



New Combination/Application of Polymer-Based Nanoparticles for Biomedical Engineering

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Abstract

Polymer-based nanoparticles (PNPs) are attractive in part due to their ultra-small size, versatility and target specificity. Therefore, PNPs have been increasingly used in a variety of biomedical applications including diagnoses and therapeutic treatment. In this chapter, we focus on the recent studies (within 5 years) with some new ideas/agent's application in biomedical field and roughly divide applications of PNPs into four categories: (1) Delivery, (2) *In vivo* imaging, (3) Therapies, and (4) Other applications. First, we introduce how PNPs can enhance the treatment and delivery efficiency of therapeutic agent. Second, how PNPs can be used to help *in vivo* imaging system for disease tracking and monitor. Then, we reveal some novel PNPs which is able to function as an agent in photodynamic, photothermal, sonodynamic and neuron capture therapy. Furthermore, we also mention some

interesting applications of PNPs for biomedical field in individual form or cluster employment, such as immunoswitch particles, surface fabrication. Finally, the challenges and future development of PNPs are also discussed. In delivery section, we focus on how polymer “can be used” as vehicles in delivery application. But, in the section of imaging and therapies, we carried on how polymer as an “adjuvant” for functional enhancement. The biodegradable property of PNPs is the feature that they can be controllable for itself degradation and drug release as a chief actor. Besides, in imaging and therapies application, PNPs can be the support role for helping contrast agent or photo/sonosensitizer to enlarge their imaging or therapeutic effect.

Keywords

Polymer-based nanoparticles (PNPs) · Delivery · Imaging · Therapeutics · PNPs cluster

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14.1 Introduction

During the past decades, the development of nanotechnology makes the generation process of polymer-based nanoparticles (PNPs) more efficient. In recent years, PNPs have been increasingly used in a variety of biomedical applications including diagnoses and therapeutic treatment.

Nanoparticles were first developed by Speiser and co-workers [1] around 1970 and are frequently defined as particles with diameters below the micron dimension (i.e., in the range 10–1000 nm) [2]. The concept of nanoscale devices has led to the development of nanoparticles. Two types of systems with different inner structures: (1) a reservoir-type system, consisting of an oily core surrounded by a polymer wall, defined here as a nanocapsule; (2) a matrix-type system composed of an entanglement of polymer units, defined here as a nanoparticle or nanosphere. The term nanoparticle can be used to refer to both systems including nanocapsules as well as nanoparticles.

Due to their ultra-small size, the PNPs have much larger surface area to volume ratio compared with bigger particles, enabling them to have unique biological behaviors. Their large functional surface area allow PNPs to interact with substances such as nucleic acids, proteins, lipids, ions, carbohydrates, probes or small molecule drugs and deliver these substances into the desired biophase via endocytosis. Furthermore, PNPs can be fabricated by different method. Their properties, such as size, shape, stability and particle composition are tunable, allowing them can be prepared to meet the requirements specific to biomedical application. For example, several pathways were involved in the endocytosis procedure of PNPs. This cellular uptake procedure can be controlled effectively by the properties of nanoparticles, such as nanoparticle size, shape, and surface chemistry [3–5]. After entering the target cell, PNPs can be made to respond to a different local stimulus, which will trigger PNPs to execute therapeutic functions, such as hyperthermia, imaging and drug release. Theses stimulus not only include biological endogenous signals like pH, glucose, enzyme, etc., but include external stimulus like alternating magnetic field (AMF), near infrared (NIR), temperature, and etc. [6, 7]. Due to their versatility and target specificity, PNPs have received much of the attention in various aspects of medicine. There are several excellent reviews on PNPs have been published, like their fabrication, characterization and strategies in the application [8–10].

In this article, we focus on the recent literature (past 5 years) and roughly divide the biomedical application of PNPs into four categories: (1)

Delivery, (2) *In vivo* imaging, (3) Therapies, (4) Other applications, and summarized in Table 14.1.

Table 14.1 Summary of current PNPs in biomedical application

Application	Indication	References
Delivery		
Dendrimers	Dendrimer with instantaneous size switching ability for tumor penetration	Li et al. [11]
Lipid Materials	PNPs as a delivery platform for CRISPR/Cas9 system	Yin et al. [12]
Polymeric materials	Combination of PNPs with CAR T-cell therapy	Smith et al. [13]
	Preventing obesity by using PNPs	Xue et al. [14]
<i>In vivo</i> imaging		
Magnetic	pH-activatable PNPs for non-invasive MRI of tumor	Mi et al. [15]
Nuclear	Quantification of the inflammation in atherosclerotic plaque using PET signal	Keliher et al. [16]
Optical	Finding a relationship between nanoparticle shapes and the ability of nanoparticle access into the nucleus	Hinde et al. [17]
	Examining the radiation effect for tumoral therapy	Miller et al. [18]
	SERS for high-precision cancer imaging	Harmsen et al. [19]
	Detection of endogenous H ₂ S within living cells via FRET	Zhao et al. [20]
	Semiconducting polymer based PNPs as probes for photoacoustic imaging	Pu et al. [21]
Therapies		
PDT	Performing PDT for deep tissue penetration	Punjabi et al. [22]
PTT	Combination of PTT and checkpoint-blockade immunotherapy to eliminate primary tumors	Chen et al. [23]
SDT	Combination of SDT and MRI for SDT monitoring	Huang et al. [24]
NCT	Incorporating Gd for MRI-guided gadolinium NCT	Mi et al. [25]

(continued)

Table 14.1 (continued)

Application	Indication	References
Other applications		
Individual application	Separation the protein biomarkers	Nehilla et al. [101]
	Simulates the immune system to inhibit tumor growth	Kosmides et al. [102]
	PNPs for neutralization of venomous biomacromolecules	O'Brien et al. [104]
	PNPs as a protein affinity reagent for inhibition of angiogenesis	Koide et al. [105]
	Predict the therapeutic efficiency of anticancer drug in a personalized manner	Yaari et al. [106]
	Identification the ideal targeted therapeutics by DNA barcoded PNPs	Dahlman et al. [107]
Cluster employment	Self-assembled nanoparticle monolayers as a cell culture tool	Wang et al. [113, 114]

Abbreviations: MRI, magnetic resonance imaging; PET, positron emission tomography; SERS, surface-enhanced Raman spectroscopy; FRET, fluorescence resonance energy transfer; PDT, photodynamic therapy; PTT, photothermal therapy; SDT, sonodynamic therapy; NCT, neutron capture therapy; Gd, gadolinium

14.2 Delivery

The ultra-small size of PNPs lead it enter cells more easily via endocytosis. Therefore, the application of PNPs in drug delivery, especially for cancer treatment, is particularly welcome. In order to grow quickly, tumor tissues tend to possess higher vascular permeability rather than normal tissues. Rapid vascularization to serve fast-growing cancerous tissue leads vessels to a leaky and defective architecture. The enhanced permeability and retention phenomenon (EPR) is based on two factors: (a) the capillary endothelium in malignant tissue is more disorderly and thus more permeable towards macromolecules than the capillary endothelium in normal tissues. This allows extravasation of circulating PNPs within the tumor interstitium, and (b) the lack of tumor lymphatic drainage in the tumor

bed results in particles' accumulation. Thus, the accumulation of PNPs in tumor tissue is much higher than normal tissues. PEGylation, coating the surface of nanocarriers with poly(ethylene glycol) (PEG), is a commonly used strategy to prolong circulation time of PNPs [26]. The EPR effect provide the PEGylation PNPs with increased opportunity to access tumors site. Due to the EPR effect, PNPs have been widely used as a vehicle to deliver the therapeutic agent, such as drug, nucleotides and protein, into tumor tissue.

