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Studies of nanoparticle delivery with *in vitro* bio-engineered microtissues

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ABSTRACT

A variety of engineered nanoparticles, including lipid nanoparticles, polymer nanoparticles, gold nanoparticles, and biomimetic nanoparticles, have been studied as delivery vehicles for biomedical applications. When assessing the efficacy of a nanoparticle-based delivery system, *in vitro* testing with a model delivery system is crucial because it allows for real-time, *in situ* quantitative transport analysis, which is often difficult with *in vivo* animal models. The advent of tissue engineering has offered methods to create experimental models that can closely mimic the 3D microenvironment in the human body.

This review paper overviews the types of nanoparticle vehicles, their application areas, and the design strategies to improve delivery efficiency, followed by the uses of engineered microtissues and methods of analysis. In particular, this review highlights studies on multicellular spheroids and other 3D tissue engineering approaches for cancer drug development. The use of bio-engineered tissues can potentially provide low-cost, high-throughput, and quantitative experimental platforms for the development of nanoparticle-based delivery systems.

1. Introduction

Nanoparticles, as a promising tool to transport molecules across tissues, have been intensively studied in recent decades. They can be utilized as an advanced delivery system, protecting therapeutic and imaging agents. The size, shape, surface characteristics, and materials used for their synthesis can be readily modified to match various applications. Nanoparticles offer a variety of functions including controlled release of therapeutic agents at a particular site or at the desired rate, improvement of drug half-life through encapsulation, increased permeability to biofilm and biological barriers, and stealth property to evade body's immune system [1–6].

As engineered nanoparticles are becoming highly functional and more complex, utilization of *in vitro* bio-engineered microtissues as a test platform to assess the efficacy of nanoparticle transport has become a powerful approach. The use of *in vitro* platforms allows for *in-situ*, real-time, high-resolution biomedical monitoring, which is often difficult with *in vivo* animal models. This paper provides a comprehensive review of nanoparticle designs and synthesis, as well as recent attempts for the evaluation of their functions using *in vitro* bioengineered microenvironment.

Fig. 1 illustrates the types of *in vitro* tissue models and nanoparticles we review in this paper. We discuss multi-cellular spheroids, hydrogels, composite tissue models that incorporate live cells and other functional structures. The use of 3D printing is an emerging technology for the preparation of tissue models. Nanoparticles tested are made of metals, metal oxides, polymers, and lipids, and their sizes ranged from a few nanometers to a few hundred nanometers with various surface functionalization and other physical characteristics such as particle rigidity [7]. Table 1 summarizes tissue types, target models and materials used, nanoparticle types and sizes, and the purposes of the studies.

This paper starts with the description of application areas of nanoparticles, which include treatment of diseases, drug release in tissue engineering, and labeling for biomedical imaging. Various nanoparticles are then described, including polystyrene beads nanoparticles (polymer nanoparticles), lipid nanoparticles, gold nanoparticles, and quantum dots. Methods of delivering nanoparticles to targeted sites and improving penetration through biological barriers will also be described.

The other important part of the paper is the review of the *in vitro* microtissues as a test platform. For efficient nanoparticle transport, several factors need to be considered such as medium density, pore sizes, porosity, concentrations of ions and other molecules. While controlling such material properties with an *in vivo* animal model is

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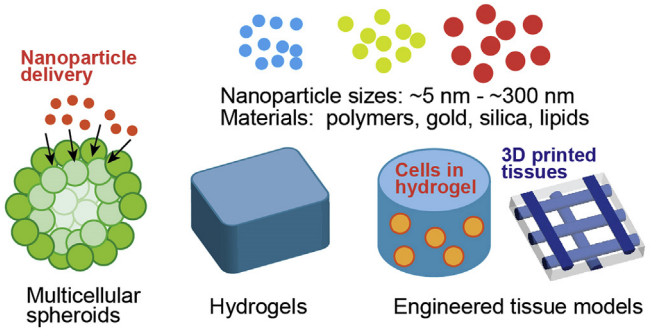

Fig. 1. Bio-engineered microtissues for *in vitro* nanoparticle delivery. The use of *in vitro* platforms allows for *in-situ*, real-time, quantitative monitoring of nanoparticle delivery.

difficult, engineered tissues provide *in vitro* platforms to study the impacts of these factors in a quantitative manner. This paper describes tumor spheroids, hydrogels, 3D printed materials, and other engineered microenvironments to study biological properties that are related to the transport and release of molecules by nanoparticles. Finally, this paper will overview future prospects.

2. Application areas

2.1. Cancer treatment

Tumors require an intense supply of oxygen and nutrients during rapid growth. This strong demand for nutrients causes a larger gap between newly formed epithelial cells in tumor tissue, which is characterized by high permeability of tumor tissue. At the same time, the tumor stroma has higher interstitial pressure and lacks a functional lymphatic network, resulting in the deformation of tumor tissue. The deformation of tumor tissue provides a convenient condition for nanoparticles penetration and accumulation, a phenomenon known as enhanced penetration and retention (EPR) effect [8–11]. Many nanoparticle anticancer drugs trying to take advantage of the EPR effect are in their clinical trial period or approved by the Food and Drug Administration (FDA). For instance, albumin-bound paclitaxel (PTX) encapsulated in 130 nm nanoparticles have been approved by the FDA for breast cancer treatment in 2005 [12]. Albumin is a material suitable for the fabrication of nanoparticles for drug delivery because it is known to be nontoxic, non-immunogenic, biocompatible and biodegradable. Another example is PTX-loaded polymer conjugates that are under investigation in the clinical trial [13].

2.2. Atherosclerosis treatment

The EPR effect is also a common phenomenon in atherosclerosis due to chronic inflammation of arterial blood vessels. Unlike tumors, the main cause for the formation of EPR in atherosclerosis is the long-term accumulation of cholesterol and cell waste in arterial blood vessels. Due to this unhealthy accumulation, the supply of oxygen and nutrients becomes ineffective and the newly-formed vessels are poorly structured. As a result, the endothelial cells in blood vessels are formed abnormally, which has a larger gap between each cell and the same leakage as we discussed in a tumor. Duivenvoorden *et al.* studied an injectable reconstituted high-density lipoprotein (rHDL) nanoparticle loaded with statins, lipid-lowering drugs. They applied statin-rHDL nanoparticles *in vivo* in a mouse atherosclerosis model and demonstrated that they accumulated in lesions to affect plaque macrophages [14]. In particular for targeted atherosclerosis imaging and therapy, the functionalization of magnetic nanoparticles for MRI with affinity linands has become an important approach [15]. Winter *et al.* [16,17] developed a method to deliver an antimicrobial agent fumagillin. Winter *et al.* used paramagnetic perfluorocarbon nanoparticles targeted to the $\alpha_v\beta_3$-integrin, an angiogenic biomarker, and demonstrated a total drug dose reduction of more than 10,000-fold. The paramagnetic nanoparticles were also used to monitor the antiangiogenic effects through MRI imaging.

2.3. Osteoarthritis treatment

Osteoarthritis (OA) is caused by the degeneration of the articular cartilage, resulting in pain and impeding mobility [18]. Currently, there is no disease-modifying drug available, and the care for the disease is limited to palliative treatment. Oxidative stress due to the reactive oxygen species (ROS) such as hydrogen peroxide can lead to inflammation and degradation of cartilage. There are two main challenges for osteoarthritis (OA) treatment [19]. First, joints are inherently leaky, and therapeutics agents are cleared away rapidly from joints within days. Second, targeted delivery of nanoparticles to chondrocytes is difficult because cartilage is avascular, meaning the population of the cells are sparse throughout the cartilage matrix [20,21].

Many types of nanoparticles have been investigated for intravenous delivery and preferential targeting/retention at pathological sites. Small (< 15 nm) cationic nanoparticles can overcome the biological barriers of the joint by binding and penetrating faster than the conventional carriers. This prevents the small cationic nanoparticles from being cleared from the joint space [22,23]. Delivery platforms such as polymeric micelles, liposomes, and dendrimers may be useful nanoparticles for osteoarthritis therapy [24–26]. When targeting the cartilage matrix, these nanoparticles can infiltrate the pores within cartilage because of their small size and highly positive surface charge that binds to cartilage matrix which is instrinsically negatively charged [27]. In addition, the functionalization of the nanoparticle surface with targeting ligands allows for increasing their targeting to cartilage matrix [27]. Also, an increase of circulation time and systemic persistence effectively promote the probability of active delivery nanoparticle or passive nanoparticle to accumulate in the inflamed/damaged joints [28].

2.4. Neuro-degenerative treatment

Brain cancer, Alzheimer's diseases, Parkinson's disease, stroke, and multiple sclerosis, are among the neuronal diseases that are prevalent, but poorly treated due to the limitations associated with drug development, administration, and the difficulty of targeting the brain [29]. The presence of the blood-brain barrier (BBB) that has a highly selective permeability system is the main problem when delivering therapeutic agents to the brain [30]. Nanoparticles can be the possible solution for delivering drugs across the barrier. The BBB is made up of specific vascular endothelial cells that bound with neurons, pericytes, and astrocytes. Nanoparticle-based drug delivery systems have the potential to deliver the desired quantity of the drug to the brain. Currently, micelles, dendrimers, liposomes, and carbon nanotubes as nanocarriers are being investigated to overcome the blood-brain barrier [31]. Size, charge, and surface ligands of the nanoparticles can potentially be considered for overcoming the BBB. Nanoparticles with a smaller size than 200 nm have more chances to cross the BBB via the clathrin-mediated mechanism [32]. Positively charged nanoparticles preferentially use the adsorptive transcytosis pathway than the neutral or negatively charged [33] In addition, coating the nanoparticles with surface ligands increases the possibilities of crossing the BBB. For example, nanoparticles coated with cell-penetrating peptides can avoid the endocytic pathway and deliver the nanoparticles directly in the cell cytoplasm [34]. Some researcher group has taken the advantage of transport-receptor mediated transcytosis (GLUT1 or albumin transporters, LF receptors, LRP1 (targeted by angiopep-2), or Tf receptors) to overcome the BBB [35–37]. An interesting example is the use of MRI-guided focused ultrasound to locally disrupt the BBB [38]. Overall,

Table 1
Summary of bio-engineered microtissues for *in vitro* nanoparticle delivery.

Tissue type	Tissue model and materials	Nanoparticle	Size	Purpose of study	Ref.
Multi-cellular spheroid	Breast cancer (MCF7 cells)	Gold NPs	2, 6, 15 nm	Size-dependent penetration	58
	Breast cancer (MCF7 cells)	polystyrene NPs	20 nm	Effect of ultrasound application	194
	Cervical cancer (SiHa cells)	polystyrene NPs	20, 40, 100, and 200 nm	Penetration in collagenase treated spheroids	166
	Cervical cancer (SiHa cells)	polymeric micelles	37 nm	Enhanced permeability and retention of DOX	167
	Cervical cancer (HeLa cells)	doxorubicin, quantum dots, and micelles	20 nm (QD)	Imaging the penetration of various NPs	168
	Pancreatic cancer (AsPC-1 cells)	albumin NPs	10, 100, 200 nm	Efficiency of drug delivery	169
Hydrogel	Crowded polymer-network hydrogels	PEGylated silica NPs	5.9 nm	Penetration in cross-linked polymers	173
	Agarose hydrogel	microgel, gold, silica, polystyrene NPs	50–260 nm (soft NP), 44–220 nm (hard NP)	Comparing the penetration of soft and hard NPs	7
	PEG and dextran solutions	Rhodamine 110, Rhodamine 6G, Alexa Fluore 488, R-phycoerytherin	Small molecules	the impact of electrostatic force on the diffusion of charged molecules	174
Engineered tissues	Colon cancer spheroid (LS174T cells) embedded in Matrigel®	Gold NPs	6 nm (with ligand)	The impact of NP surface charges on penetration	175
	Melanoma spheroids (MDA-MB-435 cells) embedded in Matrigel	PEGylated gold NPs	40, 70, 110, and 150 nm	Optical imaging in a tumor on a chip system	189
	Glioblastoma (brain tumor) spheroid (RG2 cells) coated with endothelial cells (CCL-209)	Iron oxide (Fe_3O_4) NPs	10 nm	Penetration in tumor vasculature model	203
	Prostate cancer cells (LNCap) embedded in hyaluronic acid hydrogel	Dox-loaded polymer NPs	54 nm	The efficacy of free and nanoparticle-loaded doxorubicin	176
3D printed tissues	Layer by layer skin model made of collagen hydrogel and fibroblast cells (3T3)	polystyrene NPs with hydroxyl, amine, and sulfate surface coating	100 nm	Compare penetration of NPs with different surface charges	184
	Cervical cancer cells (HeLa) embedded in gelatin/alginate/fibrinogen hydrogels	Anti-tumor drug paclitaxel	Drug small molecules	Test the drug resistance	183
	Breast cancer cells (MCF7, MDA-MB-231) and bone marrow mesenchymal stem cells in 3D printed bone matrix	Anti-tumor drug Fluorouracil (5FU)	Drug small molecules	Test the drug resistance in a 3D model	185
Others	Biofilm formed by bacteria (Pseudomonas fluorescens)	Dextran, polystyrene, and silver NPs	0.9–3.2 nm (dextran), 57 nm, 92 nm, and 135 nm (polystyrene), and 2 nm (silver)	Study diffusion coefficient of NPs in biofilms.	150

nanoparticle conjugation with therapeutic agents may prove to be a promising tool in brain drug targeting for safer therapies.

