al.* demonstrated that PEGylation of fluorescein-doped magnetic SNP (80–90 nm diameter) enhanced permeation across the BBB via transcytosis across the brain endothelial cells when compared to their non-PEGylated SNP counterparts [187]. Overall, modulation of key parameters such as anionic surface charge, small size (less than 40 nm), optimal plasma circulation time with sufficient BBB targeting and controlling the protein corona enable SNP to effectively interact with BBB for both drug delivery and diagnostic purposes. The use of ultrasmall silica nanoparticles in BBB and other CNS diseases is still in infancy and more research needs to be done to establish key parameters and the optimum physicochemical properties for faster clinical translation. Additionally, studies testing various type of SNP in overcoming BBB using *in vitro*, brain-on-chip and 3D spheroidal models (from human brain cells) could pave the way for rapid progress in this field.

5.2 MUCOSAL BARRIER

5.2.1 Gastro-intestinal

The oral route of administration is considered the most desirable as it provides a non-intrusive delivery of therapies and offers high patient acceptability [188]. However, many therapeutics and diagnostic tools suffer from poor oral absorption issues due to the hostile nature of gastro-intestinal (GI) tract. The presence of harsh gastric pH, digestive enzymes, mucus barrier and tight junctions control the movement of substances that enter the systemic circulation [189]. The GI tract mucus has been widely studied and its major component mucins are composed of 95% water, 3% highly glycosylated proteins, and 2% of other molecules [190]. The glycans on the mucin domain form gel-like domains by binding to large amounts of water present in the gastro-intestinal tract. Generally, there are two types of mucins, transmembrane mucins and gel-forming mucins. The transmembrane mucins are usually anchored at the apical side of epithelial cells (e.g., MUC1, MUC3, MUC4, MUC12, MUC13, MUC16, MUC17) while the

gel-forming mucins (e.g., MUC2, MUC5AC, MUC5B, MUC6 and MUC7) form the mucus barrier in the GI tract [191–194]. The main functions of mucins are protection, lubrication and signalling [195].

Along the GI tract, mucus is found to have different consistency depending on the location i.e., mouth, stomach, small intestine, and large intestine. The mucosa is made up of two layers in the stomach and large intestine: a luminal stirred layer that is rapidly cleared and a basal unstirred layer which is slowly cleared [196]. The outer layer loosely adheres and the literature suggests that the outer layer is the main obstacle for the oral administrated drugs [197]. However, the inner firm layer aids in the uptake of drugs [198]. In the small intestine, the mucus only has a single layer and it is loosely attached [193]. The mucus layer in the small intestine and large intestine are both mainly formed by MUC2, but the small intestine mucus layers are kept sterile. The small intestine usually flushes bacteria with unbounded loose mucus into the colon [199]. In the small intestine, the thickness of the mucus ranges from 15 to 450 μm, which usually has a turnover process lasting 4-6 hours in healthy humans [200]. The mucus is a complex dynamic hydrogel which mainly composed of cross-linked and entangled mucin fibres which have 0.5–40 MDa in size, and contains other proteins, carbohydrates, lipids, bacteria, and cellular debris [201,202]. Importantly, the dynamic viscoelastic and shear-thinning nature of mucins is one of the main reasons for negatively influencing drug absorption [203]. In addition, the negatively charged glycosylation of mucins can reduce the viscosity at high shear rates, which decreases the tight contacts between the fibres [203]. Oral SNP delivery presents a unique opportunity not only to protect the cargo in nanoporous framework from the harsh GIT conditions described above but also to alter the permeability across mucus and epithelial barrier to improve oral drug delivery especially for biologics.

Permeation enhancers are commonly used to improve efficacy and palatability of orally administrated drugs especially for macromolecules. Chemical permeation enhancer such as detergents and surfactants disrupt the lipid bilayer on the cell membrane to achieve transcellular transport, while fatty acids open the tight junctions to improve paracellular biologics transport. However, those chemicals can be irritating to gastrointestinal tract [189]. In this context, SNP have been used for oral drug delivery due to their ability as a gut permeation enhancer (Figure 4) [189,202,204]. Lamson *et al.* showed that small sized (50 nm- 200 nm) negatively charged SNP instigate the oral delivery of impermeable insulin protein by improving intestinal permeability [205]. However, when the size of SNP is reduced to 20 nm, it was found to be too small to penetrate the mucosal barrier as they interact with the mucus and subsequently these