Many recent studies, however, have described the limitations of EPR effect. The EPR effect is not suitable for metastatic liver cancers and less vascularized cancers, such as prostate cancer [27]. On the other hand, it has been reported that a phenomenon known as "accelerated blood clearance (ABC)" which repeated injections of PEGylated nanocarriers may cause of an unexpected immunogenic response, leading to lose their long-circulating characteristic [28]. Therefore, it is necessary to develop actively-targeted PNPs to specific recognize the specific cells.

To perform active targeting, specific ligands able to interact with the specific receptors localized on cell membranes can be coupled to the surface of PNPs. Several studies have summarized the various kind of ligand, such as small molecules, carbohydrates, peptides, enzymes or antibodies, have been used for active targeting [29–31]. In addition to ligation strategies, recently, a novel method called "cell membrane coating" has been developed for designing of PNPs. Cell membrane coating method is a technique, which the whole membrane will translocate from a cell to the surface of a nanoparticle, make the nanoparticle potentially perform some cell-specific functionalities, such as, immune evasion and targeting abilities. Zhang et al. [32, 33] have used this technique to address the issue of ABC phenomenon and improve PNPs functionality to achieve longer circulation time, higher tumor specificity and lower exocytosis of drug (Fig. 14.1) [34, 35]. The PNPs can be roughly classified into three types; dendrimer, lipid based PNPs and biopolymeric based PNPs.

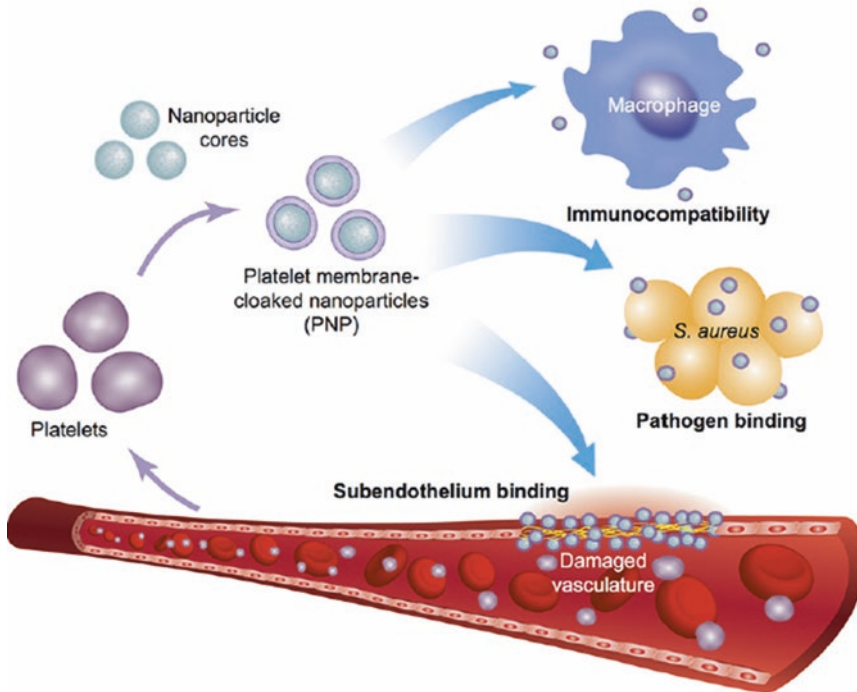


Fig. 14.1 Schematic preparation of PNPs. poly(lactico-glycolic acid) (PLGA) nanoparticles are enclosed entirely in plasma membrane derived from human platelets. The resulting particles possess platelet mimicking

properties for immunocompatibility, subendothelium binding, and pathogen adhesion. (Image adapted from Zhang et al. [33] and reprinted with permission from Springer Nature)

14.2.1 Dendrimers

Dendrimers, a large number of branching points, including precisely-defined molecular structure, numerous functional groups on the surface and cavities in the interior, have been widely used in the field of drug delivery in a three-dimensional spherical shape, nanometric size and well monodispersity [36]. With appropriate branching units and surface groups modification, dendrimers can provide the targeted delivery of gene or drugs. Kesharwani et al. [37, 38] reviewed the recent advancements in dendrimer-based PNPs for tumor-targeted delivery. They summarized the different kinds of ligands, including the biotin, folate, amino acids, peptides, aptamers and antibodies, which have been successfully conjugated to dendrimer [37]. Also, they have reviewed the different routes, such as parenteral, transdermal, oral, pulmonary, ocular and colon, can deliver the various dendrimers [38]. In the following,

several publications within these years will be discussed.

Dendrimers can be used for gene delivery. For example, Cheng et al. [39] systematically summarized the functional ligands can be modified to the dendrimers to improve the DNA- and membrane-binding affinity, transfection efficacy and biocompatibility of dendrimers. Also, dendrimers can be applied in drug delivery, including doxorubicin, 5-Fluorouracil, paclitaxel and other types of chemotherapy drugs. [40]. For example, Li et al. [11] prepared the size switching PNPs constructing from assembly of platinum-prodrug conjugated polyamidoamine dendrimers. At neutral pH, this PNPs have initial size of ~80 nm. Under acidic tumor microenvironment, however, the PNPs rapidly dissociate into the dendrimer building blocks and penetrate into tumor cell. Wei et al. [41] synthesize the amphiphilic dendrimer, which can form the supramolecular micelles to enclose the anticancer drug doxorubicin with high loading

capacity. Not only for gene transfection and chemodrug delivery, dendrimeric nanoparticles also have advantages for ocular drug delivery: nanoparticle platform of hybrid dendrimer hydrogel/poly (lactic-co-glycolic acid) with anti-glaucoma drug loading was evaluated. The hybrid dendrimer nanoparticle platform (HDNP) consists of three domains: the polyamidoamine (PAMAM) dendrimer core to encapsulate hydrophobic drugs, the poly (lactic-co-glycolic acid) (PLGA) nanoparticles to deliver either hydrophobic or hydrophilic drugs, and the PEG network to load hydrophilic drugs. The major advance of this novel dendrimer is its ability to deliver simultaneously multiple drugs in the same dosages and release them in a slow manner with sustained efficacy [42]. These platforms are very promising tools in ocular nanomedicine.