2.5. Drug release for tissue engineering

Nanoparticles provide interesting features to advance the field of regenerative medicine. Because of their size and surface addressability, nanoparticles can be used as a delivery vehicle of drugs, genes, growth factors, and imaging markers for tissue engineering [39]. In particular, the delivery of growth factors has been a topic of interest. For example, the angiogenic factors such as vascular endothelial growth factor and basic fibroblast growth factor can specifically enhance angiogenesis in a controlled release system, which is critical for maintaining sustained blood supply to developing tissues [40,41]. In the field of bone tissue regeneration, a tightly controlled supply of growth factors and cytokines is important to stimulate the right biological processes at the right time [31].

Although active biomolecules can be directly encapsulated in the 3D scaffold [42], due to the complex microenvironment formed during tissue growth, the imposition and function of these biomolecules will be significantly limited [3,43]. Therefore, the use of nanoparticles that encapsulate active molecular factors has been widely studied to provide controlled release [44]. Nanoparticles can provide high control over the pharmacokinetics of bioactive and contrast agents while protecting the therapeutic drugs from degradation and efficiently carry them to the target [30]. Richardson *et al.* reported a new polymeric drug delivery system that encapsulated two growth factors (vascular endothelial growth factor (VEGF)-165 and platelet-derived growth factor (PDGF)-BB), with controlled dose and rate of delivery, which resulted in the rapid formation of a mature vascular network [45]. Park *et al.* recently reported on PLGA biodegradable nanoparticles for co-delivery of the bone morphogenetic protein 2(BMP-2) and runt-related transcription factor 2 protein plasmid DNA to human mesenchymal stem cells (hMSCs). They showed that hMSC has significantly enhanced osteogenesis by delivering growth factors gene via the PLGA nanoparticles [46].

2.6. Probes for biomedical imaging

Good biocompatibility, tunable absorption and emission properties, and a variety of surface modification technologies of nanoparticles have made them a promising biomedical probe for disease detection and molecular imaging [47–49]. Currently, highly water-soluble small organic iodinated molecules are commonly used as contrast enhancers for X-ray computed tomography (CT). However, due to the clearance by the kidneys and vascular permeability, iodinated molecules usually have short imaging time in blood circulation [50]. Kim *et al.* recently reported a new kind of PEG-coated gold nanoparticles that could extend the blood circulation half-life. The blood circulation time of PEG-coated nanoparticles injected intravenously into rats was more than 4 h, which is much longer than that of the commonly used iodine contrast agent iopromide (less than 10 min) [51].

3. Types of particles

3.1. Polystyrene beads

Latex beads in the colloidal size range can be formed from an amorphous polymer (typically polystyrene). Latex microspheres are easy to produce and surface-modify, have good biocompatibility, and are easy to functionalize with fluorescence molecules. A variety of products are commercially available and are widely used in the study of nanoparticle penetration and biomedical imaging [52]. Goodman *et al.* used polystyrene nanoparticles with different sizes (20, 40, 100 or 200 nm) and studied the penetration in tumor spheroids (~500 μm diameter) grown from SiHa cells (human cervical cancer) with and without collagenase treatment [53].

3.2. Gold nanoparticles

The use of colloidal gold has been widely studied for cancer or arthritis treatment. The optical absorption and scattering characteristics due to the strong localized surface plasmon resonance make gold nanoparticles a unique tool for microscopic imaging and thermal therapies [54–56]. Some of the advantages of gold nanoparticles, including the small size, non-toxicity and non-immunogenicity, and the ease of functionalization, make them useful candidates of vehicles for targeted drug delivery systems [57]. As tumor-targeting delivery vehicles become smaller, the ability to by-pass the natural barriers and obstacles of the body becomes stronger. Size-dependent penetration characteristics of gold nanoparticles sized 2–15 nm have been studied with *in vitro* tumor models (multicellular tumor spheroids) [58]. To increase the specificity and efficacy of drug delivery, tumor-specific ligands may be attached to the surface of the particles along with the chemotherapeutic drug molecules to allow these molecules to circulate throughout the tumor without being redistributed into the body.

3.3. Lipid nanoparticles, liposome, micelles, and nanodiscs

Lipid nanoparticles, such as liposomes and micelles, have been utilized for drug delivery and cancer therapy. Research and development of engineered lipid-based multifunctional nanoparticles for drug delivery applications have grown rapidly since the discovery of liposomes by Bangham in 1965 [59]. A number of liposome-based drugs have been developed and entered the pharmaceutical market [60]. *Doxil* is the first lipid nanoparticle-based drug approved by the FDA (1995). It is a polyethylene glycol liposome-encapsulated form of doxorubicin. Lipid-encapsulated doxorubicin has demonstrated longer circulation time, stable remote loading, and much lower cardiotoxicity than unencapsulated doxorubicin [61]. *Doxil* is also approved by the FDA for the treatment of ovarian cancer and multiple myeloma [62]. *Ambisome®* developed by Nexstar is the first liposome-based drug to treat visceral leishmaniasis approved by FDA in 1997 [63]. It is used to treat severe deep fungal infections, such as black fever, fungal infections, and coccidioidomycosis. It can also be used to treat aggressive systemic infections caused by Aspergillus and Candida [64]. In addition, lipid nanoparticle can also be used in the formulation and preparation of vaccines. As many as almost 30 companies have sponsored clinical studies to investigate the efficacy and safety of Inflexal® V, lipid-based vaccine delivery system incorporating virus-derived proteins [65].

3.4. Quantum dots

Quantum dots (QDs) are semiconductor nanostructures that bind excitons in three spatial directions and are often called “artificial atoms.” [66] QDs are typically composed of semiconductor materials including ZnS, CdS, ZnSe, CdTe and PbSe and are sized between 5 and 50 nm. It has good fluorescence characteristics, such as the size and shape-dependent tunable emission spectrum, photostability, and relatively long fluorescence lifetime [67]. These features in fluorescence chromatography make quantum dots a very promising fluorescent probe in the biomedical field, especially in studying nanoparticle diffusion and penetration in the microenvironment. Xingyong *et al.* first demonstrated fluorescence labeling of breast tumor cells by functionalizing QDs with a cancer marker Her2 [68]. Gao *et al.* encapsulated QDs with a triblock copolymer and linked it with a cancer-specific antibody to create multifunctional probes with the functions of optical labeling and drug-delivery. They conducted an *in vivo* delivery experiment targeting human prostate cancer in mice and demonstrated enhanced permeability and retention [69]. While the toxicity of Cd contained in QDs is a concern, non-toxic QDs based on materials including

Zn, Ag, and In are also under investigation [70].

3.5. Mesoporous nanoparticle (MSN)

Mesoporous silica nanoparticles have gained great interest as a drug carrier because of their well-ordered internal mesopores with large pore volume and surface area, tunable size and morphology, and selective surface functionality [56,71,72]. Because of these morphological advantages, MSNs demonstrated high loading capacity for therapeutic agents and controlled release properties with the stimuli-responsive groups [73]. Various mesoporous nanoparticles with different structures and functionality have been developed for targeted delivery [74]. Different methods have been applied to synthesize MSNs with a tunable particle and pore sizes, including the modified Stöober method, soft and hard templating, aerosol-assisted synthesis, and dissolving reconstruction [75–77]. Manipulating the reaction parameters such as pH of the reaction, temperature, concentration of surfactant and silica source resulted in particles with different shapes, sizes and pore volumes [78]. Anchoring MSNs with targeting ligands can deliver site-specific delivery of drugs and avoid side effects. Zhang *et al.* reported an HA conjugated doxorubicin (DOX)-MSN which can actively deliver DOX through a receptor-mediated delivery system to colon cancer cells and release DOX triggered by hyaluronidase enzyme present in the tumor microenvironment. MSNs can store a high volume of chemotherapeutics in their pore and accumulate in tumor tissues via EPR [79]. To enhance the uptake by targeted cells, targeting ligands such as folic acid, mannose monoclonal antibody, galactose derivatives, hyaluronic acid, arginine-glycine-aspartate (RGD), transferrin and others, have been used for functionalization [80–82].

3.6. Biomimetic nanoparticles

3.6.1. DNA based nanoparticles

Based on the base-pairing rules, DNA molecules can function as building blocks to create programmable and bio-functional nanostructures [83]. The DNA nanostructures have demonstrated unique properties for drug delivery, such as uniform sizes and shapes, site-specific surface functionality, programmable nanostructure, and excellent biocompatibility. In addition, DNA nanostructure can obtain characteristics such as functional moieties to target tumor recognition, the capability of stimuli-responsive drug release, which makes them highly attractive vehicles [84]. A wide range of DNA nanostructures has been used to create chemotherapeutic drug loading platforms and aptamer-based delivery of DNA nanostructure for the specific uptake of drugs *in vivo* [85]. For example, DNA icosahedra nanostructure has been used as the vehicle of doxorubicin showing an efficient and specific strategy for epithelial cancer cells [86]. Chen *et al.* have developed a mimicking DNA base pairs Nanopiece(NP) based on the concept of Janus base molecules. The NP is composed of novel biomimetic molecules: 6-amino-fused guanine and cytosine. Self-assembly of the six Janus bases make a Rosette Nano-Tubes (RNT) via hydrogen bonds and further stack by π- π interaction and, when sonicated, results in novel drug delivery vehicles. Due to their unique architecture, NPs can encapsulate hydrophobic drugs and nucleotides and efficiently deliver to cells [87–89].

3.6.2. Protein-based nanoparticle

Protein-based nanoparticles have good biocompatibilities, biodegradability, and can be readily functionalized through surface modification. In addition, they show promising properties such as non-antigenicity, metabolizable, and greater stability during *in vivo* storage. A variety of protein polymers have been studied for nanoparticle-based drug delivery. Protein-based polymers can be based on albumin, gelatin, whey protein, gliadin, legumins, elastin, zein, soy protein, and milk protein, which have the ability to attach covalently with drugs and ligands [90]. Challenges to overcome include the fact that proteins are inherently unstable and complex molecules [91]. As these protein materials are improved, there will be more protein-based nanoparticles treatment.

3.6.3. Exosome based nanoparticle

One of the primary roles of exosomes is to function as chemical messengers for cellular communication. Many studies have shown that exosomes can efficiently deliver many different kinds of molecules to the target cell [92]. Therefore, they are considered to be therapeutic cargo for treatment. Due to the many similarities between liposomes and exosomes, liposome engineering technologies can be applied to engineer exosomes. Both the exosomes and liposomes are small unilamellar vesicles which have one lipid bilayer and formed vesicular structures with mean diameter ranging from 50 nm to 120 nm [93]. Because of this structure, exosomes can be engineered and modified to contain specific proteins, genetic lipids, and genetic materials, including messenger RNA (mRNA), micro RNA (miRNA), and other small non-coding RNAs, and genomic DNA (gDNA) from their progenitor cell [94,95]. The uptake of exosomes is cell-specific, based on the interaction between surface molecules from donor and recipient cells [96]. Compared to other nanoparticles, exosomes have shown many advantages such as biocompatibility, reduced clearance rates, and low long-term accumulation in tissue with low systematic toxicity and facilitated cellular uptake [97].

3.6.4. Polysaccharide based particle

Polysaccharides are based on carbohydrates, which are found abundantly in nature. Due to their biocompatibility and biodegradability, polysaccharide based nanoparticles have attracted interest as vehicles to deliver imaging and therapeutic agents [98]. In addition, the ease of facile chemical modification on the polysaccharide backbone has made a diverse group of structures [99]. These characteristics have met the requirements to be used as an effective platform for drug delivery and imaging. A variety of polysaccharides, including dextran, chitosan, and hyaluronic acid, have been utilized as the polymeric backbones to form nanoparticles, which can be provided as a valuable drug delivery vehicle [100–102]. Janes *et al.* used chitosan-based nanoparticles to entrap an anticancer drugs doxorubicin (DOX) delivered into cells in its active form [103]. Li *et al.* developed reversibly stabilized dextran nanoparticles for a triggered delivery of DOX [104]. Prepared by cross-linking dextran-lipoic acid derivatives, the nanoparticles were stable against dilution, while 90% of the DOX was released in 11 h in an *in vitro* environment that mimicked the intracellular reductive environment with 10 mM dithiothreitol.

3.7. Dendrimers

Dendrimers are made of artificial polymers with three-dimensional, highly branched, and well-defined architectures, which have been extensively explored for their applications in drug delivery [105]. Dendrimers have been regarded as promising vehicles for drug delivery due to their copious internal cavities and surface functionalities [106,107]. Drugs can be either physically encapsulated into the internal cavities or chemically conjugated to the terminal functional groups of dendrimers [108,109]. For example, several dendrimers such as poly (amino amine) (PAMAM), poly (propylene imine) (PPI) and poly (L-lysine) (PLL) have reached clinical trials on the drug delivery systems [110,111].

One of the major limitations of dendrimers as drug delivery carrier is that dendrimers do not degrade in the physiological environment, resulting in cytotoxicity [112]. Biodegradable dendrimers have been recently studied as promising candidates for drug delivery. Several biodegradable dendrimers demonstrated significant superiority and great potential for drug delivery [113].

3.8. Methods of nanoparticle synthesis

Nanoparticles that are often used for *in vitro* delivery studies include polystyrene, gold, lipid, silica nanoparticles (see also Table 1). In this section, we describe major synthesis methods of those commonly-available nanoparticles and other notable methods.

