Organotropic drug delivery: Synthetic nanoparticles and extracellular vesicles

Sara Busatto [1,2,3] · Anthony Pham [1,2] · Annie Suh [1,2] · Shane Shapiro [4,5] · Joy Wolfram [1,2,6]

Abstract

Most clinically approved drugs (primarily small molecules or antibodies) are rapidly cleared from circulation and distribute throughout the body. As a consequence, only a small portion of the dose accumulates at the target site, leading to low efficacy and adverse side effects. Therefore, new delivery strategies are necessary to increase organ and tissue-specific delivery of therapeutic agents. Nanoparticles provide a promising approach for prolonging the circulation time and improving the biodistribution of drugs. However, nanoparticles display several limitations, such as clearance by the immune systems and impaired diffusion in the tissue microenvironment. To overcome common nanoparticle limitations various functionalization and targeting strategies have been proposed. This review will discuss synthetic nanoparticle and extracellular vesicle delivery strategies that exploit organ-specific features to enhance drug accumulation at the target site.

Keywords Extracellular vesicles · Exosomes · Nanoparticles · Nanomedicine · Organotropic

1 Introduction

A major goal of medicine is to develop treatment strategies that target specific organs, tissues, and cells (Kwon et al. 2012; Tiwari et al. 2012). Therapeutic agents that are effective *in vitro* often fail *in vivo* due to biological barriers that prevent site-specific accumulation (Barua and Mitragotri 2014; Blanco et al. 2015). In fact, conventional medications are usually subject to short circulation half-lives and rapid renal clearance (Podust et al. 2016). As a consequence, only around 0.001–0.01% of the administered therapeutic dose accumulates at the target site (Li et al. 2004; Wolfram et al. 2015a). Dose limitations due to side effects further restrict drug accumulation in diseased tissues (Tamargo et al. 2015). Thus, new strategies are essential to increase site-specific delivery of therapeutic agents. Nanomedicine, which consists on the application of synthetic or biological nanoparticles (NPs) for medical purposes, is a promising approach for overcoming barriers in the body (Salata 2004; Wagner et al. 2006; Wolfram and Ferrari 2019; Wolfram et al. 2015b). In particular, nanoparticles can act as carriers for the delivery of a wide variety of drugs to diseased tissues.

Nanosized particles can be synthesized from a plethora of materials, such as lipids (Shen et al. 2017a, b; Wolfram et al. 2014a, 2016), polymers (Molinaro et al. 2013; Yang et al. 2014), metals (Mu et al. 2018; Shen et al. 2014a), silica (Shen et al. 2014b, 2017b), and silicon (Shen et al. 2013), or isolated from biological sources. NPs exhibit unique properties that are lacking on the macro and molecular scale (Baer 2018; Navya and Daima 2016). For instance, NPs have high surface area to volume ratios (Baer 2018) that can be exploited for e.g. protein (Saha et al. 2014) and polymer (Makadia and Siegel 2011; Thamake et al. 2012) functionalization to prolong circulation and bestow specific targeting abilities (Dhar et al. 2008; Jokerst et al. 2011; Paolino et al. 2014; Scavo et al. 2015). In addition to the material properties, the shape

✉ Sara Busatto
busatto.sara@mayo.edu

✉ Joy Wolfram
wolfram.joy@mayo.edu

1 Department of Transplantation Medicine, Mayo Clinic, Jacksonville, Florida 32224, USA

2 Department of Physiology and Biomedical Engineering, Mayo Clinic, Jacksonville, Florida 32224, USA

3 Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

4 Center for Regenerative Medicine, Mayo Clinic, Jacksonville, Florida 32224, USA

5 Department of Orthopedic Surgery, Mayo Clinic, Jacksonville, Florida 32224, USA

6 Department of Nanomedicine, Houston Methodist Research Institute, Houston, TX 77030, USA

(Caldorera-Moore et al. 2010; Kinnear et al. 2017; Longmire et al. 2011) and size (Betzer et al. 2017; Jiang et al. 2008; Prisner et al. 2017) of nanoparticles can confer specific transport abilities in the body. In particular, NP properties can be tailored to enhance interactions with tissue microenvironments that display distinct characteristics, including structural components, interstitial pressure (Torosean et al. 2013), pH (Du et al. 2015), and biomolecules (Friedman et al. 2013). Consequently, site-specific delivery of NPs is usually superior to that of small molecules (100 to 1000-fold improvement) (Wolfram et al. 2017).