SNP are trapped in the mucus barrier [205]. Moreover, it was also reported in the same study that SNP can exert their influence on GI permeability by binding and stimulating the integrin receptors. When these receptors undergo binding, they have the capacity to trigger diverse signalling pathways, each of which initiates the activation of the enzyme known as myosin light chain kinase (MLCK). Upon activation, MLCK phosphorylates the myosin component within the cellular cytoskeleton, thereby inducing cytoskeletal contraction. This contraction, in turn, exerts tension on tight junctions, ultimately leading to their disruption and subsequent opening.[205]. Furthermore, Abeer *et al.,* found phosphonate-modified large pore dendritic silica nanoparticles (PDSNPs) (254 ± 7 nm diameter, pore size around 11 nm) enhanced exenatide loading and penetrate through Caco-2 monolayer by 1.7 fold compared with free exenatide [19]. Ndayishimiye *et al.,* also demonstrated that vancomycin-loaded large pore SNPs (105 ± 10 nm diameter, pore size 9.2 nm) had prolonged release and negatively charged large pore SNPs enhanced the permeability of vancomycin across the epithelial cell monolayer [68]. More recently, Cao *et al.* showed nanoscale surface roughness of virus-like silica nanoparticles (anionic surface charger and 60 nm diameter) could significantly enhance the permeation of macromolecule across the gut using *in vivo* mice models [206]. These findings suggest that optimally sized SNP (50 nm- 200 nm) can be useful permeation enhancer for biologics across the gastrointestinal barrier and enable permeation into systematic circulation. Furthermore, the surface charge of silica nanoparticles (SNP) is a crucial factor to consider as a permeation enhancer. Notably, negatively charged SNPs have shown more favourable effects compared to positively charged ones, likely attributed to the negatively charged mucus layer present in the gastrointestinal tract (GIT)[206]. Positively charged SNPs have the potential to interact with the mucus layer, which could lead to entrapment. SNP also owns the advantages that stable in acidic to neutral pH. Studies showed that 45 nm SNP were stable for at least 24h in pH 3-6 [207]. This phenomenon holds particular relevance in the context of oral drug delivery involving SNP, as it ensures the stability of SNPs within the highly acidic environment of gastric juice, thereby preventing their degradation. Beside the size, the shape of silica nanoparticles also impacts the systemic circulation and renal excretion. Li *et al.* found that with the increase of MSN aspect ratios (AR) from 1, 1.75 and 5, there was a decrease of *in vivo* MSN biodegradation administered at a dose of 40 mg kg^{-1} orally to ICR mice using a volume of 100 μL [109]. The spherical shaped MSN (AR=1) was 83 nm, the short rod MSN (AR=1.75) had an 83 nm diameter and 146 nm length, and the long rod MSN (AR=5) had a 96 nm diameter and 483 nm length. They showed similar zeta potentials within the range of − 20 to − 25 mV

and similar pore size about 2.8 nm. The spherical MSN showed the highest systematic absorption by small intestine [109].

Functionalized SNP have recently been shown to overcome the GI barriers [204,208]. Gao *et al.* utilized deoxycholic acid (DC)-modified MSN coated with sulfobetaine-12 (SB-12) to deliver insulin [209]. They showed that MSN-DC could be taken up by Caco-2 cells via endocytosis and avoid entering in lysosomes. After administering insulin formulations into diabetic rats, MSN-DC@SB12 significantly induced hypoglycaemic effect and reduced the blood glucose level to 45% after 1 h administration. After 6 h, MSN-DC@SB12 reached similar blood glucose level when compared to subcutaneously administered insulin group. These results indicate that insulin was gradually released from the DC coated carrier and diffused through the mucosal barrier rapidly. These zwitterionic functionalized MSN showed improved mucus penetrating ability and higher affinity with epithelial cells [209]. Moreover, SNP with mucoadhesive coatings, such as chitosan, sodium alginate or polyethylene glycol (PEG), have also shown promising results for oral protein delivery [204,210–212]. Andreani *et al.* compared the interaction between Stöber-type SNP coated with different mucoadhesive polymers (chitosan, sodium alginate or polyethylene glycol) and insulin [210]. They found that coating could increase the stability of the delivery system, but PEGylated nanoparticles decreased the thermal stability of insulin [210]. In another study by Tan *et al.,* large pore MSN (LMSN) were loaded with therapeutic proteins and peptides (TPPs) or cell penetrating peptide (CP) and further mixed and coated with PEG [211]. The LMSN used in the study had the hydrodynamic diameter of 167 ± 44 nm, pore size distribution in the range of 2 –11 nm and zeta potential of −31 ± 5 mV. After attaching PEG (molecular weight = 10k), the size of LMSN slightly increased to 186 ± 32 nm and zeta potential shifted to −13 ± 5 mV. It was found that CPP involvement mainly affected transport and exocytosis, and the PEG polymer significantly influenced mucus penetration and cellular uptake, which could further enhance the activity of the CPP in promoting uptake and exocytosis.

SNP offer an exciting avenue for oral drug delivery due to their capacity for effective mucus penetration and their influence on gut tight junctions. It would be ideal that the size of SNP is selected which is small enough to penetrate the gut barrier (50 - 200 nm) but not so small that they are trapped in mucus (< 20 nm). Rod-shaped SNP could be considered for local delivery in the GIT while spherical shape may be superior for permeation into systemic circulation. Anionic or zwitterionic surface charge is appropriate for effective mucus penetration while mucoadhesive polymers can improve the bioavailability of SNP loaded cargos. More studies

of silica nanoparticles with different surface characteristics and porosity to overcome such barriers need to be performed to address the issue of orally delivered biologics.