Most of polystyrene nanoparticles synthesis methods are categorized as emulsion polymerization or dispersion polymerization [114]. One example of emulsion polymerization uses sodium dodecyl sulfate as an emulsifier, persulfate as an initiator, and styrene as the monomer. Polystyrene nanoparticles are synthesized under a condition of low water-oil ratio. Dispersion polymerization method is to control the injection rate and concentration of styrene solution containing an initiator or a capping material to control the size of polystyrene spheres. For example, the diameter of polystyrene spheres increases when the initiator concentration or injection rate is increased, while it decreases with an increased capping material concentration or injection rate [115].

The synthesis methods of gold nanoparticles are roughly divided into physical methods and chemical methods. Many chemical synthesis methods have been actively studied. In Kimling *et al.* [116], gold nanoparticles are formed through the reaction of a small amount of hot $HAuCl_4$ in the presence of a reducing agent such as citrate, amino acid, or ascorbic acid. The citrate ion works both as a reducing agent and a blocking agent. The Brust method [117] is used to produce gold nanoparticles in organic liquids that are not miscible with water, such as toluene. The seed-mediated method is a widely used method for producing gold nanoparticles with different shapes [118]. First, gold salts are reduced using a strong reducing agent such as $NaBH_4$ to produce seed particles. Then, in the presence of a weak reducing agent, such as ascorbic acid, and a structure-directing agent, seed particles are added to the metal salt solution to prevent further nucleation and promote the anisotropic growth of nanoparticles. Physical methods include the ultraviolet photochemical method, the ultrasonic-assisted method and the laser ablation method [119].

Among many of lipid nanoparticle synthesis methods, commonly used are the thin film method, the reverse-phase evaporation method, the solvent injection method, and the double emulsion method. The thin-film method is primitive but the most basic and widely-used liposome preparation method to date [120]. Lipid-soluble drugs such as phospholipids and cholesterol are dissolved in organic solvents, and the solution will be moved into a round-bottom flask and spun to be dried under reduced pressure, resulting in the formation of a phospholipid thin film on the inner wall. The film is then added with a buffer solution, and mixed to make the lipid membrane hydrate off the wall. The reverse-phase evaporation method [121] uses phospholipid cholesterol dissolved in organic solvents, such as ether, added to an aqueous phase with a certain oil to water ratio. Using a rotary evaporator, organic solvents are removed to obtain reverse micelles. Liposomes are then formed through ultrasound sonication. The solvent injection method [122] is to inject an oil phase solution into a water phase solution at a uniform rate. The mixture is stirred and sonicated to evaporate the organic solvent and obtain liposomes. Double emulsion method [123] is based on multiple steps. The solution of the drug is first emulsified to form a W/O colostrum, which is then added with water for another step of emulsification for a W/O/W emulsion. Liposomes can be obtained by removing organic solvents at a certain temperature.

The manufacturing methods of quantum dots include solution-based methods and epitaxial growth methods [124]. In 1993, Bawendi *et al.* synthesized quantum dots of uniform size in an organic solution for the first time [125]. They used the three oxygen group elements (sulfur, selenium, tellurium) in tri-n-octylphosphine oxide, which reacted with dimethyl cadmium in an organic solution at 200 to 300 °C to synthesize the quantum dots of corresponding materials. The epitaxial growth method refers to the growth of new crystals on a substrate [126].

The hydrothermal synthesis method uses the chemical reaction to crystallize the substance in a sealed and heated solution. Generally, reactions occur in a high-temperature aqueous solution at a high vapor pressure level. For example, a hydrothermal method has been used to fabricate Fe_3O_4 nanoparticles with a well-controlled size (31.1 ± 6.1 nm) and shape distribution [127]. Water-dispersible CdS quantum dots were made through hydrothermal synthesis using the starting materials of cadmium chloride, thiourea, and 3-mercaptopropionic acid [128].

The sol-gel process is a popular fabrication method for silica nanoparticle preparation because it is simple, inexpensive, and suitable to fine-tune nanoparticle properties. The size, morphology, and distribution of the particles can be modified based on the reaction parameters. The first stage of synthesis is to prepare a sol, which is a colloidal suspension of particles in a liquid. Then the particles react with each other to form a cross-linked 3D polymeric chain, resulting in the formation of a three-dimensionally interconnected network (gel). The gel is dried, sintered, or solidified to extract liquids to obtain porous particles [129,130].

Microfluidic systems provide a powerful platform to fabricate and optimize the characteristics of various nanoparticles, including organic nanoparticles, metal nanoparticles, and oxide nanoparticles. The process of nanoparticle formation can be tailored through the manipulation of liquids, gases, droplets, and particles within the micro-channel geometries. The flexibility to design an application-specific device can separate the nucleation and growth stages in nanoparticle formation, modulate the nanoparticles with higher uniformity, and a lower polydispersity index. Thus, microfluidic systems can be suitable used to fabricate the nanoparticles in an optimized, high-throughput manner [131].

4. Surface modification/functionalization for targeted delivery and improved penetration

Surface functionalization provides nanoparticles important properties to function as a delivery system. It allows for a targeted drug delivery that improves drug utilization efficiency and reduces adverse drug reactions. Functionalized nanoparticles can also change the mechanism of membrane transport. It can promote the penetration of drugs to biofilm and biological barriers *in vivo*. Here is described the methods of surface modification/functionalization for targeted delivery and improved penetration.

Targeted delivery is especially an important aspect of using nanoparticles for a drug delivery system. There are two kinds of delivery methods to target particular sites; passive and active [132]. In passive targeting, the designed nanoparticle circulates through the bloodstream and binds to the target site by affinity or is influenced by the environment such as pH, temperature, molecular size and shape [1]. For instance, passive targeting works through the increased permeability and retention effect, promoting the probability for tumor cells to absorb the carrier nanoparticles [133,134]. On the other hand, active targeting has moieties like antibodies and peptides on the surface of nanoparticles to anchor them to the receptor structures expressed at the target site. Ligand-attached nanoparticles have been used to target specific cancer cell lines and deliver anti-cancer agents [135]. For example, hyaluronic acid has been used as a ligand conjugated in several nanocarriers, demonstrated to increase antitumor action against melanoma stem-like cells [136].

Also to be considered to promote the efficacy of drug delivery are a number of physiological barriers that limit the delivery of nanoparticles to the desired location. These biological barriers are innately designed to act on the body's defense system, which limits the penetration of foreign materials. The first barrier is the reticuloendothelial system comprised of the liver and spleen that clear particles from the circulation when the nanoparticles are intravenously administrated [137]. The endothelium of the blood vessels into target tissues is another barrier that larger nanoparticles cannot cross under normal healthy conditions.

However, in certain pathological conditions such as inflammation or cancer, the gap between the endothelial cells is increased, and nanoparticles can extravasate from the vascular system to the diseased site [138]. After escaping from the blood capillaries, nanoparticles confront the third level of the barrier, which is the extracellular matrix (ECM) that surrounds the target cells. In some conditions, excessive rigidity of the ECM will make the nanoparticles hard to transport from capillaries to the target cells [139,140]. Finally, the nanoparticles should be internalized by the endocytic process including pinocytosis, phagocytosis or endocytosis. If the nanoparticles can escape the endosome or lysosome, they diffuse in the cell cytosol and deliver the therapeutical agents [141].

4.1. Poly (lactic acid)(PLA) and poly (ethylene glycol) PEG block polymer

Poly (lactic acid)(PLA) and poly (lactic-co-glycolic acid) microspheres are not advantageous for the release of insoluble drugs because they are highly hydrophobic, and the acidic microenvironment produced by the degradation of polymer microspheres will affect the stability of proteins and peptides [142]. The presence of PEG can dramatically improve the hydrophilicity of this copolymer, reducing the concentration of lactic acid produced after PEG-PLA degradation. In addition, the *in vivo* long-term stability and drug-loading efficiency of the protein and polypeptide drugs are enhanced [143]. Farokhzad *et al.* used poly (D,L-lactic-co-glycolic acid)-block-poly(ethylene glycol) (PLGA-b-PEG) copolymer to formulate an anti-cancer drug docetaxel (Dtxl)-encapsulated nanoparticles and surface functionalized them with the A10 2′-fluoropyrimidine RNA aptamers to target prostate-specific membrane antigen (PSMA). These drug-encapsulated nanoparticle-aptamer bioconjugates resulted in significantly enhanced *in vitro* cellular toxicity as compared with non-targeted nanoparticles that lack the PSMA aptamer [144].

4.2. Transferrin receptor, TfR-targeted polymer nanoparticles

Since the demand for iron from tumor cells is much higher than that from the normal cells, the surface of the tumor cells usually overexpresses transferrin receptors (TfR). Transferrin (Tf) can specifically bind to the transferrin receptor on the surface of tumor cells, transferring the iron bound to transferrin into cells by endocytosis [145]. Therefore, transferrin can be used to modify drug-loaded nanoparticles and provide tumor cell targeting function. Mishra *et al.* attached transferrin on the surface of the PEGylated nanoparticles as a ligand for brain targeting. The enhanced uptake compared with unmodified nanoparticles was observed by fluorescence imaging analysis [146].

4.3. Charge

Many experimental studies have shown that due to the presence of molecule interaction and van der Waals forces, the charges carried by the surface of the nanoparticles entering the ECM and being taken up by the cells affect the penetration efficiency and the cellular uptake efficiency [147,148]. Therefore, changing the surface charge potential of nanoparticles has been a promising modification method to improve nanoparticles diffusion and uptake efficiency *in vivo* or *in vitro* [149]. Peulen *et al.* reported that the charge of the nanoparticles appeared to be important in biofilm diffusion; in dense bacterial biofilms, negatively charged Ag nanoparticles showed a lower self-diffusion coefficient than positively charged and neutral nanoparticles [150].

4.4. Folic acid receptor

Folic acid is an essential component of cells for the biosynthesis of nucleotides and metabolic pathways. Cells express folic acid receptors (FR), which have a high affinity for folic acid (FA). Folic acid has been an appropriate choice when targeting cancer cells since its receptor is significantly over-expressed on cancer cells of the breast, lung, kidney, ovary, colon, brain, and myelogenous leukemias [151]. On the other hand, it is expressed in low or non-detectable levels in most normal cells. For this reason, FA has been conjugated to anticancer drugs, gene therapy vectors, immunotherapeutic agents, liposomes, dendrimers, polymeric micelles, and therapeutic siRNAs/miRNAs [152–155]. Since folic acid binds to its receptor with high affinity in nanomolar affinity ($Kd \sim 10^{-9}$ M) folate-linked nanoparticles are recognized by the folate receptor and subsequently internalized via receptor-mediated endocytosis, resulting in delivering its cargoes into the cytosol [156]. Folate-targeted nanoparticles may provide drug delivery vehicles for a variety of applications.

4.5. Arginine-glycine-aspartate (RGD)

Arginine-Glycine-Aspartate (RGD) peptides are known to bind preferentially to the $\alpha_v\beta_3$-integrin. This integrin receptor is expressed on the surface of tumor vessels and various types of cancer cells, which through activation functions as tumor growth promotion, metastasis, and angiogenesis [157,158]. Therefore, applying RGD-based strategies to target tumor cells or tumor vasculature is promising for delivering anticancer drugs for cancer therapy [79]. RGD peptide has been used as targeting ligand to functionalize the nanoparticle surface to target $\alpha_v\beta_3$-integrin [159]. Many types of RGD-conjugated nanoparticles designed to deliver therapeutic drugs have been proven to promote the efficacy of delivery to cancer cells. For example, liposomes, nanoparticles, micelles can be grafted at their surface with RGD based sequence as a targeting ligand [160,161]. RGD-conjugated nanoparticles bind to the cells overexpressing $\alpha_v\beta_3$-integrin cells and release drugs to fulfill active targeting of the tumors.

5. *In vitro* platform

The use of *in vitro* tissue models offers analyses that are difficult to conduct either with conventional 2D monolayer cultures or *in vivo* animal models. The penetration of nanoparticles and the release of drugs depend on many factors including particle size, shape, surface modification, temperature, pH, matrix porosity, and pore sizes. By using engineered tissue models, the impact of each of these factors can be quantitatively studied. In this section, we review *in vitro* tissue models designed for the study of nanoparticle transport.

Consideration of the functions of extracellular matrix (ECM) is especially important. It plays an essential role in tissue differentiation and progression. For instance, the tumor microenvironment is composed of malignant and nonmalignant cells, collagen, fiber, and blood vessels that surrounded and assisted the growth of the tumor [2]. The microenvironment of the tumor is significantly different from that of healthy tissues in morphological, physical, and chemical properties. Since the increased body fluid pressure reduces the efficiency of the mass transport through convection, diffusion is the primary mechanism of delivery into tumors. Within the tumor, the dense fiber matrix, which consists of collagen and interacting molecules, hinders the process of diffusion [162].