14.2.2 Lipid Based PNPs

Lipid based PNPs are delivery system mainly prepared from natural and/or synthetic phospho- and sphingo-lipids. Because they are absorbed more easily in human body and producing fewer toxic degradation products, therefore, lipid materials based PNPs are more suitable for clinical trial rather than other delivery system. In addition, some of them, particularly liposomes, due to their bilayer structure, can enclose and transport the both hydrophobic and hydrophilic drugs. Several researchers have comprehensive review the current state of lipid based PNPs, including development process of them, strategies of them for tumor targeting, and commercial applications of them [43–45]. Zhang et al. [46] reported that upconversion nanoparticles encapsulated Azobenzene (Azo) liposome (UCNP@Azo-Lipo) could convert near infrared (NIR) light into the UV/vis region; then UV/vis light was absorbed by the Azo molecules in the liposome. When stimulated by UV/vis light, the synthesized Azo amphiphilic derivatives created continuous rotation-inversion movement for the liposome membrane then driving drug released. The results show that they can precisely control the drug release amount via tuning the intensity and duration of light irradiation. The combination of

NIR light trigger system and liposome leads an on-off switch controllable liposome was created. Recently, lipid based PNPs and clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) systems have been combined in a new method to increase genome editing efficiencies. Yin et al. [12] use the lipid based PNPs to deliver the sgRNAs (e-sgRNA) and mRNA encoding Cas9, which can significantly decrease the specific genes such as *Pcsk9* in liver (>80% editing), and serum to undetectable levels by a single intravenous injection in mice. These lipid-Cas9 based PNPs provide a non-viral genome editing vehicles for liver genome correcting in clinical setting. The lipid based PNPs, called the liposome protamine/DNA lipoplex (LPD), was electrostatically assembled from cationic liposomes and an anionic protamine-DNA complex, it promoted efficient delivery of the retinal pigment epithelium protein 65 (*Rpe65*) gene in mice in a long-term expression and cell specific-mode leading to *in vivo* correction of blindness [47]. The efficacy of this method of restoring vision is comparable to AAV and lentiviral gene transfer of the *Rpe65* gene to *Rpe65* knockout mice [48].

14.2.3 Biopolymeric Based PNPs

Biopolymeric based PNPs, compare to other PNPs, have better storage stability and diversity of designs [49]. They can roughly divide into nanocapsules and nanospheres, based on their structure. Both of them have spherical structure comprising polymer, however, the cargo is dispersed within a matrix for nanosphere and encapsulated in central cavity for nanocapsule, respectively [50]. Nanogels are common nanosphere for delivery. Li et al. [8] summarize the method to fabricate the nanogels, based on natural and synthetic polymers, for drug and nucleic acid controlled release. Gaitzsch et al. [51] systematically review papers about how to prepare the “smart” nanocapsules for delivery system based on different techniques like self-assembly, emulsion polymerization, microfluidics, or Pickering emulsion, etc. Therefore, due to their diversity of designs, various release strategies,

including heat, light, ultrasound stimulation; pH change, oxidation process, or enzyme participation, these ways have been employed to improve the efficiency of biopolymeric based PNPs to sustained drug release within the target site [52]. In the following, we introduce some newly publications within these years.

Satchi-Fainaro et al. [53] designed a biodegradable amphiphilic polyglutamate amine PNPs (APA) PNPs that can deliver the small interfering RNA (siRNA) and microRNA (miRNA) into the tumor. After arrive the tumor site, PNPs will be degraded by an enzyme highly expressed in tumor tissues leading to release the siRNA or miRNA to silence or dysregulate the gene associated with cancer, respectively. PNPs can be used to enhance the efficiency of chimeric antigen receptor T-cell immune therapy (CAR-T). Stephan et al. [13] prepare the PNPs, based on poly(beta-amino ester) (PBAE) polymer functionalized with the microtubule-associated-nuclear localization (MTAS-NLS) peptide, can transport the leukaemia-targeting CAR genes into T-cell nuclei to rapidly program T cells with abilities of tumour-recognizing, resulting in simplifying the time consuming traditional method. Also, PNPs can be used to address obesity. Langer et al. [14] demonstrate the PNPs self-assembled from biodegradable triblock polymer composed of adipose vasculature targeted peptides conjugated poly(lactic-coglycolic acid)-b-poly(ethylene glycol) (PLGA-b-PEG) can increase the accumulation of drugs in white adipose tissue (WAT), which accelerate the transformation of WAT into brown adipose tissue (BAT) and then cause the weight loss.

Although novel polymeric based NPs for drug/gene delivery are designed/developed for biomedical application popularly; traditional biopolymer such as chitosan or gelatin also plays an important role in drug delivery system. For example, gelatin, the biodegradable polymer, exhibits excellent biocompatibility, plasticity, and adhesiveness [54]. Its degradation rate can be regulated by the degree of cross-linking. The functional groups on gelatin NPs, such as carboxyl, hydroxyl, and amino groups, are available for conjugation with ligands to bring about surface modifications. Variant gelatin nanoparticles (GPs) were synthesized by

Tseng et al. [55–57] for different application such as inhalation delivery of cisplatin loaded GPs with EGF modification for lung cancer treatment [55]; or as an efficient and safe drug carriers for ocular drug delivery in an eye-drops formula [56]; and even be a gene delivery carriers with pEGFP-C1 loading (plasmid encoded enhanced green fluorescence protein) for transgene sic chicken manipulation [57].

Nonmatter novel one or old one, biopolymeric based PNPs is the major components as delivery system for drug/gene. Beyond this, another role such as a protector for contrast agent for imaging or other therapeutic chemical application, PNPs can also participate as a supporting role for reaching final clinical requirements. These are adders thereafter.

14.3 *In vivo* Imaging

Medical imaging techniques are powerful tool allowing researchers to look inside a cell or to find difference between the normal and abnormal biological processes. However, most imaging agents, such as organic molecules and inorganic nanomaterials, do not have ability to recognize and target specific cells hindering their applications to cellular studies and biological imaging. To deal with this problem, PNPs have been introduced into the biomedical imaging field as a helper.