5.2.2 Respiratory mucosa

The respiratory system is another biological barrier which creates opportunity to bypass the first pass metabolism using conventional oral route. However, it is composed of complex system consisting of an epithelium layer that includes ciliated, goblet, brush, airway basal, and small granule cells. The airway is classified as ciliated pseudostratified columnar epithelium as all the cells lining it appear to form different layers but they all have contact with the basement membrane [213]. Respiratory mucosa provides moisture and protects the airway from inhaled dust, toxins, and pathogens. The presence of mucus and ciliary cells create a mucociliary clearance system which creates challenges for the retention of therapy in respiratory system. Conversely, in the disease state the mucociliary clearance is impaired which is also problematic as therapies are unable to access the deep lung tissues [214].

Nanoparticles, especially SNP, provide a promising solution for drug delivery to the lungs with potential to effectively avoid systemic adverse effects and drug resistance (Figure 4). Variations in the size of silica nanoparticles (SNP) dictate their penetration into distinct regions of the lung. For instance, particles measuring between 1-5 μm are capable of reaching deeper lung areas such as the bronchi, whereas those smaller than 1 μm can access the alveoli. Notably, for efficient alveolar targeting, the ideal size range for nanomaterials should be less than 500 nm[214]. In the context of lung delivery applications, silica-based nanomaterials exhibit passive accumulation within the lungs, primarily attributed to the high vascularity, permeability, and retention characteristics of these organs [215]. This inherent property positions them as promising nanocarriers for the advancement of novel therapeutic approaches in the management of lung diseases[216]. Recently, MSN (100 nm) surface-functionalised with isocyanatopropyl groups and conjugated with TNFR1 peptide were used to achieve targeted delivery of dexamethasone (glucocorticoid) to treat the sequalae of acute lung injury [217]. *In vivo* studies in CD-1 mice, demonstrated a significant reduction in inflammatory response and a marked decrease in dexamethasone associated side effects [217]. Additionally, in this study the researchers demonstrated that MSN were not only important for targeted delivery of dexamethasone but also could utilise MSN's intrinsic adjuvants properties such as ability to activate macrophages and recruit other immune cells for rapid healing at injured lung site [217]. Other researchers have used MCM-41 (160 nm diameter) with 3-mercaptopropyl and

pyridylthiol-terminated surface functionalisations for lung cancer therapy by co-delivery chemotherapies (doxorubicin and cisplatin) and siRNA (targeting MRP1 and BCL2 mRNA) to achieve suppression of cellular resistance in non-small cell lung carcinoma [218]. Surface functionalisation of the MCM-41 enabled conjugation with a siRNA and a cancer targeting ligand (LHRH peptide). In these *in vivo* experiments, NCR nude mice were administered SNP-based oncotherapy via inhalation which helped avoid the drug leaking into systemic circulation. These promising results with MSN could in future become a potential lung cancer therapy [218].

Despite the successful examples of SNP targeted lung delivery *in vitro* and *in vivo*, the safety and potential toxicity from respiratory route is not fully understood. For instance, there is some evidence which suggest that SNP can induce pulmonary fibrosis by initiating autophagic flux blockage in alveolar epithelial cells [219]. However, there is also evidence that lipid-based nanocarriers have higher accumulation and longer retention time in the lungs compared to SNP with inhalation therapy [220]. The longer retention time in respiratory mucosa may cause toxicity to the lung. As such, a balance between bioaccumulation and biodegradation of SNP in pulmonary delivery systems needs to be carefully managed. Additionally, there is a lack of direct evidence of SNP's interaction with lung mucus and epithelial barriers for infectious diseases or lung cancer. It is envisioned that SNP delivered via the nasal route could potentially accumulated in inflamed lung and could present lots of opportunities in overcoming lung barriers.

5.3 TUMOUR

In the field of oncology, nanomedicine is well established and many nanomedicines are being clinically used by cancer patients and many more undergoing clinical trials [3,221]. Because of the abnormal vasculature of tumour, nanoparticles can accumulate in tumour and subsequently be retained due to lack of lymphatic drainage [222]. Before reaching to the tumour site, intravenous injected nano-formulations need to pass through the blood circulation, sufficiently penetrate and accumulate in the tumour and finally elicit their action after intracellular drug release. To achieve this, desired nanoparticles should be able to selectively penetrate and accumulate deep into tumour tissue but not healthy tissue [223,224]. However, the complex tumour microenvironment and barriers limit the delivery efficiency. Thus,

sophisticated nanoparticles with designed size, shape, rigidity, charge, surface chemistry, have been developed for the targeted delivery of therapeutic drugs or imaging agents to solid tumours.