5.1. Multicellular spheroids

Compared to the traditional 2D cell culture method, bioengineered 3D cell culture models provide a better perspective for studying cell-cell, cell-matrix interaction, and complex process of molecule transport [58,163,164]. Multicellular cancer spheroids have emerged to be an effective 3D research model for studying solid tumor properties *in vitro* [165,166]. First of all, multicellular spheroids have several important features that are similar to solid tumors in terms of complex heterogeneous multicellular environments and physical or mechanical properties. In addition, they provide a cost-effective and time-efficient tool for screening new anti-cancer drugs and determining essential

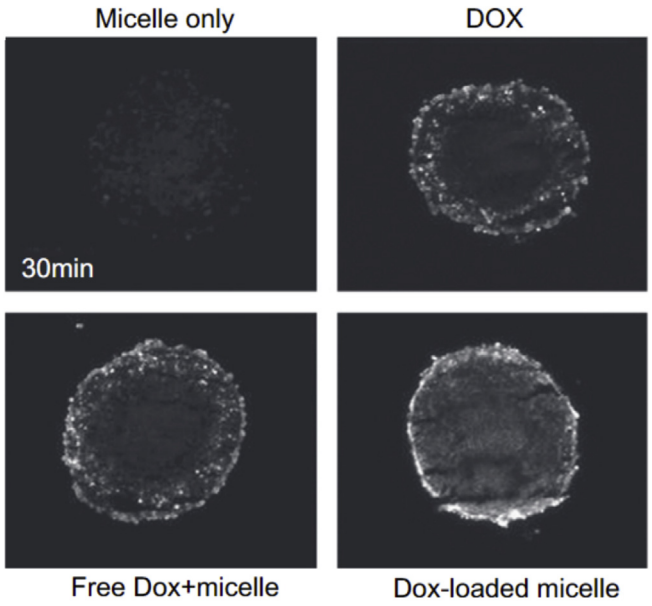

Fig. 2. Penetration of doxorubicin (DOX) to multicellular spheroids. DOX loaded on micelles reached the core of the spheroid within 30 min. Reprinted from Ref. [167] with permission from Elsevier.

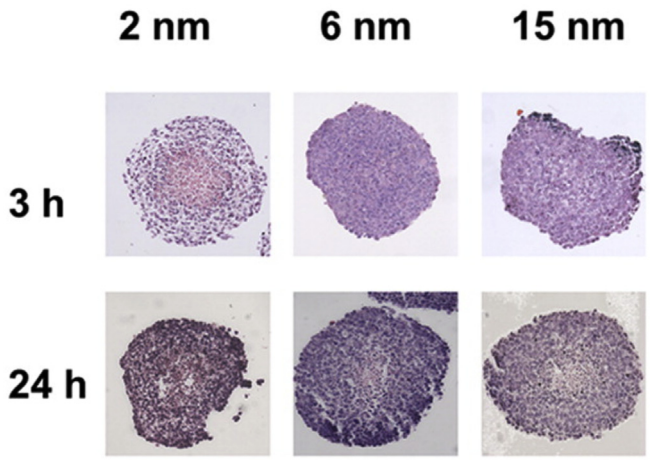

Fig. 3. Penetration of gold nanoparticles with different sizes into spheroids after incubation of 3 and 24 h. Reprinted with permission from Ref. [58] Copyright 2012 American Chemical Society. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

parameters in nanoparticle penetration. Kim *et al.* used multicellular spheroids and studied the penetration of a drug molecule doxorubicin (DOX) loaded in micelle [167]. Fig. 2 shows DOX fluorescence images of criosectioned multicellular spheroids incubated with free DOX, DOX loaded in micelles, and DOX mixed with empty micelles. DOX delivered in micelle showed an efficient delivery to the spheroid core within 30 min. Huang *et al.* studied the penetration of gold nanoparticles of 2–15 nm with identical surface coating [58]. Fig. 3 shows HE-stained sections of breast tumor spheroids incubated with gold nanoparticles after incubation of 3 and 24 h. Ma *et al.* generated HeLa-derived spheroids to study the penetration of doxorubicin, quantum dots, and polymeric micelle versus traditional 2D cell culture method. Several unique characteristics in the solid tumor can only be observed in 3D spheroid models, such as resistance to chemotherapy [168]. In another study, Hongxu Lu *et al.* compared the anti-cancer effect of albumin-based nanoparticles in 2D cultured AsPC-1 cells and 3D multicellular tumor spheroids. They also found that secreted protein acidic and rich in cysteine (SPARC) protein facilitated the penetration and drug delivery of albumin nanoparticle [169].

Goodman et al. treated SiHa (human cervical cancer cell) spheroids with collagenase and studied the penetration of polystyrene nanoparticles with different sizes [166]. Fig. 4 shows fluorescence images of criosections of collagenase untreated and treated spheroids. Treated spheroids show deeper penetration for all sizes of nanoparticles. Hoshino *et al.* [170] used force sensitive micromechanical tweezers and compared the stiffness of spheroids treated/untreated with collagenase (Fig. 5). The average Young's moduli of treated and untreated samples were 130 ± 30 Pa and 230 ± 60 Pa, respectively. This result is very interesting for the nanoparticle delivery study because it has experimentally demonstrated the correlation between mechanical stiffness and nanoparticle penetration as suggested by the concept of the enhanced penetration and retention (EPR) effect.

5.2. Hydrogels

As a biologically adaptable biomaterial, hydrogels have been widely used in biomedical engineering in recent years. Hydrogels can be roughly classified into two categories according to their physical properties: homogeneous and heterogeneous [171].

Several factors could affect the diffusion process of nanoparticles in the hydrogel, such as the pore size that should be compared to the size of nanoparticles, polymer chain mobility, and the charge group on the polymer chain [172]. Walta *et al.* studied nanoparticle penetration in crowded polymer-network hydrogels [173]. They found the penetration depends on the polymer density rather than the gel-matrix cross-linking density, and showed a correlation between nanoparticle diffusion coefficient and polymer concentrations. Zhang *et al.* studied experimental and theoretical studies to examine the impact of electrostatic

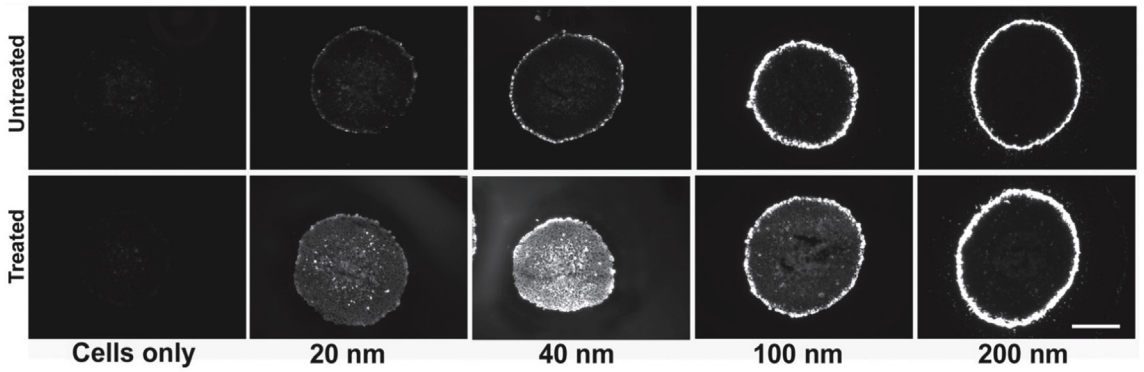

Fig. 4. Fluorescence images of collagenase-untreated and treated spheroids incubated with fluorescent nanoparticles with various sizes. Scale bar 200 μm. Reprinted from [166].

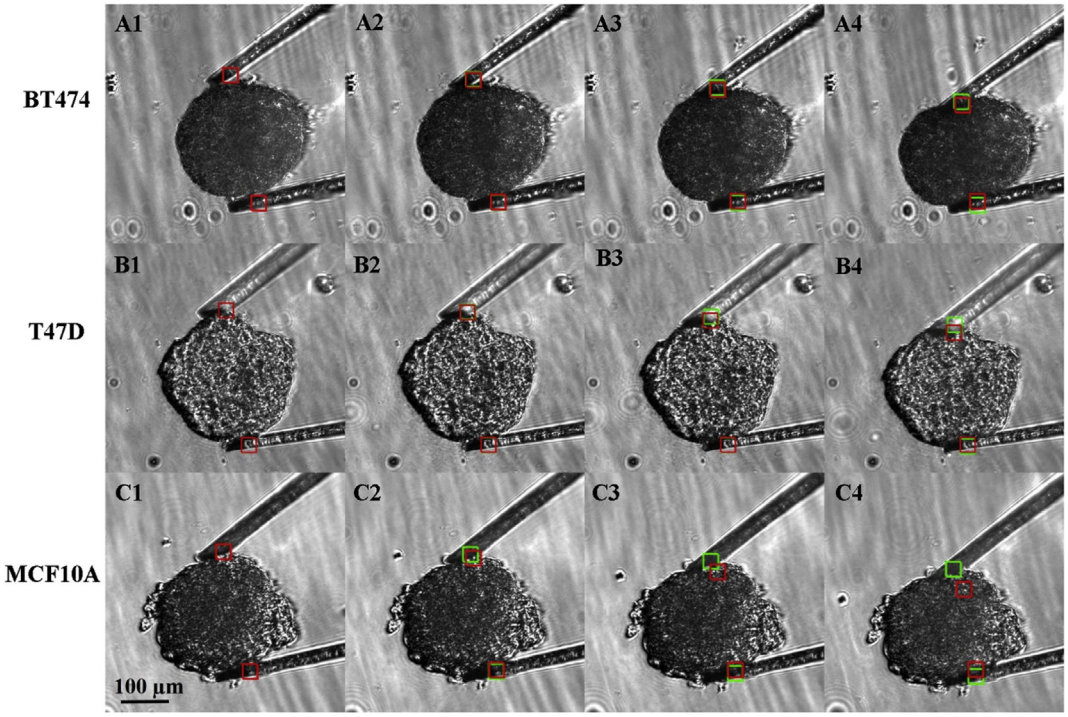

Fig. 5. Mechanical characterization of spheroids. They showed collagenase treated spheroids to be significantly softer than untreated spheroids. Reprinted from [170].

force on the diffusion of charged molecules within polymer networks. They found that particle transport was slowed down more by electrostatic attraction than by repulsion [174]. Hydrogels are often used as an *in vitro* model for extracellular matrix. Kim *et al.* developed a system where tumor spheroids are embedded in a matrigel to assess the uptake and release of fluorescent dye(FITC)-loaded gold nanoparticles [175]. They used a one-dimensional microchannel module to measure the diffusion coefficients of cationic and anionic gold nanoparticles. Xu *et al.* [176] used an engineered 3D tumor model based on hyaluronic acid (HA) hydrogel and LNCaP (prostate cancer cell line) cells to evaluate the resistance to free and nanoparticle-loaded doxorubicin. LNCaP cells cultured in the HA matrices formed ~50 μm distinct multicellular aggregates. Nanoparticles were prepared from amphiphilic block copolymers, and the average diameter was 54 nm. The 3D models prepared in HA hydrogel were more resistant to drug treatments that the 2D models.

5.3. 3D printed tissues

Three-dimensional bioprinting (3D bioprinting) is a promising technology that is developing rapidly. It has found a wide range of applications in tissue engineering and regenerative medicine [177–179]. In particular for drug discovery studies, 3D bioprinting techniques including inkjet, extrusion, and laser-based printing has provide a versatile means to fabricate and test *in vitro* engineered tissues designed for different types of tissues [180,181]. LaBonia *et al.* developed a 3D printed fluidic device to conduct dynamic dosing tests with colon cancer spheroids and tested the penetration of a cancer drug irinotecan [182].

The use of 3D printing for the fabrication *in vitro* tissues is an important application. Zhao *et al.* [183]. reported on a method of 3D printing to construct an *in vitro* cervical tumor models containing Hela cells and gelatin/alginate/fibrinogen hydrogels. They compared cell proliferation, matrix metalloproteinase protein (MMP) expression, and the resistance to the treatment of anti-tumor drug paclitaxel. It was found that the cells in the 3D printed models showed higher MMP protein expression and higher drug resistance than those in the 2D models. Xiaochun *et al.* used a bioprinting method to rebuild a 3D layer-by-layer artificial skin model [184]. They studied the penetration of silica nanoparticles with different surface charges and showed deeper penetration with positively charged particles, which is consistent with studies based on living skin tissue. The 3D bioprinting technology is especially vital because it can recreate the microenvironment to study the structural characteristics that are closely related to drug sensitivity. Zhu *et al.* used a stereolithography-based 3D bioprinter to create a hydrogel-based nanocomposite *in vitro* tissue model for the study of breast cancer bone metastasis [185] Their model mimicked bone-specific environment to evaluate the bone invasion by breast cancer cells. The 3D matrix showed higher drug resistance of breast cancercells than 2D culture.

6. *In Vitro* imaging methods

Biomedical imaging is an essential step to have a better understanding of nanoparticle penetration and diffusion process [186]. Fluorescent microscopy can provide the most intuitive and convenient way to observe fluorescence-labeled nanoparticle distribution and penetration *in vitro* [187]; confocal microscopy is able to improve the optical resolution and contrast through rebuilding 3D structure within objective [188,189]. Fluorescence correlation spectroscopy (FCS) analyzes the fluorescence intensity fluctuation caused by nanoparticle Brownian motion to calculate the diffusion coefficient of the nanoparticles in fluid liquid [190]. On the other hand, electron microscope, such as Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM), are advantageous when characterizing the size and shape of nanoparticles at nano-size level [191]. The use of magnetic resonance imaging (MRI) is not restricted by material thickness and is suitable for relatively large tissue samples. Time-resolved MicroMRI has been conducted to evaluate the penetration of with Gold nanoparticles in a hydrogel-based *in vitro* systems [192].