Currently, most studies using PNPs for molecular imaging focus on designing of nanoparticles with a structure of combination of imaging agent core and polymer shell. This designing has some advantages. First, it can decrease the cytotoxicity of imaging agent. Second, various targeting ligands can be modified to the polymer shell allowing imaging agent can active bind to cellular target moieties. In addition, it will promote imaging agent accumulation in the tumor, increasing the signal-to-noise ratio, highlighting tumor tissue within the body. Third, compare to imaging agent alone, polymer shell can make it with larger size, allowing them to have longer circulation time, decreasing in the time to agent's administration. Zheng et al. [58] discuss the clearance pathways and tumor targeting of imaging nanoparticles, including ultra-small inorganic core modified

with protein-adsorption-resistant zwitterionic or PEGylated surface. Lammers et al. [59] summarize the advantages and limitations of imaging modalities, such as magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computed tomography (SPECT) and optical imaging. Also, they provide principles for the rational use of PNPs in noninvasive imaging. Smith et al. [60] divide the imaginable nanomaterials based on the fundamental physicochemical properties. In addition, carrier, such as micelle, liposome, dendrimer and polymeric materials, for delivery of these nanomaterials are discussed.

In this section, we discuss the recent applications of PNPs in bioimaging. Based on imaging modalities, we have roughly divided imaging into three parts: magnetic, nuclear, and optical. The research we discussed here will mainly focus on how polymers are utilized to coat on the inorganic nanoparticles for *in vivo* distribution/biocompatibility. However, several studies, which utilize as polymers, like conjugated polymer, as co-component of imaging agents, will also be examined.

14.3.1 Magnetic

Magnetic resonance imaging (MRI), rather than use of damaging radiation, is based on the tiny magnetic moments produced by the spin of certain atomic nuclei within the body. MRI is a valuable technique for the clinical diagnosis that producing the non-invasive three-dimensional detailed anatomical images of soft tissues. Because soft tissue is present all over the human body, therefore, in medical diagnosis, MRI has a wide range of applications, such as detection of peripheral neuropathy, systemic cancer and cardiovascular disease. Gadolinium (Gd) and iron oxide based nanoparticles is common MRI contrast agent [61]. Recently, MRI agent made by Gd hexanedione nanoparticle has been developed to label and track the stem cell with low toxicity but higher image enhancement capacity [62].

Zhang et al. [63] describes the way to improve the biocompatibility and reflexivity of Gd-based contrast agents by conjugation of natural or synthetic polymers to it. Bakhtary et al. [64] sum-

marize the work of using PNPs with super paramagnetic iron oxide nanoparticles (SPION)-based contrast agents to early detect and image different major cancer types, including liver, prostate, brain, breast and cervical.

In addition to Gd and SPION, manganese (Mn)-based materials have been used for MRI contrast agent in recent years. Mi et al. [15] prepared pH-activatable PNPs for non-invasive imaging of tumor. They confined Mn^{2+} within calcium phosphate (CaP) core enveloping by a PEG shell, which can avoid aggregation of core. After arrive the tumor, the acidic tumor environment make pH-sensitive CaP core to release the Mn^{2+} ion, which bind to proteins increasing the MRI contrast (Fig. 14.2).

14.3.2 Nuclear

Nuclear imaging is a technique using radioactive agents to observe metabolic processes in the body, which can be used to diagnose abnormalities in the bodily functions, especially effective in identification of cancerous tumors. The most common used nuclear imaging techniques are positron emission tomography (PET) and single photon emission computed tomography (SPECT). Both of them can provide accurate three-dimensional images, but the main difference between their imaging is the type of radioactive agents used. The decay of the radioactive agents used with PET measure positron emission, whereas the SPECT detect gamma ray emission. Radioactive agents can be used for imaging have been discussed earlier [60, 65, 66].

However, some clinical radioactive agents, such as ^{18}F -fluorodeoxyglucose, do not have the ability to conduct themselves to target site. Moreover, due to low molecular weight, their circulation time is short. Therefore, to overcome these problems, several studies have incorporated the radioactive agents into PNPs [67]. For example, Phillips et al. [68] prepared the silica particles labeling with ^{124}I for PET and coating the nanoparticle with cRGDY peptide and PEG to improve the accuracy of metastatic melanoma diagnostic. Chen et al. [69] encapsulate vana-

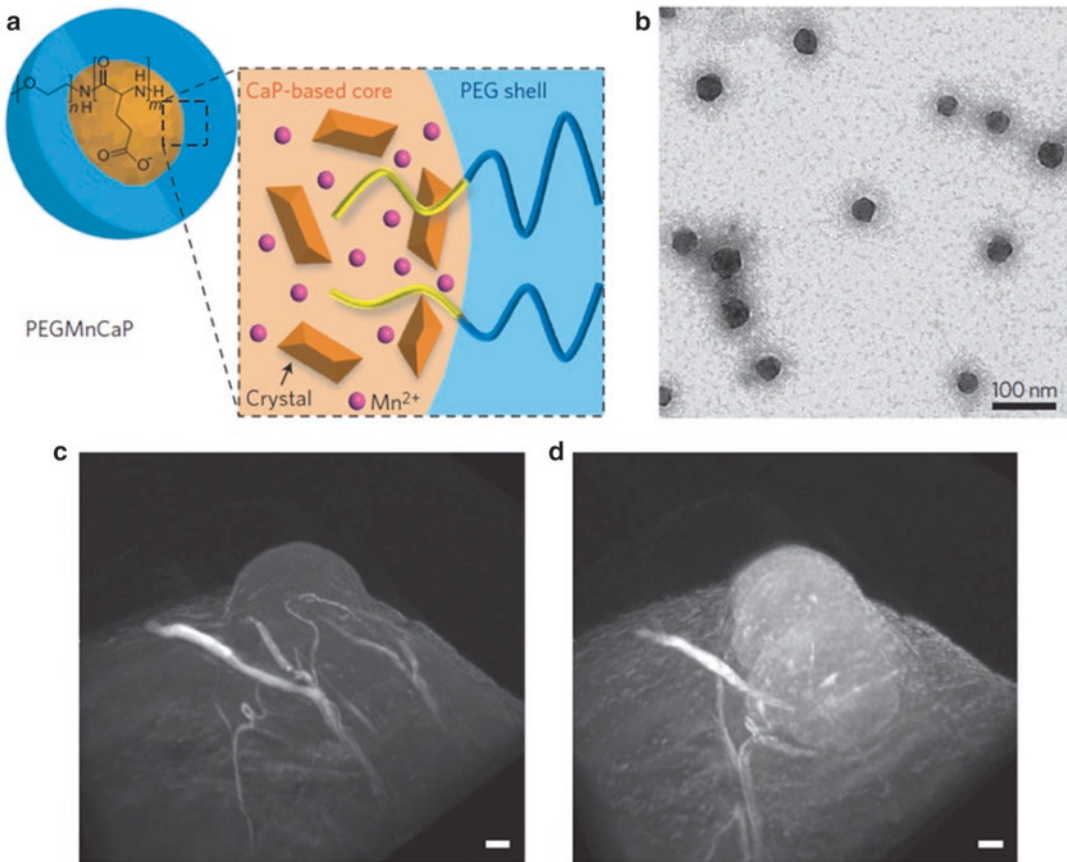


Fig. 14.2 (a) Illustration of the PEGMnCaP structure. (b) TEM image of PEGMnCaP. (c and d) 3D MRI of C26 tumors before (c) and 1 h after (d) the intravenous injection