Tumour-penetrating ability was proven to be inversely proportional to the sizes of the nanoparticles. In general, due to enhanced permeability and retention (EPR) effect, nanoparticles with size between 10 nm to 200 nm could homogeneously penetrate deeply and passively accumulate at the tumour site [225]. For example, Tang *et al*. showed that 50 nm monodisperse silica nanoconjugates (Cpt–NCs) had better antitumor efficacy than 200 nm in C57BL/6 and BALB/c nude mice models [226]. This might be achieved by the faster tumour penetration, accumulation, and cellular internalization of smaller sizes SNP. Bouchoucha *et al.* further showed the size effect of SNP for effective drug delivery to tumour. The researchers developed dispersible phosphonated MSNs loaded with doxorubicin. Using an *in vivo* chicken embryos HT1080 fibrosarcoma tumour model, it was shown that after single intravenous administration, the tumor growth was inhibited significantly more with 45 nm MSN when compared to 150 nm MSN [227].

The surface charge of SNP also needs to be considered to meet the varying requirements in therapeutic agents' transportation. The negatively charged nanoparticles avoid the immune clearance but it can be repelled from negatively charged cell membrane and lead to low cellular uptake [223,228]. Thus, a neutral surface charge or charge-switchable nanoparticles are desired. Luo *et al.* coated a charge reversal polyanion poly(ethylene glycol)-blocked-2,3-dimethylmaleic anhydride-modified poly(L-lysine) (PEG-PLL(DMA)) on the surface of cationic MCM-41 nanoparticles (diameter 120 nm, pore size 3 nm) as a shielding layer [228]. The shielding layer can be degraded in acidic tumour microenvironment. With this design, the anionic SNP have the benefit of prolonged circulation time. When the switched cationic SNP reach the designed tumour site, they exhibit high affinity to the tumour cells.

Moreover, SNP can easily be modified to generate stimuli-controlled drug release formulations. Many researchers have shown that by controlling SNP's interaction with specific tumour microenvironment, SNP can prevent the release of anti-cancer drug in the normal tissues and thereby limit systematic side effects [172,229,230]. Tumour microenvironment differs from healthy tissues in regard to redox reactivity, acidic pH and altered enzymatic activity. SNP can be modified to capitalise on these differences to achieve targeted drug release in the tumour. PEGylation has been widely used in improving stability, biocompatibility, and

biodistribution of nanoparticles [231]. For example, Cheng *et al*. attached a targeting polymer poly(ethylene glycol)–folic acid (PEG–FA) on the surface of polydopamine (PDA)-modified MSN (MSNs@PDA–PEG–FA) (diameter 140–190 nm, pore size 2.5 nm), which PDA acted as a pH-sensitive gatekeeper, folic acid was a targeting moity against folate receptor on overexpressed cancerous cells, and PEG was able to improve the long-term blood circulation of nanoparticles [232]. With doxorubicin loading, MSNs@PDA–PEG–FA achieved higher antitumor efficacy *in vivo* in Hela tumour-bearing nude model mice, compared with free doxorubicin or MSN without folic targeting ligand [232]. The peptide iRGD is another effective cancer-targeting moiety when attached to SNP. In a study by Wang *et al*., MSN (diameter 100 -200 nm, pore size 4 nm) conjugated with iRGD were shown to penetrate deep into the tumour [233]. iRGD/MSN-encapsulated indocyanine green (ICG) showed capability to escape endosomal digestion, and concurrently deliver siRNA (siPlk1) and miRNA (miR-200c) in orthotopic MDA-MB-231 breast tumours models *in vivo*, and to significantly suppress the growth of the primary tumour when administered intravenously [233].

While many researchers have attempted to deliver chemotherapies with SNP to treat cancer, others have used SNP to provide photodynamic therapy and photothermal ablative therapy against cancer. These therapies involve the use of photosensitiser which can be excited to generate reactive oxygen species or hyperthermia which leads to tumour destruction. The properties of SNP with large specific surface area and porosity features are ideal for protecting the photosensitiser agents. Moreover, SNP can easily be coated with gold and other metals which have been used for ablative therapies for various cancers. Notable example includes SNP-based photothermal ablative therapy such as Auroshell and Aurolase (Table 2), which have now advanced into clinical trials [5]. Nonetheless, the efficacy of treating tumours located beyond the reach of external radiation remains challenging due to the limited tissue penetration depth of light. The frequent utilization of near-infrared light at wavelengths around 808 nm, while effective for superficial tissues, presents a considerable obstacle when addressing deep-seated tumours, as it can only penetrate depths of 1-2 mm. Currently, alternative strategies are being explored to overcome this limitation. One such approach is sonodynamic therapy (SDT), a combined therapeutic modality that involves the delivery of sonosensitive agents to the tumour site, followed by ultrasound application to induce tumour cell death. This technique holds the potential to access deep-seated tissues to activate sonosensitizers, thereby generating reactive oxygen species (ROS) to effectively eliminate cancer cells. [234] The SNP-tumour interactions are complex and dynamic in nature. However, based on available data use of

particles with sizes in the range of 20 to 50 nm is most optimal for tumour penetration and accumulation.