6.1. Optical microscopy

The optical properties of nanoparticles highly rely on the nanoparticle size, shape, and composition [193]. Confocal microscopy is one of the most common methods of 3D fluorescence imaging. However, as we have already seen in a few examples, physically-sectioned tissue samples are often preferred because they provide more accurate assessment of nanoparticle penetration than optically-sliced images of confocal microscopy. Examples of the use of confocal microscopy include Grainger *et al.*, where they used a confocal microscope to observe the penetration of fluorescein isothiocyanate (FITC)-loaded polystyrene particles in MCF-7 spheroids with or without an application of ultrasound. With ultrasound treatment, 20 nm nanoparticles showed a 6–20 fold higher penetration to the spheroid core compared to the untreated test [194]. In another work, Walta *et al.* employed confocal dual-focus fluorescence correlation spectroscopy (2fFCS) to study the probe tracer diffusion in heterogeneous hydrogels [173].

6.2. Electron microscopy: TEM, SEM

The resolution limit of optical microscopy is defined by the wavelengths of light used for imaging. Since the wavelength of high-speed electrons is much shorter than the wavelengths of visible lights, the resolution of the electron microscope (about 0.2 nm) is much higher than that of the optical microscope [195]. Zheng *et al.* observed the motion of inorganic nanoparticles during fluid evaporation using TEM [196]. Mu *et al.* employed SEM to examine the morphological properties of a PLGA nanoparticle delivery system that enables the transport of a cancer drug paclitaxel into the tumor site *in vivo* [197]. Fig. 6 shows SEM images taken to study the efficacy of cyclic RGD peptide-functionalized PEGylated poly(trimethylenecarbonate) nanoparticles (c(RGDyK)-nanoparticle) [187]. Spheroids were treated with conventional nanoparticles-based Paclitaxel (NP/PTX), Taxol, c(RGDyK)-NP/PTX, demonstrating the strongest penetration with c(RGDyK)-NP. Fig. 7 shows TEM images showing gold nanoparticle penetration in Huang *et al.* [58].

6.3. MRI

MRI is another imaging modality that is used in addition to fluorescence chromatography for the study of penetration and diffusion of Gold nanoparticles. In the work of Xiaoling *et al.*, microMRI was used to monitor polymer nanoparticle diffusion in biological gel in real-time and compared the result to that obtained using established fluorescence microscopy and found that the two results corresponded to each other [192]. The MRI technology is advantageous because it can monitor nanoparticle diffusion in thick samples which cannot be imaged using fluorescent microscopy.

7. Conclusion and perspective

We have reviewed studies of nanoparticle-based drug delivery with the emphasis on the use of *in vitro* engineered microtissues. Currently, one of the most commonly used *in vitro* models is the multi-cellular spheroid, which can be easily prepared from different types of cells. Since spheroids are already widely used in the pharmaceutical industry for drug development and screening, they serve as an excellent reference model to assess the efficacy of new delivery systems employing novel nanoparticles. They are compatible with standard cell culture protocols and model many key cellular parameters such as cell-cell attachment, proliferation, mechanical rigidity, and, most importantly, the transport of molecules and nanoparticles. However, the formation of spheroids highly relies on the biological characteristics of the cells used, and it is still a complex problem to regulate quantitative parameters such as porosity and matrix cross-linking. Gene modification or an application of enzymes such as collagenase followed by quantitative imaging or mechanical characterizations will be the future direction to provide quantitative measures for the evaluation of nanoparticle

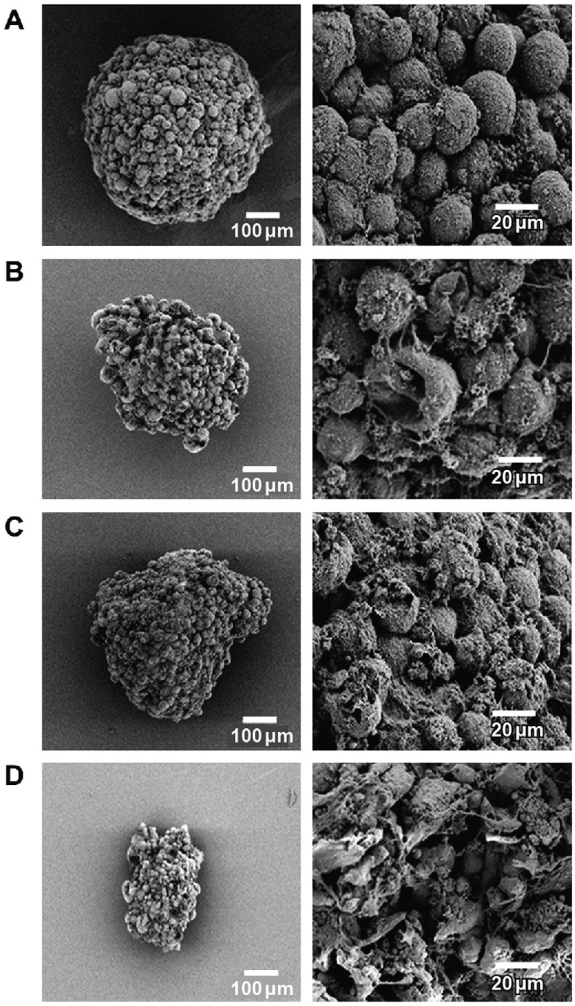

Fig. 6. SEM images of spheroids at day 3 after treatment of (A) Control (B) Taxol (C) NP/PTX and (D) c(RGDyK)-NP/PTX (D). Reprinted from Refs. [187] with permission from Elsevier.

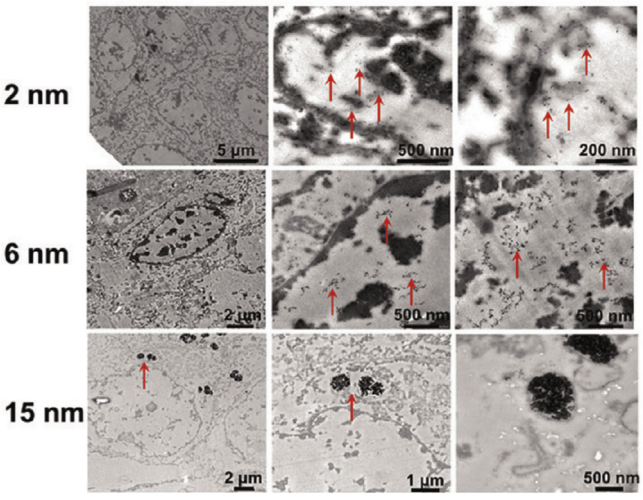

Fig. 7. TEM images of spheroids treated with gold nanoparticles for 24 h. Reprinted with permission from Ref. [58] Copyright 2012 American Chemical Society. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

delivery. For simplification, the use of hydrogel is advantageous because it allows for easy modification of polymer density, cross-linking, and porosity. For the cases where more detailed functional structures of organs or tumors are of concern, testing with bio-engineered tissues, including 3D printed materials, will be beneficial. For example, simple spheroids do not model vasculature, or the formation of blood vessels, which are key aspects that characterize tumors. More complexed engineered 3D tissue models are emerging as a new trend as the technology of 3D bioprinting has gained popularity. It has been demonstrated that *in vitro* models can be effectively used as a tool for studying disease mechanisms and assessing drug sensitivity. To understand the roles of specific tissue characteristics, such as biological barriers, in nanoparticle delivery, the use of microfluidic channels to mimic tissue miroenvironment is also an important direction [198,199].

While nanoparticle penetration has been studied through a number of imaging modalities, not many studies have focused on mechanical characteristics of tissues. As we discussed, the structural composition is one of the crucial factors defining tissue characteristicssuch as drug resistance. Methods to correlate mechanical characteristics and nanoparticle transport properties are yet to be fully investigated. In particular, the measurement of the elastic modulus allows for a simple, straight forward means for quantitative tissue analysis. Since the first successful report of the Young's moduli of multi-cellular spheroids by Jaiswal *et al.*, a few studies have reported on the mechanical characteristics of spheroids [200,201]. Measurements of elastic moduli or more detailed structural analysis of tissues will be a new direction for the study of nanoparticle penetration and transport.

As more complex bio-engineered tissues being proposed, understanding and characterization of tissue heterogeneity are becoming a more important subject. For example, it is known that the interaction between tumor cells and tumor-associated fibroblast cells is one of the factors that define the cancer drug resistance. Fibroblast cells promote ECM remodeling and stiffening which may reduce the penetration of nanoparticles and induce drug resistance. Jaiswal *et al.* studied a method of imaging the elasticity map of a tumor cell-fibroblast co-culture spheroid [202]. Another example as we discussed earlier is the presence of the blood-brain barrier (BBB) that affects the delivery of therapeutic agents to the brain. Ho *et al.* studied nanoparticle delivery in a brain tumor spheroid coated with endothelial cells to study the role of a blood vessel as a barrier [203]. *In vitro* assays that can investigate structural heterogeneity in 3D tumor models will be a useful tool to study the interaction between different types of cells.

Declaration of competing interest

The authors declare no conflict of interest.

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References

[1] J.K. Patra, G. Das, L.F. Fraceto, et al., Nano based drug delivery systems: recent developments and future prospects, J. Nanobiotechnol. 16 (1) (2018) 71.
[2] I. Brigger, C. Dubernet, P. Couvreur, Nanoparticles in cancer therapy and diagnosis, Adv. Drug Deliv. Rev. 64 (2012) 24–36.
[3] J. Panyam, V. Labhasetwar, Biodegradable nanoparticles for drug and gene delivery to cells and tissue, Adv. Drug Deliv. Rev. 55 (3) (2003) 329–347.
[4] A. des Rieux, V. Fievez, M. Garinot, Y. Schneider, V. Préat, Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach, J. Contr. Release 116 (1) (2006) 1–27.
[5] F.X. Gu, R. Karnik, A.Z. Wang, et al., Targeted nanoparticles for cancer therapy, Nano Today 2 (3) (2007) 14–21.
[6] P.D. Reddy, D. Swarnalatha, Recent advances in novel drug delivery systems, Int. J. PharmTech. Res. 2 (3) (2010) 2025–2027.
[7] P. Latreille, V. Adibnia, A. Nour, et al., Spontaneous shrinking of soft nanoparticles boosts their diffusion in confined media, Nat. Commun. 10 (1) (2019) 1–8.
[8] D.P. O'Neal, L.R. Hirsch, N.J. Halas, J.D. Payne, J.L. West, Photo-thermal tumor ablation in mice using near infrared-absorbing nanoparticles, Canc. Lett. 209 (2) (2004) 171–176.
[9] K.N. Sugahara, T. Teesalu, P.P. Karmali, et al., Tissue-penetrating delivery of compounds and nanoparticles into tumors, Canc. Cell 16 (6) (2009) 510–520.
[10] H. Maeda, J. Wu, T. Sawa, Y. Matsumura, K. Hori, Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review, J. Contr. Release 65 (1-2) (2000) 271–284.
[11] J. Kim, Y. Kim, K. Park, et al., Antitumor efficacy of cisplatin-loaded glycol chitosan nanoparticles in tumor-bearing mice, J. Contr. Release 127 (1) (2008) 41–49.
[12] H. Cho, Z. Dong, G.M. Pauletti, et al., Fluorescent, superparamagnetic nanospheres for drug storage, targeting, and imaging: a multifunctional nanocarrier system for cancer diagnosis and treatment, ACS Nano 4 (9) (2010) 5398–5404.
[13] J. Gong, M. Chen, Y. Zheng, S. Wang, Y. Wang, Polymeric micelles drug delivery system in oncology, J. Contr. Release 159 (3) (2012) 312–323.
[14] R. Duivenvoorden, J. Tang, D.P. Cormode, et al., A statin-loaded reconstituted high-density lipoprotein nanoparticle inhibits atherosclerotic plaque inflammation, Nat. Commun. 5 (2014) 3065.
[15] J.R. McCarthy, R. Weissleder, Multifunctional magnetic nanoparticles for targeted imaging and therapy, Adv. Drug Deliv. Rev. 60 (11) (2008) 1241–1251.
[16] P.M. Winter, A.M. Morawski, S.D. Caruthers, et al., Molecular imaging of angiogenesis in early-stage atherosclerosis with αvβ3-integrin–targeted nanoparticles, Circulation 108 (18) (2003) 2270–2274.
[17] P.M. Winter, S.D. Caruthers, H. Zhang, T.A. Williams, S.A. Wickline, G.M. Lanza, Antiangiogenic synergism of integrin-targeted fumagillin nanoparticles and atorvastatin in atherosclerosis, JACC (J. Am. Coll. Cardiol.): Cardiovas. Imag. 1 (5) (2008) 624–634.
[18] C.G. Helmick, D.T. Felson, R.C. Lawrence, et al., Estimates of the prevalence of arthritis and other rheumatic conditions in the United States: Part I, Arthritis Rheum. 58 (1) (2008) 15–25.
[19] N. Gerwin, C. Hops, A. Lucke, Intraarticular drug delivery in osteoarthritis, Adv. Drug Deliv. Rev. 58 (2) (2006) 226–242.
[20] C.H. Evans, V.B. Kraus, L.A. Setton, Progress in intra-articular therapy, Nat. Rev. Rheumatol. 10 (1) (2014) 11.
[21] C. Larsen, J. Østergaard, S.W. Larsen, et al., Intra-articular depot formulation principles: role in the management of postoperative pain and arthritic disorders, J. Pharmaceut. Sci. 97 (11) (2008) 4622–4654.
[22] A.G. Bajpayee, M. Scheu, A.J. Grodzinsky, R.M. Porter, Electrostatic interactions enable rapid penetration, enhanced uptake and retention of intra-articular injected avidin in rat knee joints, J. Orthop. Res. 32 (8) (2014) 1044–1051.
[23] N.J. Shah, B.C. Geiger, M.A. Quadir, et al., Synthetic nanoscale electrostatic particles as growth factor carriers for cartilage repair, Bioeng. Transl. Med. 1 (3) (2016) 347–356.
[24] B.J. Crielaard, C.J. Rijcken, L. Quan, et al., Glucocorticoid-loaded core-cross-linked polymeric micelles with tailorable release kinetics for targeted therapy of rheumatoid arthritis, Angew. Chem. Int. Ed. 51 (29) (2012) 7254–7258.
[25] Jolanda M. van den Hoven, S.R. Van Tomme, J.M. Metselaar, B. Nuijen, J.H. Beijnen, G. Storm, Liposomal drug formulations in the treatment of rheumatoid arthritis, Mol. Pharm. 8 (4) (2011) 1002–1015.
[26] V. Gajbhiye, V.K. Palanirajan, R.K. Tekade, N.K. Jain, Dendrimers as therapeutic agents: a systematic review, J. Pharm. Pharmacol. 61 (8) (2009) 989–1003.
[27] D.A. Rothenfluh, H. Bermudez, C.P. O'Neil, J.A. Hubbell, Biofunctional polymer nanoparticles for intra-articular targeting and retention in cartilage, Nat. Mater. 7 (3) (2008) 248.
[28] R. Haag, F. Kratz, Polymer therapeutics: concepts and applications, Angew. Chem. Int. Ed. 45 (8) (2006) 1198–1215.
[29] W. Barnabas, Drug targeting strategies into the brain for treating neurological diseases, J. Neurosci. Methods 311 (2019) 133–146.
[30] J.J. Alexander, Blood-brain barrier (BBB) and the complement landscape, Mol. Immunol. 102 (2018) 26–31.
[31] D. Teleanu, C. Chircov, A. Grumezescu, A. Volceanov, R. Teleanu, Blood-brain delivery methods using nanotechnology, Pharmaceutics 10 (4) (2018) 269.
[32] O. Betzer, M. Shilo, R. Opochinsky, et al., The effect of nanoparticle size on the ability to cross the blood–brain barrier: an *in vivo* study, Nanomedicine 12 (13) (2017) 1533–1546.
[33] H. Ou, T. Cheng, Y. Zhang, et al., Surface-adaptive zwitterionic nanoparticles for prolonged blood circulation time and enhanced cellular uptake in tumor cells, Acta Biomater. 65 (2018) 339–348.
[34] G. Guidotti, L. Brambilla, D. Rossi, Cell-penetrating peptides: from basic research to clinics, Trends Pharmacol. Sci. 38 (4) (2017) 406–424.
[35] T. Lin, P. Zhao, Y. Jiang, et al., Blood–brain-barrier-penetrating albumin nanoparticles for biomimetic drug delivery via albumin-binding protein pathways for antiglioma therapy, ACS Nano 10 (11) (2016) 9999–10012.
[36] D. Liu, Y. Cheng, R. Cai, et al., The enhancement of siPLK1 penetration across BBB and its anti glioblastoma activity *in vivo* by magnet and transferrin co-modified nanoparticle, Nanomed. Nanotechnol. Biol. Med. 14 (3) (2018) 991–1003.
[37] A.J. Clark, M.E. Davis, Increased brain uptake of targeted nanoparticles by adding an acid-cleavable linkage between transferrin and the nanoparticle core, Proc. Natl. Acad. Sci. U. S. A. 112 (40) (2015) 12486–12491, https://doi.org/10.1073/pnas.1517048112 [doi].
[38] E. Nance, K. Timbie, G.W. Miller, et al., Non-invasive delivery of stealth, brain-penetrating nanoparticles across the blood− brain barrier using MRI-guided focused ultrasound, J. Contr. Release 189 (2014) 123–132.