Traditionally, NP drug delivery approaches have been divided into active and passive targeting strategies (Bertrand et al. 2014; Li et al. 2017). Active targeting involves utilization of NP surface molecules that preferentially bind to the tissue of interest (Bazak et al. 2015), while passive targeting involves exploitation of physical properties, such as NP shape and size (Bertrand et al. 2014; Gentile et al. 2013). An example of active targeting is the coupling of transferrin to NPs to target pathological cells that express high levels of transferrin receptors (Gan and Feng 2010). The most common example of passive targeting is exploitation of immature leaky vasculature and impaired lymphatic drainage for intratumoral accumulation of NPs, known as the enhanced permeability and retention (EPR) effect (Greish 2010). The EPR effect can be enhanced through NP pegylation, which attracts a protective water shell that hides NPs from macrophages and enhances circulation half-life (Jokerst et al. 2011; Pasut et al. 2015; Wolfram et al. 2014b). NP shapes can also be optimized for improved biodistribution and site-specific delivery (Kinnear et al. 2017). For example, discoidal particles pioneered by Dr. Mauro Ferrari mimic the dimensions of platelets to exploit fluid dynamics for adherence to inflamed vasculature (Decuzzi et al. 2010; Mi et al. 2016a, b; Shen et al. 2015; Venuta et al. 2017).

Despite major nanoparticle-mediated improvements in the biodistribution of drugs, analysis of more than 100 nanodelivery studies demonstrated that changing the material, shape, size, and charge of NPs results in minimal improvements in intratumoral accumulation (Wilhelm et al. 2016). Notably, tumor type, stage, and model had a greater impact on site-specific delivery compared to nanoparticle properties, indicating that the macro and microenvironment play a crucial role in promoting NP adhesion, extravasation, and tissue penetration (Wilhelm et al. 2016). Additionally, the success of nanomedicine is mainly hampered by the immune system (Boraschi et al. 2017) and anatomical obstacles, such as the endothelium (Blanco et al. 2015). One of the first barriers encountered upon intravenous injection is the mononuclear phagocyte system (MPS), which is composed of specialized phagocytic cells in the lymph nodes, spleen, and liver. The phagocytes are responsible for rapid clearance of nanosized endogenous and exogenous circulating material (Samuelsson

et al. 2017). Additionally, the adaptive immune system produces antibodies against immunogenic molecules, leading to a potent immune response against subsequent exposure, which can also compromise NP therapy. For example, the formation of antibodies against molecules used for NP functionalization, such as polyethylene glycol (PEG), has been reported in animal models (Kierstead et al. 2015). Therefore, nanoparticle design strategies can be combined with other approaches that aid in overcoming biological barriers. For instance, optimization of the administration route can substantially improve biodistribution, as exemplary demonstrated by intranasal administration of nanoparticles to overcome the blood brain barrier (BBB) (Patel et al. 2018). Additionally, temporary inactivation of resident macrophages in the liver through pretreatment with pharmacological agents was shown to reduce immunological clearance of NPs, leading to improved site-specific delivery (Khalid et al. 2016; Pelt et al. 2018; Wolfram et al. 2017).