5.4 SKIN

Skin is the largest organ and forms the most extensive biological barrier in the human body. The skin barrier is composed of multiple layers (stratum corneum, epidermis, dermis, and hypodermis) along with many associated appendages (hair follicles, sweat glands, sebaceous glands, and nails) [235]. Among these, stratum corneum plays an important role to create the barrier function of skin as it is made up of many sheets of flattened corneocytes connected via protein and lipids [235]. In addition, epidermis forms an important barrier, as it is poorly vascularised and relies on diffusion from dermis to access nutrients and oxygen. In contrast, the dermis and hypodermis contain more blood vessels. Therefore, if a topically administered drug can permeate the epidermis layer, it can readily be absorbed by the capillaries in the dermis [174]. For the pharmaceutical industry, skin presents both an obstacle due to its barrier function and a prospect owing to the large surface area that can be utilised for the delivery of therapies. To overcome the different skin barriers, smart silica nanoparticulate drug carriers have been developed to transport the drugs into sweat glands or hair follicles to achieve local delivery. The skin appendages have also been proven to be an important reservoir to store nanoparticles and allow drug to diffuse across the capillary walls, and avoid the interaction with stratum corneum [236–238]. Consequently, the transdermal and topical drug delivery systems that circumvent skin barrier and bypass oral route of administration are of particular interest. Different types of SNP have been used as a topical delivery system for variety of therapies including local anaesthetics [239], immunosuppressants [240,241], antibiotics [242,243] and antifungals [244,245].

Penetration of SNP in human skin is dependent on the size and surface chemistry. In a study by Rancan *et al.,* the effect of size and surface charge of Stöber-like SNP on skin permeation was investigated [246]. Rancan *et al.* reported that larger than 75 nm size are efficiently blocked by the skin, while the cationic amino-functionalised SNP had better penetration into the skin due to electrostatic interaction with anionic skin membrane [246]. Nafisi *et al.* used amino-functionalized MCM-41-type SNP for formulation of lidocaine to improve its skin bioavailability for enhanced local anaesthetic effects [239]. They found that these particles had a steady *in vitro* drug release profile and it was better at permeating the skin *ex vivo* when

compared to free lidocaine or bare MCM-41 loaded with lidocaine [239]. Similarly, in another study, amino-functionalised cationic MSN (90–230 nm) were shown to enhance the permeability of 5-flurouracol and dexamethasone in rat skin *ex vivo*, and the formulation improved cytotoxicity against melanoma cells *in vitro* when compared to pure drug alone [247]. However, an anionic surface on MSN can be considered if it improves the solubility and bioavailability of its cargo. For instance, in a study by Parekh *et al.,* it was shown that the solubility of hydrophobic drugs such as tacrolimus was significantly improved using the anionic phosphate-functionalised MSN when compared to cationic amino-functionalised MSN [240]. The data from this study demonstrated that in an *in vivo* model of atopic dermatitis skin lesion using BALB/c mice, the phosphate-functionalised MSN led to improvement in skin histological scores [240].

Interestingly, SNP can also be designed to penetrate different depths of skin [237]. Larger SNP around 200-300 nm have been used for localised delivery for conditions such as atopic dermatitis while smaller SNP (50 nm) can be utilised for systemic delivery [240,246,248]. Surface functionalisation is another tool to modulate skin penetration kinetics of SNP. Mahrooqi *et. Al* generated thiolated SNP which could bind to the hair follicle and the stratum corneum layer, then tested their penetration *ex vivo* on pig flank skin [237]. Thiolation of SNP enabled binding with hair keratin protein; but after PEGylation there was about 50% reduction in SNP surface thiolation which reduced keratin binding efficiency. Consequently, the PEGylated SNP showed deeper skin penetration [237]. Overall, SNP hold considerable promise as vehicles for drug delivery through the dermal route, offering a range of advantageous attributes. Their ability to retain drugs within the skin ensures targeted and localized drug delivery at the intended administration site[249]. Moreover, the ease with which their surfaces can be modified allows for fine-tuning of drug release profiles, affording control and selectivity in the release [250]. Leveraging the high porosity and pore volume characteristic of MSNs, these nanoparticles can effectively store and facilitate long-term drug release, facilitating smart and precise therapeutic interventions [251]. Nevertheless, more work on SNP translocation across skin barriers is required to identify risk factors associated and to further investigate the capacity of SNP for dermal therapy.