of PEGMnCaP measured with 7T MRI. Scale bars, 50 μm . (Image adapted from Kataoka et al. [15] and reprinted with permission from Springer Nature)

dium disulfide (VS_2) nanodots, a MRI agent, labeling with $^{99\text{m}}\text{Tc}^{4+}$, a SPECT agent, inside PEG modified lipid micelle leading to fabricate the VS_2 @lipid-PEG PNPs. In addition to MRI and SPECT, the high NIR absorbance of VS_2 @lipid-PEG PNPs provides the PNPs with strong photoacoustic (PA) contrasts, resulting in multiple imaging functions. Keliher et al. [16] synthesize the ^{18}F labeled polyglucose nanoparticles with size around 5 nm, can be excreted out of kidney and used to quantify the inflammation in atherosclerotic plaque using PET signal. Combination of PNPs with variant nuclear signaling agent enhances its image quality in cancer diagnosis and expands its application in other filed such as atherosclerotic detection.

14.3.3 Optical

Optical imaging technique is typically faster and cheaper than magnetic and nuclear imaging. In addition, it can be used in high-throughput analysis in numerous cells which's interested Though the limited penetration deep of optical light hinders its clinical deployment, optical imaging can achieve highest spatial resolution in all of imaging modalities [60]. Therefore, currently, optical imaging has been widely used to observe molecular processes or detailed features of physiologic structures.

The most commonly used optical imaging agents are inorganic nanomaterials, such as semi-conductors, metals and metal oxides. To provide

these inorganic agents biocompatible and enhance the solubility/suspension in buffer media, several strategies have been studied to functionalize the surface of them with various amphiphilic block copolymers and to specific interact with target biomolecules [70].

In addition to inorganic nanomaterials, organic nanomaterials, such as conjugated polymer and fluorescent dye, can be also applied to bioimaging. Unlike other imaging modality, the unique opto-electronic properties of conjugated polymer can provide PNPs with intrinsic imaging ability without incorporation of imaging agent. Therefore, conjugated PNPs have widely been studied both *in vitro* and *in vivo* imaging [71].

On the other hand, current developments in the organic dyes design provide the new strategy in tuning the imaging properties of PNPs. Traditionally, the way to achieve high brightness in dye loaded PNPs depend on confining a large amount of dyes within the PNPs matrix. However, the aromatic structure of dye prefer to aggregate leading to fluorescence quenching. Recently, several studies have shown that PNPs can be used to increase the quantum yields of certain organic dyes, called aggregation-induced emission dyes (AIE dyes), by restricting the intramolecular rotation of these dyes within PNPs [72]. Klymchenko et al. [73] have reported that the brightness enhancement of AIE dyes-based PNPs are higher than quantum dots (QDs) for a comparable size, and have nearly reaching level of the brightest conjugated polymer-based nanoparticles.

Due to improve the brightness, biocompatibility, and selective targeting capability of these optical imaging agents, the development of PNPs for biomedical imaging have drawn attention [74, 75]. For example, Kim et al. [76] used the PEGylated Cornell dots (C dots) to observe the difference of propagation from cell to cell between the ferroptosis and other types of death. Hinde et al. [17] synthesize the fluorescein-labelled PNPs, based on poly(oligoethylene glycol methacrylate)- block-poly(styrene-co-vinylbenzaldehyde) P(OEGMA)-b-P (ST-co-PVBA) block copolymer, with different shapes but identical surface chemistries to understand

how nanoparticle shapes will affect itself to access into the nucleus. Weissleder et al. [18] used the fluorescein-labelled dextran coated nanoparticles to examine the relationship between radiation effects and tumoral therapeutic nanoparticles concentration. Moreover, recently, several techniques have been used to enhance the accuracy and efficiency of imaging, including the surface-enhanced Raman spectroscopy (SERS) [19, 77], fluorescence resonance energy transfer (FRET) [20, 78], and photoacoustic effect [21, 79]. AIE dye, SERS or FRET etc. all these methodologies are developed for getting high image quality from optical signals. Combination of PNPs provides a way to enhance signal or improve biocompatibility of dye/metal/metal oxides contents to reach the goal.

14.4 Therapies

Incorporation of metal nanoparticles or organic molecule into PNPs not only can ensure a precise assessment of imaging for biomedical purposes, but also use in treating cancer, such as inhibition of tumor promotion or induction of cell death. Phototherapies based on nanotechnologies have attracted tremendous attention for their potential in biomedical application. Systematic classification of nanomaterials for phototherapies has been studied by Liu et al. [80]. Also, they review the different types of PNPs to deliver these nanomaterials to cancer cells. Zheng et al. [81] discuss about the photophysical relaxation pathways of organic molecules for phototheranostic techniques including the radiative emission, intersystem crossing/triplet-state relaxation and vibrational relaxation.

To monitor the treatment efficiency of therapy, most of researches will combine therapeutic PNPs with imaging agents enhancing the ability to visualize the targeted tissue. Elsabahy et al. [82] review the way to use PNPs to overcome challenges in imaging and therapy. The principle to design PNPs, such as components, type of structure and cross-linking, for delivery of diagnostic and therapeutic agents will be discussed.

In the following, we will focus on the application of PNPs in photodynamic therapy (PDT) and photothermal therapy (PTT). In addition, we will also discuss the sonodynamic therapy (SDT) and neutron capture therapy (NCT), the promising strategies combining PNPs with low-intensity ultrasound or epithermal neutrons.

14.4.1 Photodynamic Therapy (PDT)

A promising biomedical application of PNPs based therapy is photodynamic therapy (PDT). PDT kill the cells via singlet oxygen or reactive oxygen species (ROS) generated from light-activated chemical called a photosensitizer (PS). PS can transfer the absorbed light energy to either oxygen molecules to produce singlet oxygen or to surrounding molecules to form free radicals, leading to generation of different radical oxidizing agents, such as superoxide, hydrogen peroxide, and the hydroxyl radical [83]. Though, singlet oxygen only causes a destruction in nanometers, they will lead to activation of significant and complex cascade, resulting in local, regional, and systemic alteration of both cell and immune response [84]. Therefore, PDT has been extensively studied in the treatment of various disease, especially in cancers [10].