Another approach to overcome barriers in the body is exploitation of biological components that have been optimized through evolution for effective navigation within and between compartments in the body. One example is Abraxane, a United States Food and Drug Administration (FDA) approved NP, that contains albumin and paclitaxel (Peer et al. 2007). Albumin is the most abundant protein in the plasma and a carrier for endogenous hydrophobic molecules (Fanali et al. 2012). Cell-secreted nanoparticles, known as extracellular vesicles (EVs), are another example of natural carriers for short and long-distance transportation of biomolecules (Busatto et al. 2018; Théry et al. 2018; Vader et al. 2016). It has been shown that EVs exhibit organ-specific targeting abilities that are at least partially due to integrins, but most likely attributed to the interplay of several EV components (Hoshino et al. 2015). In particular, Hoshino et al. isolated EVs from tumor cell lines that colonized to specific organs during the metastatic process. Notably, each EV population mirrored the organ tropism of the secreting cell line promoting the formation of the pre-metastatic niche (Hoshino et al. 2015). EVs were also able to educate specific tumor cells to colonize to organs that were not preferred sites of metastasis (Hoshino et al. 2015; Nogues et al. 2018). Consequently, exploitation of EVs as drug carriers presents a promising opportunity for obtaining site-specific delivery. The vast majority of synthetic and EV-based nanodelivery strategies have focused on exploiting common characteristics of the tumor microenvironment, and several reviews have discussed this topic in detail (Chen et al. 2016a, b; Chulpanova et al. 2018; Wilhelm et al. 2016; Yang and Gao 2017).

Furthermore, innovative approaches for synthetic NP targeting and novel advances to deliver therapy in utero are emerging. Even though NP modifications augmenting site specific drug delivery has advanced, differences in macro environments make some organs (liver and lungs) more suitable

for NP uptake than others (brain, eyes, lymphatics, and bone). For example, resident macrophages in the liver promote organotropic homing of NPs, making hepatic tissue an ideal target for NP-based therapeutics (Borrelli et al. 2018), while the brain and eyes are limited by specialized endothelial barriers (Del Amo and Urtti 2008; Hersh et al. 2016). The purpose of this review is to provide an overall perspective of NP-based drug delivery strategies designed to exploit organ-specific properties (Fig. 1).

2 Liver delivery

A large portion of systemically injected nanoparticles accumulate in the liver due to resident macrophages (Gustafson et al. 2015). Despite this hepatic tropism, the intra-organ biodistribution is often unfavorable for the treatment of liver diseases. Studies have focused on specifically delivering NPs to hepatocytes for improved therapeutic effects. For example, cationic solid lipid nanoparticles (CSLN) were designed to

have a similar lipid composition as natural low-density lipoprotein (LDL), enabling hepatocyte recognition and intake of nanoparticles via lipoprotein receptors (Kim et al. 2008; Kong et al. 2013). The CSLN can form a complex with negatively charged siRNA that can silence pro-fibrogenic growth factors genes. This complex protects siRNA from enzymatic degradation during circulation, while increasing specificity for hepatocytes. *In vitro*, the CSLN/siRNA complex displayed higher uptake and improved gene silencing in HepG.2.2.15 cells compared to transfection with the commercially available agent, Lipofectamine. CSLN/siRNA treatment of rats with liver fibrosis reduced connective tissue growth factor (CTGF) expression and reversed the hepatic cell structure and lobular architecture to resemble that of non-fibrotic rats (Kong et al. 2013).