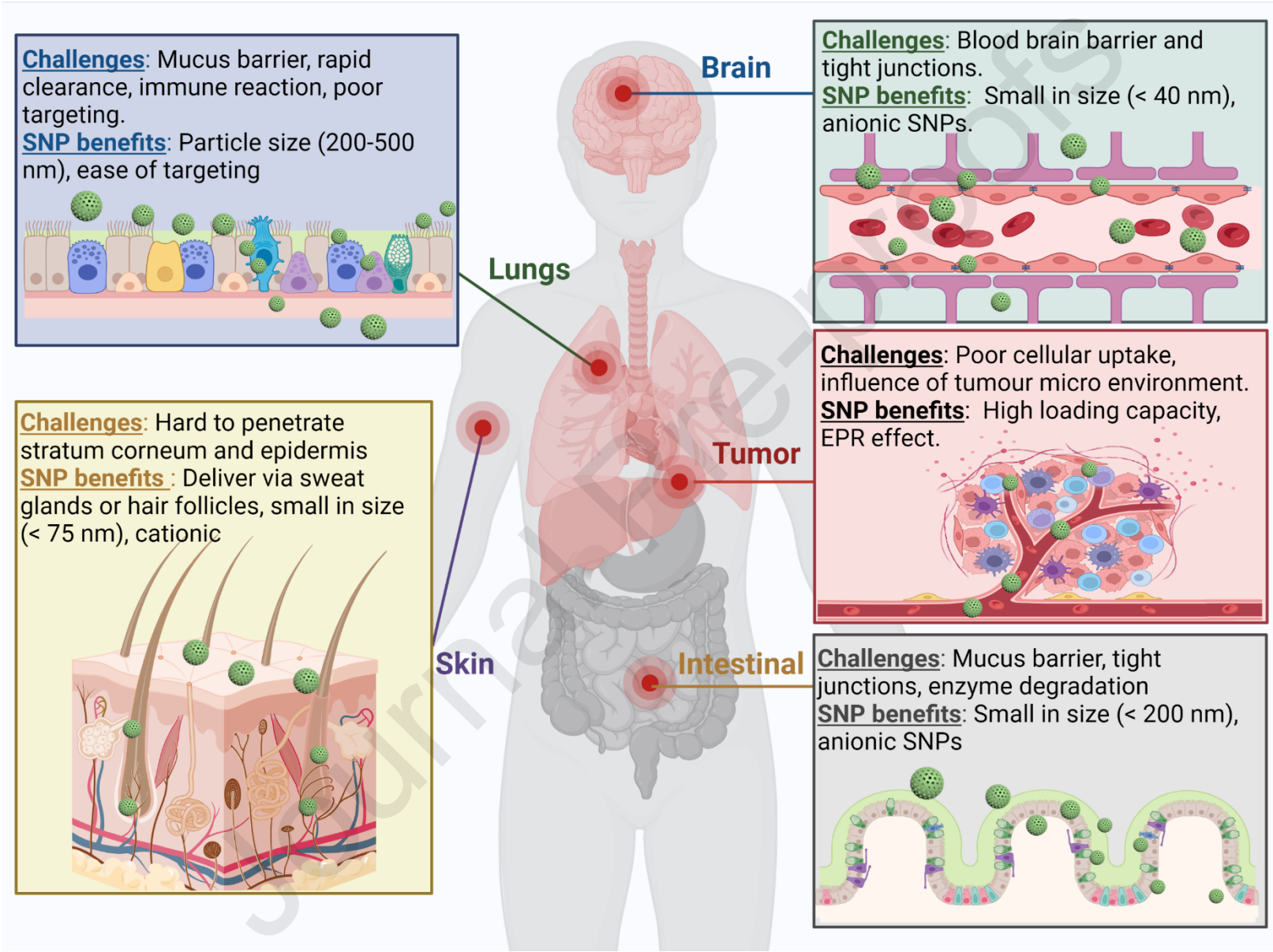
Challenges: Mucus barrier, rapid clearance, immune reaction, poor targeting.
SNP benefits: Particle size (200-500 nm), ease of targeting
Lungs
Brain
Challenges: Blood brain barrier and tight junctions.
SNP benefits: Small in size (< 40 nm), anionic SNPs.
Challenges: Poor cellular uptake, influence of tumour micro environment.
SNP benefits: High loading capacity, EPR effect.
Tumor
Challenges: Hard to penetrate stratum corneum and epidermis
SNP benefits : Deliver via sweat glands or hair follicles, small in size (< 75 nm), cationic
Skin
Intestinal
Challenges: Mucus barrier, tight junctions, enzyme degradation
SNP benefits: Small in size (< 200 nm), anionic SNPs

Figure 4. Summary of challenges associated with targeting different biological barriers and how with modulation of SNP's structural and physiochemical properties, these barriers can be overcome. In the ***lung****, delivery of therapeutics is difficult due to the mucus barrier, rapid mucociliary and immune clearance. In the* ***brain****, the presence of blood-brain barrier (BBB) and the tight junctions pose a significant challenge preventing the permeation of substances. SNP can be synthesised with negative surface charge and ultra-small size (< 50 nm) allowing it to permeate charge selective BBB. In the* ***tumour,*** *the microenvironment is acidic, hypoxic and heterogenic which regulates the uptake of anti-cancer therapies. SNP can be designed to high loading capacities for chemotherapies and with tumour homing properties (e.g., folic acid conjugation) which increase tumour specificity and reduce off target side effects. In addition, enhanced permeation, and retention effect (EPR) increases passive accumulation of SNP in solid tumour due to leaky tumour vasculature and SNP remain in the tumour due to poor lymphatic drainage. In the* ***gastrointestinal (GI) tract,*** *many substances cannot be delivered orally because of the highly acidic environment in stomach, mucus barrier in intestines, tight junctions between epithelial cell and rapid enzymatic degradation. SNP can protect cargo from harsh environment in GI tract and locally deliver to designed site. To increase the* ***intracellular*** *uptake, the modification of SNP aspect ratio and addition of targeting moieties can improve receptor mediated transport. Created with BioRender.com.*