[39] S. van Rijt, P. Habibovic, Enhancing regenerative approaches with nanoparticles, J. R. Soc. Interface 14 (129) (2017) 20170093.
[40] J. Shi, A.R. Votruba, O.C. Farokhzad, R. Langer, Nanotechnology in drug delivery and tissue engineering: from discovery to applications, Nano Lett. 10 (9) (2010) 3223–3230.
[41] M. Goldberg, R. Langer, X. Jia, Nanostructured materials for applications in drug delivery and tissue engineering, J. Biomater. Sci. Polym. Ed. 18 (3) (2007) 241–268.
[42] S. Stratton, N.B. Shelke, K. Hoshino, S. Rudraiah, S.G. Kumbar, Bioactive polymeric scaffolds for tissue engineering, Bioact. Mater. 1 (2) (2016) 93–108.
[43] M. Biondi, F. Ungaro, F. Quaglia, P.A. Netti, Controlled drug delivery in tissue engineering, Adv. Drug Deliv. Rev. 60 (2) (2008) 229–242.
[44] S. Zhang, H. Uludağ, Nanoparticulate systems for growth factor delivery, Pharm. Res. 26 (7) (2009) 1561.
[45] T.P. Richardson, M.C. Peters, A.B. Ennett, D.J. Mooney, Polymeric system for dual growth factor delivery, Nat. Biotechnol. 19 (11) (2001) 1029.
[46] J.S. Park, S.W. Yi, H.J. Kim, S.M. Kim, K. Park, Regulation of cell signaling factors using PLGA nanoparticles coated/loaded with genes and proteins for osteogenesis of human mesenchymal stem cells, ACS Appl. Mater. Interfaces 8 (44) (2016) 30387–30397.
[47] S.K. Nune, P. Gunda, P.K. Thallapally, Y. Lin, Laird forrest M, berkland CJ. Nanoparticles for biomedical imaging, Expet Opin. Drug Deliv. 6 (11) (2009) 1175–1194.
[48] S. Lee, X. Chen, Dual-modality probes for *in vivo* molecular imaging, Mol. Imag. 8 (2) (2009) 7290.2009. 00013.
[49] R. Weissleder, Molecular imaging in cancer, Science 312 (5777) (2006) 1168–1171 doi: 312/5777/1168 [pii].
[50] H. Lusic, M.W. Grinstaff, X-ray-computed tomography contrast agents, Chem. Rev. 113 (3) (2012) 1641–1666.
[51] D. Kim, S. Park, J.H. Lee, Y.Y. Jeong, S. Jon, Antibiofouling polymer-coated gold nanoparticles as a contrast agent for *in vivo* X-ray computed tomography imaging, J. Am. Chem. Soc. 129 (24) (2007) 7661–7665.
[52] J. Panyam, W. Zhou, S. Prabha, S.K. Sahoo, V. Labhasetwar, Rapid endo-lysosomal escape of poly (DL-lactide-co-glycolide) nanoparticles: implications for drug and gene delivery, Faseb. J. 16 (10) (2002) 1217–1226.
[53] S.A. Kulkarni, S. Feng, Effects of particle size and surface modification on cellular uptake and biodistribution of polymeric nanoparticles for drug delivery, Pharm. Res. 30 (10) (2013) 2512–2522.
[54] I.H. El-Sayed, X. Huang, M.A. El-Sayed, Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer, Nano Lett. 5 (5) (2005) 829–834.
[55] I.H. El-Sayed, X. Huang, M.A. El-Sayed, Selective laser photo-thermal therapy of epithelial carcinoma using anti-EGFR antibody conjugated gold nanoparticles, Canc. Lett. 239 (1) (2006) 129–135.
[56] J.X. Zhang, K. Hoshino, Molecular Sensors and Nanodevices: Principles, Designs and Applications in Biomedical Engineering, Academic Press, 2018.
[57] G.F. Paciotti, D.G. Kingston, L. Tamarkin, Colloidal gold nanoparticles: a novel nanoparticle platform for developing multifunctional tumor-targeted drug delivery vectors, Drug Dev. Res. 67 (1) (2006) 47–54.
[58] K. Huang, H. Ma, J. Liu, et al., Size-dependent localization and penetration of ultrasmall gold nanoparticles in cancer cells, multicellular spheroids, and tumors *in vivo*, ACS Nano 6 (5) (2012) 4483–4493.
[59] A. Puri, K. Loomis, B. Smith, et al., Lipid-based nanoparticles as pharmaceutical drug carriers: from concepts to clinic, Crit. Rev. Ther. Drug Carrier Syst. 6 (2009) 26.
[60] R.H. Müller, K. MaÈder, S. Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery–a review of the state of the art, Eur. J. Pharm. Biopharm. 50 (1) (2000) 161–177.
[61] Y.C. Barenholz, Doxil®—the first FDA-approved nano-drug: lessons learned, J. Contr. Release 160 (2) (2012) 117–134.
[62] Y. Ning, K. He, R. Dagher, et al., Liposomal doxorubicin in combination with bortezomib for relapsed or refractory multiple myeloma, Oncology 12 (2007) 21.
[63] A. Meyerhoff, US food and drug administration approval of AmBisome (liposomal amphotericin B) for treatment of visceral leishmaniasis, Clin. Infect. Dis. 28 (1) (1999) 42–48.
[64] Z. Merchant, G. Buckton, M.G. Taylor K, et al., A new era of pulmonary delivery of nano-antimicrobial therapeutics to treat chronic pulmonary infections, Curr. Pharmaceut. Des. 22 (17) (2016) 2577–2598.
[65] C. Herzog, K. Hartmann, V. Künzi, et al., Eleven years of inflexal® v—a virosomal adjuvanted influenza vaccine, Vaccine 27 (33) (2009) 4381–4387.
[66] M. Sabaeian, A. Khaledi-Nasab, Size-dependent intersubband optical properties of dome-shaped InAs/GaAs quantum dots with wetting layer, Appl. Optic. 51 (18) (2012) 4176–4185.
[67] I.L. Medintz, H.T. Uyeda, E.R. Goldman, H. Mattoussi, Quantum dot bioconjugates for imaging, labelling and sensing, Nat. Mater. 4 (6) (2005) 435–446.
[68] X. Wu, H. Liu, J. Liu, et al., Immunofluorescent labeling of cancer marker Her2 and other cellular targets with semiconductor quantum dots, Nat. Biotechnol. 21 (1) (2003) 41.
[69] X. Gao, Y. Cui, R.M. Levenson, L.W. Chung, S. Nie, *In vivo* cancer targeting and imaging with semiconductor quantum dots, Nat. Biotechnol. 22 (8) (2004) 969.
[70] P. Subramaniam, S.J. Lee, S. Shah, S. Patel, V. Starovoytov, K. Lee, Generation of a library of non-toxic quantum dots for cellular imaging and siRNA delivery, Adv. Mater. 24 (29) (2012) 4014–4019.
[71] M. Vallet-Regí, F. Balas, D. Arcos, Mesoporous materials for drug delivery, Angew. Chem. Int. Ed. 46 (40) (2007) 7548–7558.
[72] M. Rosenholm J, C. Sahlgren, M. Linden, Multifunctional mesoporous silica nanoparticles for combined therapeutic, diagnostic and targeted action in cancer treatment, Curr. Drug Targets 12 (8) (2011) 1166–1186.
[73] A. Baeza, M. Colilla, M. Vallet-Regí, Advances in mesoporous silica nanoparticles for targeted stimuli-responsive drug delivery, Expet Opin. Drug Deliv. 12 (2) (2015) 319–337.
[74] M. Martínez-Carmona, M. Colilla, M. Vallet-Regí, Smart mesoporous nanomaterials for antitumor therapy, Nanomaterials 5 (4) (2015) 1906–1937.
[75] M. Grün, I. Lauer, K.K. Unger, The synthesis of micrometer-and submicrometer-size spheres of ordered mesoporous oxide MCM-41, Adv. Mater. 9 (3) (1997) 254–257.
[76] X. Wang, J. Feng, Y. Bai, Q. Zhang, Y. Yin, Synthesis, properties, and applications of hollow micro-/nanostructures, Chem. Rev. 116 (18) (2016) 10983–11060.
[77] T. Fontecave, C. Boissiere, N. Baccile, F.J. Plou, C. Sanchez, Using evaporation-induced self-assembly for the direct drug templating of therapeutic vectors with high loading fractions, tunable drug release, and controlled degradation, Chem. Mater. 25 (23) (2013) 4671–4678.
[78] J. Blin, M. Impéror-Clerc, Mechanism of self-assembly in the synthesis of silica mesoporous materials: *in situ* studies by X-ray and neutron scattering, Chem. Soc. Rev. 42 (9) (2013) 4071–4082.
[79] F. Danhier, A. Le Breton, V. Préat, RGD-based strategies to target alpha (v) beta (3) integrin in cancer therapy and diagnosis, Mol. Pharm. 9 (11) (2012) 2961–2973.
[80] G. Villaverde, V. Nairi, A. Baeza, M. Vallet-Regí, Double sequential encrypted targeting sequence: a new concept for bone cancer treatment, Chem., A Eur. J. 23 (30) (2017) 7174–7179.
[81] V. López, M.R. Villegas, V. Rodriguez, et al., Janus mesoporous silica nanoparticles for dual targeting of tumor cells and mitochondria, ACS Appl. Mater. Interfaces 9 (32) (2017) 26697–26706.
[82] Y. Li, H. He, X. Jia, W. Lu, J. Lou, Y. Wei, A dual-targeting nanocarrier based on poly (amidoamine) dendrimers conjugated with transferrin and tamoxifen for treating brain gliomas, Biomaterials 33 (15) (2012) 3899–3908.
[83] N.C. Seeman, Nucleic acid junctions and lattices, J. Theor. Biol. 99 (2) (1982) 237–247.
[84] Y. Ke, C. Castro, J.H. Choi, Structural DNA nanotechnology: artificial nanostructures for biomedical research, Annu. Rev. Biomed. Eng. 20 (2018) 375–401.
[85] Y. Sakai, M. Islam, M. Adamiak, S.C. Shiu, J.A. Tanner, J.G. Heddle, DNA aptamers for the functionalisation of DNA origami nanostructures, Genes 9 (12) (2018) 571.
[86] M. Chang, C. Yang, D. Huang, Aptamer-conjugated DNA icosahedral nanoparticles as a carrier of doxorubicin for cancer therapy, ACS Nano 5 (8) (2011) 6156–6163.
[87] Y. Chen, S. Song, Z. Yan, H. Fenniri, T.J. Webster, Self-assembled rosette nanotubes encapsulate and slowly release dexamethasone, Int. J. Nanomed. 6 (2011) 1035–1044, https://doi.org/10.2147/IJN.S18755 [doi].
[88] S. Song, Y. Chen, Z. Yan, H. Fenniri, T.J. Webster, Self-assembled rosette nanotubes for incorporating hydrophobic drugs in physiological environments, Int. J. Nanomed. 6 (2011) 101–107, https://doi.org/10.2147/IJN.S11957 [doi].
[89] X. Sun, Y. Chen, H. Yu, et al., Anti-miRNA oligonucleotide therapy for chondrosarcoma, Mol. Canc. Therapeut. 18 (11) (2019) 2021–2029, https://doi.org/10.1158/1535-7163.MCT-18-1020 [doi].
[90] J.E. Gagner, W. Kim, E.L. Chaikof, Designing protein-based biomaterials for medical applications, Acta Biomater. 10 (4) (2014) 1542–1557.
[91] S. Moein Moghimi, Recent developments in polymeric nanoparticle engineering and their applications in experimental and clinical oncology, Anti Canc. Agents Med. Chem. 6 (6) (2006) 553–561.
[92] J.S. Schorey, C.V. Harding, Extracellular vesicles and infectious diseases: new complexity to an old story, J. Clin. Invest. 126 (4) (2016) 1181–1189.
[93] S. Antimisiaris, S. Mourtas, A. Marazioti, Exosomes and exosome-inspired vesicles for targeted drug delivery, Pharmaceutics 10 (4) (2018) 218.
[94] A. Aryani, B. Denecke, Exosomes as a nanodelivery system: a key to the future of neuromedicine? Mol. Neurobiol. 53 (2) (2016) 818–834.
[95] W.J. Goh, S. Zou, W.Y. Ong, et al., Bioinspired cell-derived nanovesicles versus exosomes as drug delivery systems: a cost-effective alternative, Sci. Rep. 7 (1) (2017) 14322.
[96] C. He, S. Zheng, Y. Luo, B. Wang, Exosome theranostics: biology and translational medicine, Theranostics 8 (1) (2018) 237–255, https://doi.org/10.7150/thno.21945 [doi].
[97] E.J. Bunggulawa, W. Wang, T. Yin, et al., Recent advancements in the use of exosomes as drug delivery systems, J. Nanobiotechnol. 16 (1) (2018) 81.
[98] M. Swierczewska, H. Han, K. Kim, J. Park, S. Lee, Polysaccharide-based nanoparticles for theranostic nanomedicine, Adv. Drug Deliv. Rev. 99 (2016) 70–84.
[99] Y. Chung, G. Tae, S.H. Yuk, A facile method to prepare heparin-functionalized nanoparticles for controlled release of growth factors, Biomaterials 27 (12) (2006) 2621–2626.
[100] S. Kim, J. Kim, D.G. You, et al., Self-assembled dextran sulphate nanoparticles for targeting rheumatoid arthritis, Chem. Commun. 49 (88) (2013) 10349–10351.
[101] C. Lim, J. Shin, I.C. Kwon, S.Y. Jeong, S. Kim, Iodinated photosensitizing chitosan: self-assembly into tumor-homing nanoparticles with enhanced singlet oxygen generation, Bioconjugate Chem. 23 (5) (2012) 1022–1028.
[102] H.S. Han, T. Thambi, K.Y. Choi, et al., Bioreducible shell-cross-linked hyaluronic acid nanoparticles for tumor-targeted drug delivery, Biomacromolecules 16 (2) (2015) 447–456.
[103] K.A. Janes, M.P. Fresneau, A. Marazuela, A. Fabra, M.J. Alonso, Chitosan nanoparticles as delivery systems for doxorubicin, J. Contr. Release 73 (2-3) (2001) 255–267.
[104] Y. Li, L. Zhu, Z. Liu, et al., Reversibly stabilized multifunctional dextran nanoparticles efficiently deliver doxorubicin into the nuclei of cancer cells, Angew. Chem. Int. Ed. 48 (52) (2009) 9914–9918.
[105] C.C. Lee, J.A. MacKay, J.M. Fréchet, F.C. Szoka, Designing dendrimers for