A study by Liang et al. explored the use of lyophilized conditioned media from mesenchymal stem cells (MSCs) in PLGA NPs encased by a red blood cell membrane for treatment of liver failure (Fig. 2a) (Liang et al. 2018). MSCs and the MSC-derived secretome have previously shown to be
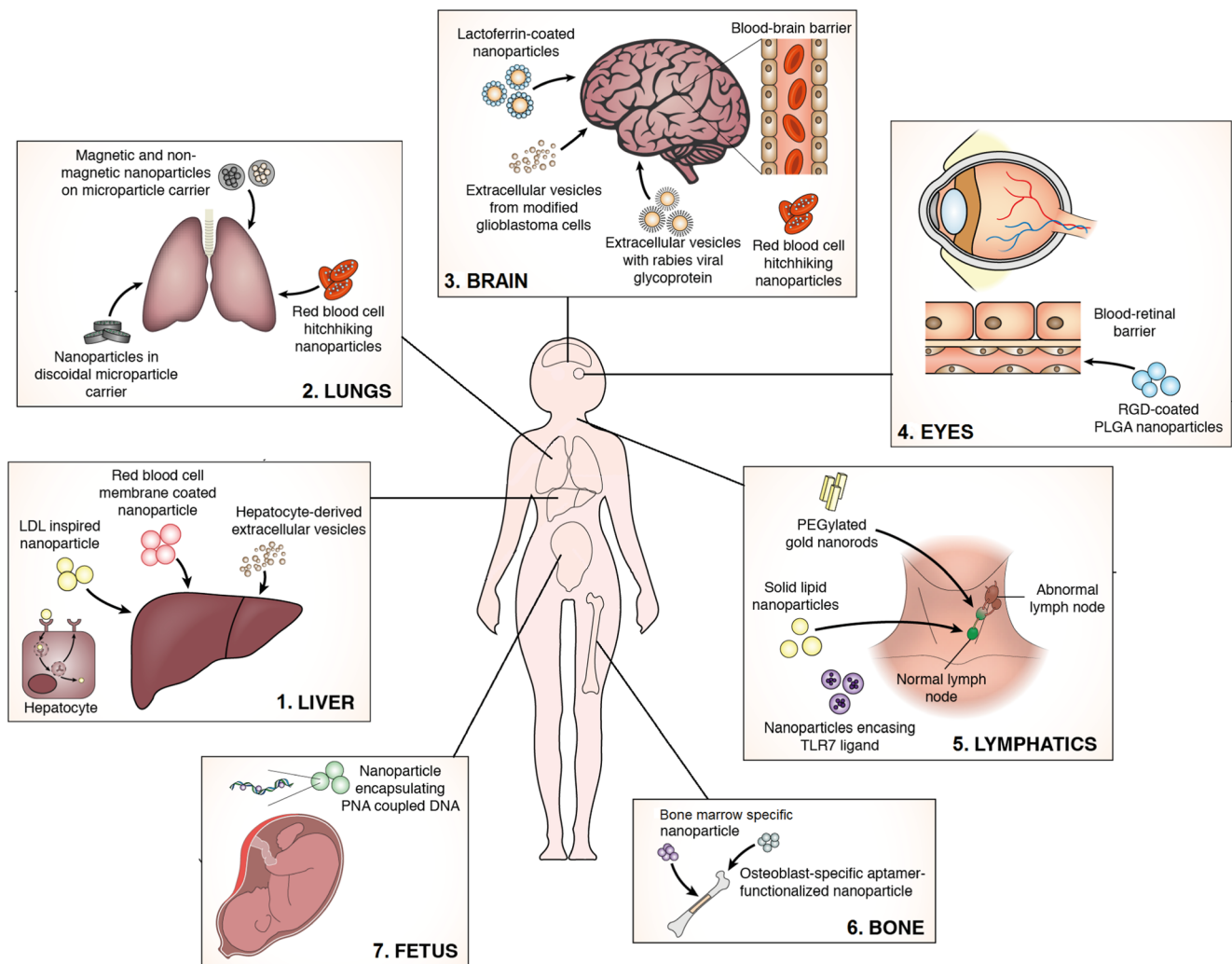