6 FUTURE OPPORTUNITIES AND CHALLENGES

Based on the results of various clinical studies of SNP in humans, it is evident that they have tremendous potential within the biomedical field. However, the rate of clinical translation is still terribly slow. For instance, gold shell SNP were first developed in 1990's by Rice University but this technology only advanced into clinical trials with help of Nanospectra Biosciences company in late 2000's [252]. In the future, it is imperative to further investigate the unknowns of SNP such as the effect of chronic exposure of SNP and long-term toxicological profiles using clinically relevant models. Despite better stability and other numerous advantages to overcome biological barriers, there is still reluctance to trial inorganic nanomaterials, such as SNP, compared to organic nanomaterials, such as liposomes or lipid nanoparticles. This is mainly due to lack of long-term safety and efficacy data on silica nanoparticles in various diseases. Additionally, large number of studies on the use of silica nanoparticles are focused on development of new type of structures, chemistry and focus on delivery via parenteral route[23,92,172,253–256]. It would be prudent to consider using existing materials which are scalable and have comprehensive pre-clinical safety profiles for delivery via alternate route such as mucosal or topical. So far, no comparative clinical trials have been conducted to understand the influence of route of administration on SNP degradation and clearance rates. The pharmacokinetic profiles of SNP is also dependent on various factors which need to be sufficiently characterised such as the particle size, shape, surface charge and porosity [159]. This warrants further investigation to understand any potentially detrimental influence of SNP on the human body and substantiate the safety data for SNP.

Biological barriers such as mucus barrier in lungs and gastro-intestinal track pose significant challenge for penetration of imaging and therapeutics to reach the target (Figure 4). SNP's versatility and ease of modification allows them to efficiently penetrate across different biological barriers (Figure 4) compared to many polymeric and lipid carriers. The clinical trials of SNP, although limited, provide a proof-of-concept from human data confirming their safety and viability (Table 2). However, many of the clinical trial studies for SNP have conducted single dose toxicity studies which can often return desirable results for most nanomaterials. Moreover, the clinical trials conducted thus far have only tested either solid SNP without pores or with small pores which do not have ability to load cargo especially with large molecular weights. Newer generation of SNP's have tuneable surface chemistry and pore volume which has high loading capacity to carry multiple payloads with complex size such as proteins

[172,257–264] but their pre-clinical safety and degradation data are missing. With evolution in synthetic chemistry of SNP, preclinical models have already demonstrated improvements in the delivery and efficacy of these difficult to deliver molecules [25,265–267]. It is now pertinent that the nexus between the clinical translation of these SNP is bridged.

From *in vivo* models, there is sufficient evidence to predict no long-term ramifications of SNP with repeated administration (Table 1). However, no animal model can entirely foresee long term SNP safety in human. With advances in biomedical fields, sophisticated organs-on-a-chip and humans-on- a-chip can be used as reliable preclinical models compared to conventionally used animal models [268,269]. For instance, multiple organ-on-chip systems are now even available commercially and they can more accurately represent the biochemical and dynamic human biological cross talk between different organ system over period of multiple months. High throughput screening of various silica nanoparticles using such innovative materials is urgently needed to narrow down selective candidates for human trials.

In biomedical settings, one of the main advantages of SNP is the ability to achieve targeted delivery. The success of Cornell dots in phase I and II clinical trials for detecting tumours can be attributed to the design considerations of using ultra-small SNP with tumour targeting RGD peptide conjugation (NCT03465618, NCT01266096 and NCT02106598). As αvβ3 and αIIbβ3 integrin receptors are highly expressed in numerous cancers, therefore the selection of tumour targeting RGD peptide as ligand for these receptors is ideal. Moreover, RGD peptide is easy to synthesise, has minimal immunogenicity, offers high stability and it has a small size (346.34 g/mol) which do not impact the overall size of nanoformulation [270,271]. To accelerate the clinical translation of SNP for applications in other disease states, selection of better targeting ligands is urgently sought. In this regard, a shift towards personalised nanomedicine with carefully stratifying patients based on the expression of disease specific markers may help eventuate the potential for targeted delivery of SNP in clinical trials.

Biodistribution and clearance are crucial factors to consider when evaluating the safety and efficacy of new substances, particularly in the context of clinical studies. Quantifying these aspects will pave the way to understand how SNP are distributed throughout the body and how they are eliminated over time. Lack of sufficient data on biodistribution and clearance has hindered the progress of SNP into clinical studies. Part of the reason for Cornell dot's success from translation perspective includes thorough assessment and quantification of clearance at specific time intervals. Without this data, it becomes challenging to assess the potential risks

and benefits associated with the use of specific SNP in humans. Therefore, it is essential to gather relevant information about the *in vivo* biodistribution and clearance profiles of SNP of interest before proceeding to clinical trials.