biological applications, Nat. Biotechnol. 23 (12) (2005) 1517.
[106] P. Kesharwani, A. Gothwal, A.K. Iyer, K. Jain, M.K. Chourasia, U. Gupta, Dendrimer nanohybrid carrier systems: an expanding horizon for targeted drug and gene delivery, Drug Discov. Today 23 (2) (2018) 300–314.
[107] L. Wu, M. Ficker, J.B. Christensen, P.N. Trohopoulos, S.M. Moghimi, Dendrimers in medicine: therapeutic concepts and pharmaceutical challenges, Bioconjugate Chem. 26 (7) (2015) 1198–1211.
[108] Omayra L. Padilla De Jesús, H.R. Ihre, L. Gagne, J.M. Fréchet, F.C. Szoka, Polyester dendritic systems for drug delivery applications: *in vitro* and *in vivo* evaluation, Bioconjugate Chem. 13 (3) (2002) 453–461.
[109] C. Kojima, K. Kono, K. Maruyama, T. Takagishi, Synthesis of polyamidoamine dendrimers having poly (ethylene glycol) grafts and their ability to encapsulate anticancer drugs, Bioconjugate Chem. 11 (6) (2000) 910–917.
[110] R. Esfand, D.A. Tomalia, Poly (amidoamine)(PAMAM) dendrimers: from biomimicry to drug delivery and biomedical applications, Drug Discov. Today 6 (8) (2001) 427–436.
[111] N. Shao, Y. Su, J. Hu, J. Zhang, H. Zhang, Y. Cheng, Comparison of generation 3 polyamidoamine dendrimer and generation 4 polypropylenimine dendrimer on drug loading, complex structure, release behavior, and cytotoxicity, Int. J. Nanomed. 6 (2011) 3361–3372, https://doi.org/10.2147/IJN.S27028 [doi].
[112] K. Jain, P. Kesharwani, U. Gupta, N. Jain, Dendrimer toxicity: let's meet the challenge, Int. J. Pharm. 394 (1-2) (2010) 122–142.
[113] D. Huang, D. Wu, Biodegradable dendrimers for drug delivery, Mater. Sci. Eng. C 90 (2018) 713–727.
[114] H. Kawaguchi, Functional polymer microspheres, Prog. Polym. Sci. 25 (8) (2000) 1171–1210.
[115] S.Y. Yoon, Y. Park, J.S. Lee, Controlled synthesis of spherical polystyrene beads and their template-assisted manual assembly, Bull. Kor. Chem. Soc. 35 (8) (2014) 2281.
[116] J. Kimling, M. Maier, B. Okenve, V. Kotaidis, H. Ballot, A. Plech, Turkevich method for gold nanoparticle synthesis revisited, J. Phys. Chem. B 110 (32) (2006) 15700–15707.
[117] L.M. Liz-MarzÂ¡n, Gold nanoparticle research before and after the Brustâ €"Schiffrin method, Chem. Commun. 49 (1) (2013) 16–18.
[118] N.R. Jana, L. Gearheart, C. Murphy, Seed-mediated growth approach for shape-controlled synthesis of spheroidal and rod-like gold nanoparticles using a surfactant template, Adv. Mater. 13 (18) (2001) 1389–1393.
[119] S. Iravani, H. Korbekandi, S.V. Mirmohammadi, B. Zolfaghari, Synthesis of silver nanoparticles: chemical, physical and biological methods, Res. Pharm. Sci. 9 (6) (2014) 385–406.
[120] S. Xia, S. Xu, Ferrous sulfate liposomes: preparation, stability and application in fluid milk, Food Res. Int. 38 (3) (2005) 289–296.
[121] Szoka F, Jr, Papahadjopoulos D. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation, Proc. Natl. Acad. Sci. U. S. A. 75 (9) (1978) 4194–4198, https://doi.org/10.1073/pnas.75.9.4194 [doi].
[122] C. Jaafar-Maalej, R. Diab, V. Andrieu, A. Elaissari, H. Fessi, Ethanol injection method for hydrophilic and lipophilic drug-loaded liposome preparation, J. Liposome Res. 20 (3) (2010) 228–243.
[123] S. Giovagnoli, P. Blasi, A. Schoubben, C. Rossi, M. Ricci, Preparation of large porous biodegradable microspheres by using a simple double-emulsion method for capreomycin sulfate pulmonary delivery, Int. J. Pharm. 333 (1-2) (2007) 103–111.
[124] D. Bera, L. Qian, T. Tseng, P.H. Holloway, Quantum dots and their multimodal applications: a review, Materials 3 (4) (2010) 2260–2345.
[125] C. Murray, D.J. Norris, M.G. Bawendi, Synthesis and characterization of nearly monodisperse CdE (E= sulfur, selenium, tellurium) semiconductor nanocrystallites, J. Am. Chem. Soc. 115 (19) (1993) 8706–8715.
[126] N.N. Ledentsov, D. Bimberg, Z.I. Alferov, Progress in epitaxial growth and performance of quantum dot and quantum wire lasers, J. Lightwave Technol. 26 (11) (2008) 1540–1555.
[127] S. Ge, X. Shi, K. Sun, et al., Facile hydrothermal synthesis of iron oxide nanoparticles with tunable magnetic properties, J. Phys. Chem. C 113 (31) (2009) 13593–13599.
[128] A. Aboulaich, D. Billaud, M. Abyan, et al., One-pot noninjection route to CdS quantum dots via hydrothermal synthesis, ACS Appl. Mater. Interfaces 4 (5) (2012) 2561–2569.
[129] C.J. Brinker, G.W. Scherer, Sol-gel science: the physics and chemistry of sol-gel processing, Acad.Press (1990) 1–18.
[130] G.J. Owens, R.K. Singh, F. Foroutan, et al., Sol-gel based materials for biomedical applications, Prog. Mater. Sci. 77 (2016) 1–79.
[131] S. Teh, R. Lin, L. Hung, A.P. Lee, Droplet microfluidics, Lab Chip 8 (2) (2008) 198–220.
[132] N. Bertrand, J. Wu, X. Xu, N. Kamaly, O.C. Farokhzad, Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology, Adv. Drug Deliv. Rev. 66 (2014) 2–25.
[133] H. Maeda, T. Sawa, T. Konno, Mechanism of tumor-targeted delivery of macromolecular drugs, including the EPR effect in solid tumor and clinical overview of the prototype polymeric drug SMANCS, J. Contr. Release 74 (1-3) (2001) 47–61.
[134] F. Yuan, M. Dellian, D. Fukumura, et al., Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size, Canc. Res. 55 (17) (1995) 3752–3756.
[135] J. Varshosaz, M. Farzan, Nanoparticles for targeted delivery of therapeutics and small interfering RNAs in hepatocellular carcinoma, World J. Gastroenterol. 21 (42) (2015) 12022–12041, https://doi.org/10.3748/wjg.v21.i42.12022 [doi].
[136] H. Shen, S. Shi, Z. Zhang, T. Gong, X. Sun, Coating solid lipid nanoparticles with hyaluronic acid enhances antitumor activity against melanoma stem-like cells, Theranostics 5 (7) (2015) 755–771, https://doi.org/10.7150/thno.10804 [doi].
[137] J. Yoo, E. Chambers, S. Mitragotri, Factors that control the circulation time of nanoparticles in blood: challenges, solutions and future prospects, Curr. Pharmaceut. Des. 16 (21) (2010) 2298–2307.
[138] H.F. Galley, N.R. Webster, Physiology of the endothelium, Br. J. Anaesth. 93 (1) (2004) 105–113.
[139] R.K. Jain, T. Stylianopoulos, Delivering nanomedicine to solid tumors, Nat. Rev. Clin. Oncol. 7 (11) (2010) 653.
[140] R.K. Jain, Transport of molecules in the tumor interstitium: a review, Canc. Res. 47 (12) (1987) 3039–3051.
[141] L.M. Bareford, P.W. Swaan, Endocytic mechanisms for targeted drug delivery, Adv. Drug Deliv. Rev. 59 (8) (2007) 748–758.
[142] B.S. Zolnik, D.J. Burgess, Effect of acidic pH on PLGA microsphere degradation and release, J. Contr. Release 122 (3) (2007) 338–344.
[143] C. Perez, A. Sanchez, D. Putnam, D. Ting, R. Langer, M. Alonso, Poly (lactic acid)-poly (ethylene glycol) nanoparticles as new carriers for the delivery of plasmid DNA, J. Contr. Release 75 (1-2) (2001) 211–224.
[144] O.C. Farokhzad, J. Cheng, B.A. Teply, et al., Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy *in vivo*, Proc. Natl. Acad. Sci. U. S. A. 103 (16) (2006) 6315–6320 doi: 0601755103 [pii].
[145] P. Ponka, C.N. Lok, The transferrin receptor: role in health and disease, Int. J. Biochem. Cell Biol. 31 (10) (1999) 1111–1137.
[146] V. Mishra, S. Mahor, A. Rawat, et al., Targeted brain delivery of AZT via transferrin anchored pegylated albumin nanoparticles, J. Drug Target. 14 (1) (2006) 45–53.
[147] C. Schleh, M. Semmler-Behnke, J. Lipka, et al., Size and surface charge of gold nanoparticles determine absorption across intestinal barriers and accumulation in secondary target organs after oral administration, Nanotoxicology 6 (1) (2012) 36–46.
[148] T. Osaka, T. Nakanishi, S. Shanmugam, S. Takahama, H. Zhang, Effect of surface charge of magnetite nanoparticles on their internalization into breast cancer and umbilical vein endothelial cells, Colloids Surf. B Biointerfaces 71 (2) (2009) 325–330.
[149] F. Lu, S. Wu, Y. Hung, C. Mou, Size effect on cell uptake in well-suspended, uniform mesoporous silica nanoparticles, Small 5 (12) (2009) 1408–1413.
[150] T. Peulen, K.J. Wilkinson, Diffusion of nanoparticles in a biofilm, Environ. Sci. Technol. 45 (8) (2011) 3367–3373.
[151] N. Parker, M.J. Turk, E. Westrick, J.D. Lewis, P.S. Low, C.P. Leamon, Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay, Anal. Biochem. 338 (2) (2005) 284–293.
[152] G.A. Mansoori, K.S. Brandenburg, A. Shakeri-Zadeh, A comparative study of two folate-conjugated gold nanoparticles for cancer nanotechnology applications, Cancers 2 (4) (2010) 1911–1928.
[153] T. Yoshizawa, Y. Hattori, M. Hakoshima, K. Koga, Y. Maitani, Folate-linked lipid-based nanoparticles for synthetic siRNA delivery in KB tumor xenografts, Eur. J. Pharm. Biopharm. 70 (3) (2008) 718–725.
[154] H.S. Yoo, T.G. Park, Folate-receptor-targeted delivery of doxorubicin nano-aggregates stabilized by doxorubicin–PEG–folate conjugate, J. Contr. Release 100 (2) (2004) 247–256.
[155] O. Taratula, R. Savla, H. He, T. Minko, Poly (propyleneimine) dendrimers as potential siRNA delivery nanocarrier: from structure to function, Int. J. Nanotechnol. 8 (1-2) (2010) 36–52.
[156] J. Sudimack, R.J. Lee, Targeted drug delivery via the folate receptor, Adv. Drug Deliv. Rev. 41 (2) (2000) 147–162.
[157] K.M. Hodivala-Dilke, A.R. Reynolds, L.E. Reynolds, Integrins in angiogenesis: multitalented molecules in a balancing act, Cell Tissue Res. 314 (1) (2003) 131–144.
[158] J.S. Desgrosellier, D.A. Cheresh, Integrins in cancer: biological implications and therapeutic opportunities, Nat. Rev. Canc. 10 (1) (2010) 9.
[159] X. Jiang, X. Sha, H. Xin, et al., Self-aggregated pegylated poly (trimethylene carbonate) nanoparticles decorated with c (RGDyK) peptide for targeted paclitaxel delivery to integrin-rich tumors, Biomaterials 32 (35) (2011) 9457–9469.
[160] S. Meng, B. Su, W. Li, et al., Integrin-targeted paclitaxel nanoliposomes for tumor therapy, Med. Oncol. 28 (4) (2011) 1180–1187.
[161] C. Zhan, B. Gu, C. Xie, J. Li, Y. Liu, W. Lu, Cyclic RGD conjugated poly (ethylene glycol)-co-poly (lactic acid) micelle enhances paclitaxel anti-glioblastoma effect, J. Contr. Release 143 (1) (2010) 136–142.
[162] M.W. Pickup, J.K. Mouw, V.M. Weaver, The extracellular matrix modulates the hallmarks of cancer, EMBO Rep. 15 (12) (2014) 1243–1253.
[163] A.I. Minchinton, I.F. Tannock, Drug penetration in solid tumours, Nat. Rev. Canc. 6 (8) (2006) 583.
[164] L. Kunz-Schughart, Multicellular tumor spheroids: intermediates between monolayer culture and *in vivo* tumor, Cell Biol. Int. 23 (3) (1999) 157–161.
[165] O. Trédan, C.M. Galmarini, K. Patel, I.F. Tannock, Drug resistance and the solid tumor microenvironment, J. Natl. Cancer Inst. 99 (19) (2007) 1441–1454.
[166] T.T. Goodman, P.L. Olive, S.H. Pun, Increased nanoparticle penetration in collagenase-treated multicellular spheroids, Int. J. Nanomed. 2 (2) (2007) 265–274.
[167] T. Kim, C.W. Mount, W.R. Gombotz, S.H. Pun, The delivery of doxorubicin to 3-D multicellular spheroids and tumors in a murine xenograft model using tumor-penetrating triblock polymeric micelles, Biomaterials 31 (28) (2010) 7386–7397.
[168] H. Ma, Q. Jiang, S. Han, et al., Multicellular tumor spheroids as an *in vivo*–like tumor model for three-dimensional imaging of chemotherapeutic and nano material cellular penetration, Mol. Imag. 11 (6) (2012) 7290.2012. 00012.
[169] H. Lu, L. Noorani, Y. Jiang, A.W. Du, M.H. Stenzel, Penetration and drug delivery of albumin nanoparticles into pancreatic multicellular tumor spheroids, J. Mater. Chem. B 5 (48) (2017) 9591–9599.