Fig. 1 Schematic of site-specific delivery strategies for nanotherapeutics to the brain, liver, bone, eye, lymphatic system, and fetus
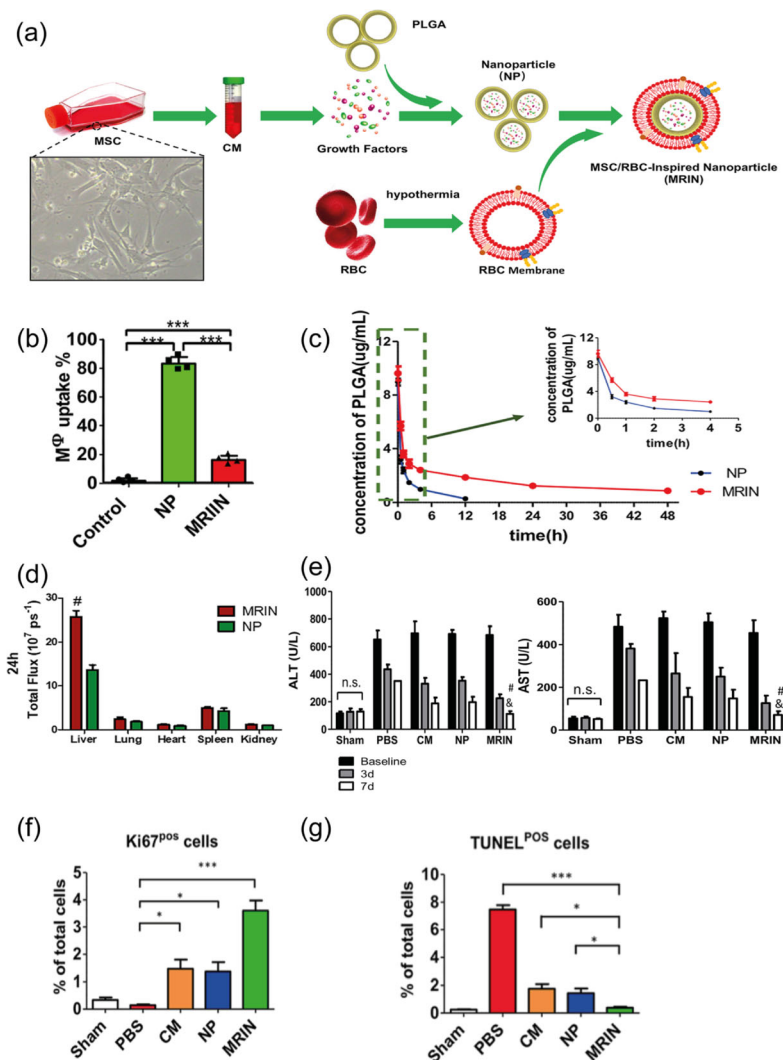
Fig. 2 Liver delivery of intravenously injected mesenchymal stem cell/ red blood cell (RBC)-inspired nanoparticle (MRIN) in a mouse model of acute liver failure. **a** Schematic of MRIN fabrication: growth factors are isolated from mesenchymal stem cell-conditioned media (CM) and encapsulated in poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs). NPs are then coated with cell membranes obtained from RBCs resulting in the final MRIN product. **b** Macrophage uptake of fluorescently labeled particles ($n = 4$). **c** Concentration of uncoated NPs and MRINs in blood ($n = 5$). **d** Analysis of particle accumulation in organs 24 h post treatment ($n = 3$). Data expressed as mean values ± s.d. #, $P < 0.01$ compared to uncoated NPs. **e** Serum concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) following carbon tetrachloride (CCl$_4$)-induced liver injury ($n = 3$). #, $P < 0.05$ compared to uncoated NP group; &, $P < 0.05$ compared to CM group. **f** Quantitation of proliferative cells via Ki67 staining ($n = 3$). *. $P < 0.05$; ***, $P < 0.001$. **g** Quantitation of apoptotic cells via terminal deoxynucleotidyl transferase-mediated 2′-deoxyuridine, 5′-triphosphate (dUTP) nick end labeling (TUNEL) ($n = 3$). *, $P < 0.05$; ***; $P < 0.001$. Reproduced from Liang et al. 2018 with permission

effective in the treatment of acute liver failure in animal models and in clinical trials (Lin et al. 2017; Shi et al. 2017). Previous studies using RBC-coated NPs have demonstrated an increased circulation half-life due to the preservation of membrane proteins essential for long-term RBC blood circulation (Mohandas and Gallagher 2008; Piao et al. 2014). The RBC membrane prevents detection and uptake of NPs by macrophages, while the nanoscale size (200 nm) enables the NPs to bypass filtration in the lungs (Liang et al. 2018). In cell culture, it was shown that 20% of the red blood cell membrane-coated NPs were endocytosed by macrophages, while the corresponding value was 80% for non-coated ones

(Fig. 2b) (Liang et al. 2018). In animal models, the coated NPs had a prolonged circulation time (Fig. 2c) and increased accumulation in the liver (Fig. 2d) compared to uncoated NPs (Liang et al. 2018). Treatment with the coated NPs also lead to a greater improvement in survival of mice with liver failure induced by carbon tetrachloride (CCl$_4$) (Liang et al. 2018). The coated NPs were also superior at improving the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicators of liver functionality (Fig. 2e), as well as promoting a normal liver architecture (Fig. 2f, g) (Liang et al. 2018).