Current clinical trials are mainly focused on using SNP for cancer diagnostics or for investigation of the biodistribution of SNP in humans [5]. Other applications such as oral delivery of SNP with therapeutic proteins have been widely studied in pre-clinical models but have not been studied in clinical trials [19,205,208,272]. For example, insulin is a life-saving protein for diabetes patients but suffers from poor patient compliance [188]. It is evident from the many clinical trials being conducted that there is a dire need for formulations that orally deliver insulin (NCT03392961, NCT00521378 and NCT00814294)[189]. There are successful reports of pre-clinical formulations with glucose responsive drug release from SNP which would be suitable for treatment of diabetes [273,274]. However, there have been no clinical trials conducted as of yet to deliver insulin orally using SNP. The data from two preliminary clinical studies suggest that SNP have the potential to orally deliver drugs and showing significant improvement in bioavailability [9,12]. Considering the established safety, stability and improvements in bioavailability when using SNP, it is pertinent to further explore it clinically for oral delivery of macromolecules such as insulin.

Another aspect to consider for the application of SNP in clinical trials is the need for synthesis protocol to be consistent and under precise conditions. SNP are often synthesised in small batches for the trials and the scale up required for large scale clinical trials could pose a challenge. Therefore, it is very important to investigate reliable scale-up methods and synthesise reproducible SNP with minimal batch to batch variation. Despite of significant costs associated with manufacturing large scale SNP, some researchers have shown that SNP synthesis is indeed scalable [14,15,275]. For instance, recently Liu *et al*., developed a two-fold strategy of decreasing ethanol to water ratio and addition of co-solvent ethylene glycol in order to yield kilogram range upscaled synthesis of SNP [14]. Moreover, attention must be paid to ensuring adequate characterisation of SNP as per the minimum reporting standards for nanomaterials [159]. In this regard, the prerequisite for clinical translation of SNP includes the need to establish reproducibility and scale-up synthesis protocol. Recently, many companies have started selling SNPs on commercial level. Therefore, while new syntheses protocols should be investigated, commercially available SNP could be assessed first since they are available in different sizes and functions, on a reproducible scale.

As the SNP progresses into clinical studies, the next aspect to be addressed is the shelf stability of SNP under various environmental conditions. Early pioneering investigations have demonstrated that through the implementation of suitable storage conditions, such as low-temperature storage following the freeze-drying process for drug-loaded silica nanoparticles (SNP), the formulation can retain both its efficacy and colloidal stability even after extended periods of storage. A recent study investigated the stability of SNP SBA-15 under different temperatures and humidity levels. They found that low humidity would help the storage for at least 6 months. While in high humidity, SBA-15 tend to lose its surface area because of the collapse of pores structure. Interestingly, the SNP could maintain the pore structure before calcination (with micelles support the pore structure). However, it could be challenge from a commercial perspective with micelles in the SNP [276]. Many other studies also investigated the shelf stability of drug loaded SNP. Moore *et al*. improved the colloidal stability of silica nanoparticles by conjugating with linkers [277]. The antibody-coated nanoparticles could easily resuspend by adding solvent and shaking hand after freeze-drying. Ngamcherdtrakul *et al*. discovered with proper freeze-dry procedures and buffers, PEG-PEI-silica nanoparticles with siRNA could store 2 months at 4°C and at least 6 months at −20°C [278]. Moreover, Hosseinpour *et al*. freeze-dry miRNA loaded SNP (rno-miRNA-26a-5p@MSN-CC-PEI) with 5% trehalose and stored under 3 and 6 months, and the enhancement effect of miRNA wasn't altered by lyophilization and storage [261]. However, it is important to acknowledge that these studies are currently confined to the realm of theoretical potential and have not yet progressed to the stage of commercially viable products or practical applications. Further in-depth investigations and research endeavours are required to bridge this gap and facilitate the transition towards commercially appealing products and applications.

7 CONCLUSIONS

In conclusion, SNP have tremendous clinical potential as emerging bionanomaterials because of the capacity to modulate their surface chemistry, shape, size and pore size to achieve desired function. SNP offer key advantages in improving the bioavailability of variety of cargos including biologics and can be tailored to offer controlled release of drugs. Preliminary evidence from both long-term animal studies and single dose human studies suggests that SNP have good biocompatibility, safety and low toxicity profile (Table 1 and 2). Clinical trials have now progressed to phase II which alludes to their promising potential [5]. In future, systematic

pre-clinical and clinical studies are needed to understand the long-term impact of SNP in humans. Importantly, in future there is a compelling need to focus on SNP's application in overcoming biological barriers for variety of clinical indications such as Diabetes, Inflammatory Disorders, Infectious Diseases and Cancer. A customised and tailored approach for a specific biological barrier is needed by modulation of size, shape, and surface chemistry for the advancement of the next generation of clinically relevant SNP formulations. Finally, a close collaboration between materials scientists, pharmacists, biologists and industry is a key to the success of these materials benifiting patients in the future.

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