The development of PS has been comprehensive review by Zhang et al. [85]. Many photosensitizers used in PDT for cancer treatment are based on the tetrapyrrole backbone, a hydrophobic structure which is only slightly soluble and exhibits certain tendency to aggregate in aqueous resulting in a low therapeutic efficiency [86]. The combination of PS with PNPs not only decrease cytotoxicity of PS, but improve the solubility and stability of PS in water, which increase tumor accumulation of PS, which enhance the effect of PDT. Thus, many studies focused on design/synthesis different PNPs for effectively transport PS into specific tumor regions and then conduct the PDT by external light irradiation [80, 85]. Near-Infrared (NIR), due to relatively low, compared to visible light, absorption coefficient resulting the deep penetration in tissue, has been widely used in PDT treatment [87].

To increase the depth of PDT, recently, PS combined with upconversion nanoparticles (UCNPs) has attracted great attention. UCNPs is a materials converting low energy NIR light into high energy UV/visible light, which can activate the PS creating a photodynamic reaction [88]. Punjabi et al. [22] prepared the PNPs containing the PS (aminolevulinic acid) to convert it into protoporphyrin IX in the UCNPs treated by a biocompatible laser, which perform PDT for deep tissue penetration (>1.2 cm) than others with <1.0 cm depth).

To further penetrate into the deeper layer, scintillating nanoparticles (SCNP) have emerged as promising candidates for PDT application. After exposure to ionizing radiation, SCNP absorb the radiation and emit energy as visible light, which can trigger the PDT more efficiency. Cai et al. [89] summarized recent developments of SCNP. They also discuss about the strategies to loading SCNP, including the way to wrap it with polymer. Although the incorporation of SCNP within PNPs can overcome the penetration limit, the application of scintillating nanoparticles is still in infant stage. The relevant techniques for this strategy need to find ways to improve further.

14.4.2 Photothermal Therapy (PTT)

Another type for phototherapy is photothermal therapy (PTT). Different from PDT killing the cell by ROS, PTT use the heat to treat tumors. After light irradiation, the PTT agents will produce the photothermal effects, which can transform the energy of light into local heat. The heat not only can be used to cause thermal ablation of tumor cell, leading to cell membrane disruption and protein denaturation, but also can be helpful to address some of the limitations of nanodelivery systems, such as endosomal escape or cargo release. Therefore, to improve the therapeutic efficiency, PTT therapy has been combined with other therapeutic approaches. Kim et al. [90] revealed synergistic therapeutic systems combining gene and PTT ablation, they focus on how PTT effect enhanced cellular uptake, facilitate endosomal escape and induce

gene release for transfection. Zou et al. [91] showed when PTT combined with PNPs, it can be used alone or synergize with the imaging, radiotherapy, chemotherapy and immunotherapy to improve the efficiency of cancer treatment. For example, El-Sayed et al. [92] prepared the gold nanoparticles containing PNPs that can induce PPT cell death and molecular changes of single cell through real-time surface enhanced Raman spectroscopy (SERS). Chen et al. [23] design the PNPs, which combine the PTT with checkpoint-blockade immunotherapy, could eliminate primary tumors and inhibit metastases.

Different types of agents for PTT, including inorganic and organic nanomaterials, have been reported in various works [80, 93]. Inorganic nonmaterial, such as metallic nanomaterials, carbon nanostructures, quantum dots, and heavy metal nanocrystals and porous silicon nanomaterials, have shown the ability to efficiently convert light into heat. Shao et al. produced the biodegradable poly (lactic-co-glycolic acid) (PLGA) nanoparticle loaded with black phosphorus quantum dots (BPQDs), these hydrophobic PLGA polymer shell not only increased the photothermal stability via separation the BPQDs from oxygen and water, but mediated the BPQDs degradation [94]. On the other hand, organic nanomaterials, including organic compound and conjugated polymer based PNPs, have considered as potential agents in PTT due to their biodegradability. Among organic agents, conjugated polymer based PNPs have drawn great attention arising for their photothermal efficiencies, which is similar to gold nanoparticles [93]. Zhou et al. [95] prepare the conjugated polymer based PNPs, which can release the heat shock protein inhibitor. This inhibitor could reduce the cellular tolerance to heat resulted in better PTT effect.

14.4.3 Sonodynamic Therapy (SDT)

Different to the phototherapies conducting by light, sonodynamic therapy (SDT) executes the remedy by “sound”. After activated by low-intensity ultrasound stimulation, specialized chemical agents, sonosensitizers, can produce

ROS leading to cell damage. Due to ultrasound can non-invasive penetrate deeper to internal organs, compared to traditional phototherapies, SDT has attracted more attention in recent years.

The sonosensitizer, however, suffer the same problems as photosensitizer, such as low biological stability, tumor-accumulation. Therefore, to increase the SDT efficiency, several researchers have explored ways to combine PNPs with sonosensitizer, such as inorganic nanoparticles or organic compound. Xu et al. [96] confirmed the design of PNPs as carrier for sonosensitizer nanoparticles, including gold, Fe_3O_4 , silver, porous silicon, and carbon fluoroxide, they introduce different methods to encapsulate the sonosensitizer into PNPs. Qian et al. [97] provides systematic description about the development of amplified SDT performance assisted by PNPs. A recent study demonstrated that, with proper design, MRI imaging can be displayed by sonosensitizers. Huang et al. [24] chelate MRI agents, Mn ion, to the sonosensitizers, protoporphyrinc (PpIx), and anchored the Mn chelateing sonosensitizers, Mn-protoporphyrin (MNPpIx), in to the inner mesoporous organosilica nanoparticles (HMONS-MnPpIX); then, the surface of HMONS-MnPpIX was also covalently modified with PEG, these PNPs made by HMONS-MnPpIX not only increased the SDT efficiency, but also augmented the Mn^{2+} ions chelating on protoporphyrin, these make this composite sonosensitizers with good MRI performance combined with SDT monitoring.