EVs from hepatocytes have also been used to promote post-injury liver regeneration by delivery of endogenous bio-molecules, such as sphingosine kinase 2 (SK2), which stimulates cell proliferation (Nojima et al. 2016). Treatment with these self-tropic EVs led to an increase in hepatocyte proliferation in mouse models of ischemia/reperfusion (I/R) injury and partial hepatectomy. In addition to utilizing targeting properties and endogenous therapeutic biomolecules in EVs, exogenous drugs have been loaded into EVs to further improve therapeutic efficacy (Borrelli et al. 2018). Taken together, various biomimicry approaches have shown promise for liver and hepatocyte-specific targeting.

3 Lung delivery

Lung cancer and other pulmonary disorders affect a large portion of the population and have high mortality rates. As is the case for most solid tumors, a small fraction of intravenously injected drugs reach lung cancer cells (Primeau et al. 2005). The pulmonary administration route is a promising alternative for delivery of NPs to lung tissue, as this method substantially increases site-specific exposure to therapeutic agents while reducing systemic side effects (De Backer et al. 2015; Rahhal et al. 2016; Tewes et al. 2016). In particular, NPs have shown promise as inhaled drug carriers for chemotherapeutic agents (Chen et al. 2016a, b; Nagesh et al. 2016; Nørgaard et al. 2010). A study by Abbas et al. sought to improve inhalation of NPs by incorporating them into larger microparticles (MPs), as previous research has demonstrated the failure of NPs to deposit deep into lung tissue due to size-dependent ease of exhalation. The use of aerodynamically favorable MPs as a carrier can aid in lung deposition (Ali and Lamprecht 2014; Rytting et al. 2008). Aerodynamics can be evaluated with a Next Generation Impactor (NGI), in which particles are dispersed in an air stream traveling through eight stages of NGI based on aerodynamic cutoff diameters. The results of this model suggest that approximately 10% of the MP dose would be retained in the upper respiratory track, while 90% would travel to the lower conducting and respiratory tracts, demonstrating the value of this model in aiding particle design for improved pulmonary delivery (Abbas et al. 2016). In addition to improving lung delivery, the authors sought to improve drug release through the use of magnetic and non-magnetic NPs, both containing epidermal growth factor receptor (EGFR) antibodies for targeting cancer cells. By administering both nanoparticles via MPs, a sustained basal drug release with intermittent increases in drug release via application of an alternating high frequency magnetic field (HFMF) could be established. This method of combined sustained and intermittent drug release has been shown improve therapeutic results in cancer treatment (Celikoglu et al. 2008; Howell 2001). Other studies have examined how

to optimize NP design for lung targeting trough intravenous administration. A study by Decuzzi et al. examined how the size and shape of NPs and MPs affects their accumulation in specific organs. Mice were injected with particles with varying shapes and sizes. After two to six hours, the mice were euthanized, and organs (liver, spleen, heart, lung, kidneys, and brain) were harvested and examined. The authors noted that discoidal particles were found to be advantageous for lung treatment with the results showing discoidal particle accumulation in the lungs to be four times higher than that of spherical particles (Decuzzi et al. 2010). Mathematical modeling and experimental studies with discoidal particles have demonstrated improved adherence to the blood vessel wall when compared to other particle shapes (Decuzzi and Ferrari 2006; Gentile et al. 2008; Lee et al. 2009). Additionally, discoidal particles have been shown to avoid internalization by various cell types, possibly reducing uptake by liver macrophages (Champion and Mitragotri 2006; Gratton et al. 2008). A study by Mi et al. examined the use of discoidal MPs as carriers for therapeutic agents encapsulated into NPs for the treatment of melanoma lung metastases (Mi et al. 2016a). The discoidal MPs were selected in order to increase lung accumulation and enable simultaneous delivery of multiple types of NPs for combination therapy. Specifically, liposomes carrying small interfering RNA (siRNA) and docetaxel-encapsulated poly(lactide-co-glycolide) (PLGA) PEG NPs were incorporated in the same MP (Mi et al. 2016a). Mice studies revealed that liposome delivery resulted in siRNA retention solely in the kidneys, whereas MP-liposome delivery led to siRNA accumulation predominantly in the lungs after 24 h (Mi et al. 2016a). Similar results were observed when comparing PLGA-PEG NPs to NPs complexed with MPs; the NP only treatment resulted in significant accumulation in the liver, while the MP delivery system resulted in high lung retention. Analysis of cell-specific accumulation demonstrated that siRNA uptake in lung cancer cells was ten times higher with the MP platform compared to liposome delivery. Treatment with MPs resulted in longer survival times and significant reductions in metastatic nodules (Mi et al. 2016a). MPs were shown to be superior in treating mice when compared to other treatment groups, including combination therapy with siRNA-loaded liposomes and docetaxel-loaded PLGA-PEG NPs.