[170] D. Jaiswal, N. Cowley, Z. Bian, G. Zheng, K.P. Claffey, K. Hoshino, Stiffness analysis of 3D spheroids using microtweezers, PloS One 11 (2017) 12 e0188346.
[171] B. Amsden, Solute diffusion within hydrogels. mechanisms and models, Macromolecules 31 (23) (1998) 8382–8395.
[172] F. Alexis, E. Pridgen, L.K. Molnar, O.C. Farokhzad, Factors affecting the clearance and biodistribution of polymeric nanoparticles, Mol. Pharm. 5 (4) (2008) 505–515.
[173] S. Walta, F. Di Lorenzo, K. Ma, U. Wiesner, W. Richtering, S. Seiffert, Diffusion of rigid nanoparticles in crowded polymer-network hydrogels: dominance of segmental density over crosslinking density, Colloid Polym. Sci. 295 (8) (2017) 1371–1381.
[174] X. Zhang, J. Hansing, R.R. Netz, J.E. DeRouchey, Particle transport through hydrogels is charge asymmetric, Biophys. J. 108 (3) (2015) 530–539.
[175] B. Kim, G. Han, B.J. Toley, C. Kim, V.M. Rotello, N.S. Forbes, Tuning payload delivery in tumour cylindroids using gold nanoparticles, Nat. Nanotechnol. 5 (6) (2010) 465.
[176] X. Xu, C.R. Sabanayagam, D.A. Harrington, M.C. Farach-Carson, X. Jia, A hydrogel-based tumor model for the evaluation of nanoparticle-based cancer therapeutics, Biomaterials 35 (10) (2014) 3319–3330.
[177] S.V. Murphy, A. Atala, 3D bioprinting of tissues and organs, Nat. Biotechnol. 32 (8) (2014) 773–785.
[178] A. Do, B. Khorsand, S.M. Geary, A.K. Salem, 3D printing of scaffolds for tissue regeneration applications, Adv. Healthc. Mater. 4 (12) (2015) 1742–1762.
[179] N.E. Fedorovich, J. Alblas, J.R. de Wijn, W.E. Hennink, A.J. Verbout, W.J. Dhert, Hydrogels as extracellular matrices for skeletal tissue engineering: state-of-the-art and novel application in organ printing, Tissue Eng. 13 (8) (2007) 1905–1925.
[180] W. Peng, P. Datta, B. Ayan, V. Ozbolat, D. Sosnoski, I.T. Ozbolat, 3D bioprinting for drug discovery and development in pharmaceutics, Acta Biomater. 57 (2017) 26–46.
[181] J. Goole, K. Amighi, 3D printing in pharmaceutics: a new tool for designing customized drug delivery systems, Int. J. Pharm. 499 (1-2) (2016) 376–394.
[182] G.J. LaBonia, S.Y. Lockwood, A.A. Heller, D.M. Spence, A.B. Hummon, Drug penetration and metabolism in 3D cell cultures treated in a 3D printed fluidic device: assessment of irinotecan via MALDI imaging mass spectrometry, Proteomics 16 (11-12) (2016) 1814–1821.
[183] Y. Zhao, R. Yao, L. Ouyang, et al., Three-dimensional printing of hela cells for cervical tumor model *in vitro*, Biofabrication 3 (2014) 6 035001.
[184] X. Hou, S. Liu, M. Wang, et al., Layer-by-layer 3D constructs of fibroblasts in hydrogel for examining transdermal penetration capability of nanoparticles, SLAS Technol.: Transl. Life Sci. Innov. 22 (4) (2017) 447–453.
[185] W. Zhu, B. Holmes, R.I. Glazer, L.G. Zhang, 3D printed nanocomposite matrix for the study of breast cancer bone metastasis, Nanomed. Nanotechnol. Biol. Med. 12 (1) (2016) 69–79.
[186] J. Lee, Y. Huh, Y. Jun, et al., Artificially engineered magnetic nanoparticles for ultra-sensitive molecular imaging, Nat. Med. 13 (1) (2007) 95.
[187] X. Jiang, H. Xin, J. Gu, et al., Solid tumor penetration by integrin-mediated pegylated poly (trimethylene carbonate) nanoparticles loaded with paclitaxel, Biomaterials 34 (6) (2013) 1739–1746.
[188] T. Wang, J. Bai, X. Jiang, G.U. Nienhaus, Cellular uptake of nanoparticles by membrane penetration: a study combining confocal microscopy with FTIR spectroelectrochemistry, ACS Nano 6 (2) (2012) 1251–1259.
[189] A. Albanese, A.K. Lam, E.A. Sykes, J.V. Rocheleau, W.C. Chan, Tumour-on-a-chip provides an optical window into nanoparticle tissue transport, Nat. Commun. 4 (1) (2013) 1–8.
[190] O. Krichevsky, G. Bonnet, Fluorescence correlation spectroscopy: the technique and its applications, Rep. Prog. Phys. 65 (2) (2002) 251.
[191] A. Verma, O. Uzun, Y. Hu, et al., Surface-structure-regulated cell-membrane penetration by monolayer-protected nanoparticles, Nat. Mater. 7 (7) (2008) 588.
[192] X. Wang, Y. Chen, L. Xue, et al., Diffusion of drug delivery nanoparticles into biogels using time-resolved micromri, J. Phys. Chem. Lett. 5 (21) (2014) 3825–3830.
[193] A.B. Chinen, C.M. Guan, J.R. Ferrer, S.N. Barnaby, T.J. Merkel, C.A. Mirkin, Nanoparticle probes for the detection of cancer biomarkers, cells, and tissues by fluorescence, Chem. Rev. 115 (19) (2015) 10530–10574.
[194] S.J. Grainger, J.V. Serna, S. Sunny, Y. Zhou, C.X. Deng, M.E. El-Sayed, Pulsed ultrasound enhances nanoparticle penetration into breast cancer spheroids, Mol. Pharm. 7 (6) (2010) 2006–2019.
[195] E. Betzig, J.K. Trautman, Near-field optics: microscopy, spectroscopy, and surface modification beyond the diffraction limit, Science 257 (5067) (1992) 189–195 doi: 257/5067/189 [pii].
[196] H. Zheng, S.A. Claridge, A.M. Minor, A.P. Alivisatos, U. Dahmen, Nanocrystal diffusion in a liquid thin film observed by *in situ* transmission electron microscopy, Nano Lett. 9 (6) (2009) 2460–2465.
[197] L. Mu, S. Feng, A novel controlled release formulation for the anticancer drug paclitaxel (taxol®): PLGA nanoparticles containing vitamin E TPGS, J. Contr. Release 86 (1) (2003) 33–48.
[198] R. Booth, H. Kim, Characterization of a microfluidic *in vitro* model of the blood-brain barrier (Î¼BBB), Lab Chip 12 (10) (2012) 1784–1792.
[199] N.S. Bhise, J. Ribas, V. Manoharan, et al., Organ-on-a-chip platforms for studying drug delivery systems, J. Contr. Release 190 (2014) 82–93.
[200] N.P. Omelyanenko, P.A. Karalkin, E.A. Bulanova, et al., Extracellular matrix determines biomechanical properties of chondrospheres during their maturation *in vitro*, Cartilage (2018) 1947603518798890.
[201] L. Guillaume, L. Rigal, J. Fehrenbach, C. Severac, B. Ducommun, V. Lobjois, Characterization of the physical properties of tumor-derived spheroids reveals critical insights for pre-clinical studies, Sci. Rep. 9 (1) (2019) 1–9.
[202] D. Jaiswal, Z. Moscato, Y. Tomizawa, K.P. Claffey, K. Hoshino, Elastography of multicellular spheroids using 3D light microscopy, Biomed. Optic Express 10 (5) (2019) 2409–2418.
[203] D.N. Ho, N. Kohler, A. Sigdel, et al., Penetration of endothelial cell coated multicellular tumor spheroids by iron oxide nanoparticles, Theranostics 2 (1) (2012) 66–75, https://doi.org/10.7150/thno.3568 [doi].