14.4.4 Neutron Capture Therapy (NCT)

Neutron capture therapy (NCT) is a treatment based on the nuclear reaction. Unlike aforementioned therapies which destroy the cells by ROS or heat, NCT kill the cell by gamma ray. After penetrate into the tissue, the epithermal neutrons would slow down and be captured by NCT agents, causing lethal radiation to injury in tumor cells. Boron (B) has been extensively

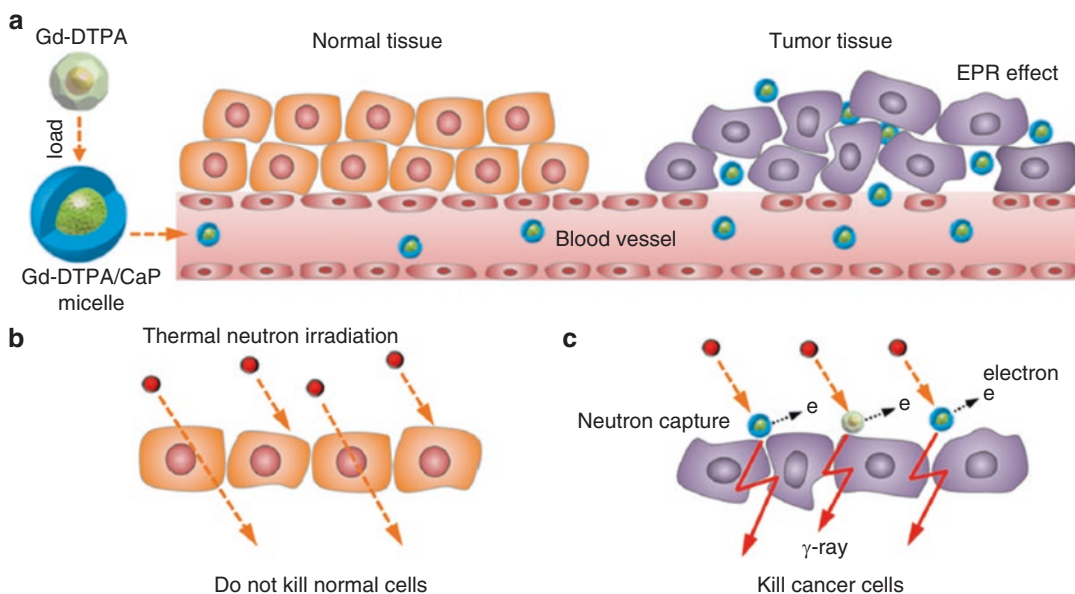


Fig. 14.3 Scheme of Gd-DTPA/CaP hybrid micelles targeting tumors for gadolinium neutron capture therapy (GdNCT). (a) The accumulation of Gd-DTPA delivered by Gd-DTPA/CaP in tumors through the EPR effect. (b) Low energy thermal neutron irradiation does not kill normal cells

without NCT agents. (c) Thermal neutron irradiation could kill or cause hazardous damage to cancer cells by the γ -rays emitted from the Gd nuclides after nuclear reaction with captured thermal neutrons. (Image adapted from Mi et al. [25] and reprinted with permission from ACS publish)

studied as NCT agent. Subsequent to the capture of neutron, the nuclear fission of B could produce high energy alpha particles and lithium-7 nuclei [98].

To achieve sufficient deposition of NCT agents in a tumor site, NCT agents could be delivered by PNPs. For example, Huang et al. [99] synthesize the amphiphilic carborane-conjugated polycarbonates, which can self-assemble into different sizes in water, after irradiation of thermal neutron, the boron content of the carboranes conducts the NCT and suppresses tumor growth. In Kataoka's study [25], they replace the B with Gd, and loaded Gd into calcium phosphate core with hybridizing of PEG-polyanion block copolymers. There's two reasons to use Gd in their study: first, Gd has the largest capture cross-section, among NCT agents for thermal neutron absorption (larger than B) resulting in the emission of high energy gamma rays; the other reason is Gd can helpful for guiding the NCT under MRI (Fig. 14.3).

14.5 Other Applications

The development of nanotechnology has been successfully selected as a alternative way to design, synthesize, and apply to present materials in biomedical filed. In addition to the above-mentioned application, such as diagnosis and therapy, PNPs have been addressed in various applications. In the following, we roughly divide the application of PNPs into two categories by its condition; individual application and cluster employment.

14.5.1 PNPs Individual Application

In the preceding sections, we described how metal nanoparticles can be incorporated into PNPs to adhere to cells for imaging or treatment of them. However, the use of PNPs is not only limited to these. Recent studies have shown excellent performance of PNPs in mediation of

cell behavior, biomolecular isolation, immunotherapy, protein affinity reagents and personalized medicine. In the following, we will introduce these applications.

PNPs with magnetic nanoparticle can control the mechanical stimulation of single-cell behavior. Tseng et al. [100] manipulate the dextran-magnetic nanoparticles, via magnetic fields, within HeLa cells. They apply localized nanoparticle-mediated forces producing the tension on the cortex of cells and observe the responses in cellular behavior. This technique offer a tool to analysis of molecular, such as proteins or nucleic acids, localization and its functional role in cell.

In addition to mediation of cell behavior, magnetic PNPs can be employed for separation the protein biomarkers. Nehilla et al. [101] use the classical temperature-responsive polymer poly (N-isopropylacrylamide), or pNIPAAm, to create the stimuli-responsive binary reagent system, including polymer coating magnetic nanoparticles (PNPs) and polymer-antibody conjugates (Ab), to capture the antigen. When the stimulus is applied, the PNPs and Ab with captured antigen will aggregate to form magnetically separable species. After removal of a stimulus, the captured antigen will release from PNPs. This system can customize for types of affinity reagents, providing a potential platform for biomarker discovery and diagnostics.

By combining immunotherapy with PNPs, nanoparticle platform can simulates the immune system to inhibit tumor growth is developed Kosmides et al. [102] prepare the “immunoswitch particle” by coating dextran-magnetic nanoparticles with two different antibodies that switch off the inhibitory checkpoint PD-L1 pathway on tumor cells and switch on CD8+T cells via the 4-1BB co-stimulatory pathway. These immunoswitch PNPs can increase effectiveness of immunotherapy over soluble antibody, resulting in reduction in cost and complexity of therapeutic.

PNPs are used for protein affinity reagent in biomedical application. Because the biological affinity reagents, such as antibody, have some disadvantage, including high cost, difficulties in production and storage. Several researchers have

tried to try to find out alternatives. Hoshino et al. [103] prepare the PNPs, which interact strongly and weakly with denatured and native lysozyme respectively, to refold the aggregated lysozyme. Shea et al. [104] develop a PNPs can neutralize venomous biomacromolecules, phospholipase A₂ (PLA₂), inhibiting the hemolytic reaction. Koide et al. [105] synthesis the PNPs with affinity to a key vascular endothelial growth factor, VEGF₁₆₅, leading to inhibit VEGF-mediated angiogenesis without exhibition off-target activity.

PNPs can apply to personalized medicine to match specific patient with the most effective treatment. Schroeder et al. [106] load the different cancer drug and corresponding DNA barcodes into PNPs. Through the analysis of DNA barcodes, the correlation between the drug and cell viability is revealed finding out the most efficient drug. Similar to Schroeder et al., Dahlman et al. [107] use DNA barcoding to analyze the biodistribution of PNPs with varying PEG characteristics. The DNA barcoding can facilitate the researcher to understand the relationships between treatment and disease.

14.5.2 PNPs Cluster Employment

PNPs, especially spherical particles, have been used as building blocks to form close-packed two-dimensional (2D) or 3D structures so called self-assembled colloidal crystals [108, 109]. Various methods have been proposed such as evaporation induced colloidal self-assembly or self-assembly of colloids at air-water interfaces [109, 110]. The chosen method is often dependent on the particle material and size. The method and particle used also often determines the ultimate quality of crystal structure (i.e. the size of defect). High quality of 2D or 3D crystals is a common goal in various applications.

Colloidal crystal (CC) monolayers provide unique property which can be utilized in different fields such as photonics, sensors, and biology [111]. Recently, CC monolayers or CC-derived substrates have been utilized as a cell culture tool for modulating cell behavior [112, 113]. CC monolayers can be composed of single, binary,

and even more types of particles. For example, binary colloidal crystal (BCC) monolayers have been fabricated on glass substrate in large area using large silicon particles and small polymer particles. Both 2D single and binary CCs have been used in cell culture. BCCs provide higher complexity on the surface than single CC such as heterogeneous chemistry. The ability of present more chemical or physical cues on cell adhering surface such as ternary CCs may elicit useful bioinformation.

Wang et al. [109] first used BCCs as a substrate for stem cell culture. BCCs were fabricated using evaporation-induced colloidal self-assembly. The detailed mechanism was described using this method. Depending on the BCC combinations, long ranged BCC can form on a 2D surface. Mammalian cells including MG63 osteoblasts, L929 fibroblasts, and human adipose stem cells (hASCs) were cultured on those long ranged BCCs, and cell spreading area was found to be inhibited on BCCs compared to flat controls. However, once cell adhesive protein, fibronectin,

was pre-adsorbed onto BCCs, a synergic effect of BCCs and cell adhesive protein was found that cell spreading was significantly increased. Based on this result, hASCs were expanded on BCCs for longer term [113]. Interestingly, osteo- (BSP, RUNX2, and OPN) and chondro-genes (AGG, SOX9, and COL2) were upregulated on BCCs, but not adipogenes (PPAR γ and adiponectin). Thus, it has been demonstrated that BCCs can induce early osteochondral differentiation during stem cell expansion.

In another study, BCCs have been demonstrated that can replace the cell adhesive protein, vitronectin, during cell reprogramming of fibroblasts into induced pluripotent stem cells (iPSCs) (Fig. 14.4) [114]. Currently, the standard protocol for *in vitro* cell reprogramming needs pre-coating of extracellular proteins which support not only cell attachment but also provides abundant bio-signals during cell growth. The result showed that human iPSC colonies formed on BCCs without vitronectin coating with high percentage while the colony was difficult to form on flat sur-

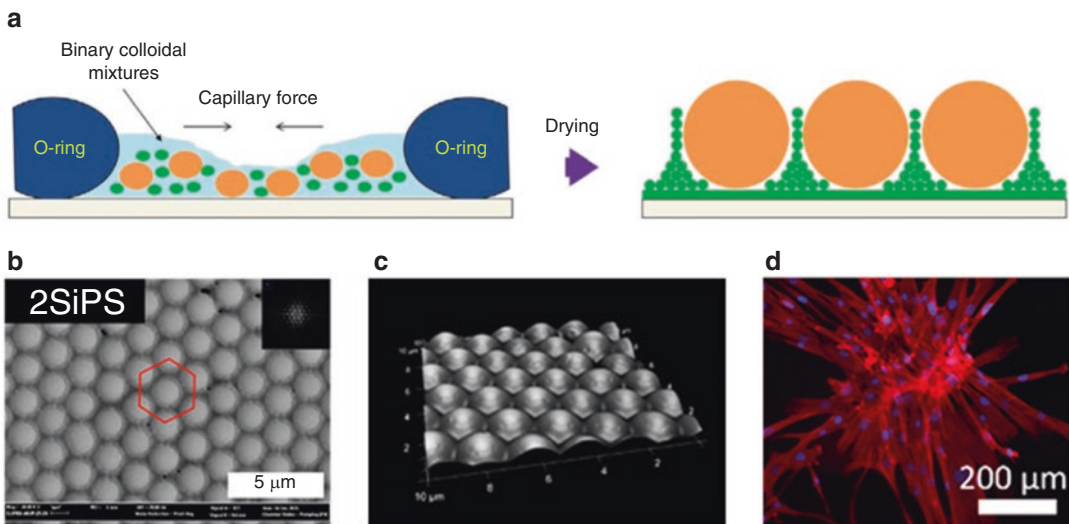


Fig. 14.4 PNPs self-assembly into monolayers as cell culture substrates. (a) Binary colloidal mixture self-assembled into crystal monolayer. (b) Scanning electron micrograph of one example, 2 micron silica and 0.1 micron polystyrene (2SiPS). (c) Atomic force microscopy

of 2SiPS. (d) Human adipose stem cells (hASCs) grew on 2SiPS for 4 days and formed clumps on the surface. (Image adapted from Wang et al. [114] and reprinted with permission from Springer Nature)

face without vitronectin. This result implies that BCCs regulates the cytoskeleton of fibroblasts and/or promotes extracellular matrix synthesis by fibroblasts which in turns facilitate cell reprogramming process. Overall, PNPs can be self-assembled into monolayer as a mask to producing biochemical patterns or substrate for *in vitro* cell expansion.

14.6 Conclusions

Although negative impacts of nanoparticle on patient tissue, such as liver [115], lung [116] and cardiovascular [117] were reported, due to some PNPs have capacity to cause inflammatory condition of lung, or accumulated lung to weaken the immune system, then activate a portion of latent virus in body, therefore, the proper design of PNPs can help decrease the risk of problems we mention.

Due to their good biocompatibility, large surface area to volume ratio, and versatile design, PNPs are considered competitive potential system for biomedical application. As a delivery system, PNPs not only can protect and transport of cargo, such as drug, plasmid or protein, more specific into the desired target site but can “smart” control of cargo release rate from PNPs. Also, via entrapment of metallic nanoparticles or fluorescent organic compounds, PNPs could serve as imaging or therapy platform to observe or destroy the specific tissue. Moreover, PNPs could be used to control of cell behavior, biomolecule isolation, protein affinity reagents and surface fabrication. Recently, by associating specific PNPs with photoreactive agents as therapeutics, PNPs become a powerful tool for the application of personalized medicine to treat and improve cancer care.

In this chapter, we introduce some recent publications, including original and review articles with new agent or nanoparticles combination. All of them show that PNPs offer the new possibilities to develop both new diagnostic and therapeutic ways for biomedical application. We believe that PNPs could create more medical breakthroughs in the future.

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