Another study explored the use of red blood cells (RBCs) as NP carriers for lung delivery. This RBC-hitchhiking (RH) strategy involved intravenous injection of NPs adhered to RBCs through mixing (RH) (Brenner et al. 2018). As the RBCs pass through the first capillary bed downstream of the injection, i.e. the pulmonary capillary bed, NPs transfer to the blood vessel wall (Brenner et al. 2018). Mice studies showed a significant improvement in lung accumulation when comparing RH NPs to NP alone. Several NPs were tested using the RH method, all of which showed a substantial increase in lung uptake when compared to free NPs. The fold improvement

ranged from 4-fold for albumin-NPs and 40-fold for liposomes (Brenner et al. 2018). The efficacy of this treatment was demonstrated across multiple species: mice, rats, pigs, and humans. The experiment that determined the performance of RH treatment in humans was carried out ex vivo through injection into the pulmonary artery in human lungs that were unsuitable for transplantation. Analysis of the human lungs revealed that 41% of the injected RH NPs was deposited in the lung compared to 15–20% of the free NPs (Brenner et al. 2018).

Overall, these studies suggest that injection route and particle dimensions play a major role in lung delivery. In particular, inhaled MPs have been shown to increase drug bioavailability in the lungs, while discoidal MPs and RH improve intravenous treatment of lung pathologies.

4 Brain delivery

The BBB separates and protects the central nervous system (CNS) from the systemic blood circulation (Daneman and Prat 2015). This barrier is composed of endothelial cells with tight junctions that line the lumen of cerebral capillaries forming a physical obstacle that permits passage of a select few small molecules (Ballabh et al. 2004; Pandey et al. 2016). The BBB is an example of a biological barrier that substantially limits organotropic drug delivery (Hersh et al. 2016). Emphasis has been placed on designing drug delivery vehicles to enable therapeutic agents to reach the CNS. For example, engineered EVs have shown promise in delivering small interfering RNA (siRNA) to the brain following intravenous injection. Dendritic cells were transfected with a plasmid encoding for a fusion protein composed of the EV membrane protein, lysosome-associated membrane protein 2 (Lamp2b), the CNS-specific rabies viral glycoprotein (RVG), and a FLAG epitope (Fig. 3a,b) (Alvarez-Erviti et al. 2011). EVs with the RVG peptide were purified from cell media and loaded with siRNA through electroporation (Alvarez-Erviti et al. 2011). The siRNAs were against beta-amyloid cleaving enzyme 1 (BACE1) protease that is involved in the enzymatic cascade that causes ß-amyloid peptide aggregation in Alzheimer's pathogenesis. *In vitro* results showed that RVG-EVs had a delivery efficiency comparable to cationic liposomes, resulting in an equal and dose-dependent gene knock down without evident cytotoxic effects (Alvarez-Erviti et al. 2011). Notably, *in vivo* studies demonstrated that intravenously injected EVs efficiently targeted brain tissue delivering exogenous genetic material without inducing inflammatory responses (Alvarez-Erviti et al. 2011). Specifically, the EVs caused a substantial decrease in BACE1 mRNA (61%) (Fig. 3c) and protein (62%) (Fig. 3d) levels in cortical tissue (Alvarez-Erviti et al. 2011). RVG-EV therapy also decreased the concentration of the ß-amyloid 1–42 peptide (55%), which

is one of the major components of amyloid plaques (Fig. 3e) (Alvarez-Erviti et al. 2011).

In addition to the use of viral peptides, NPs can be functionalized with endogenous ligands that recognize receptors that are overexpressed in the CNS. For example, neuronal cells of patients affected by age related neurodegenerative pathologies have increased levels of lactoferrin receptors (Qian and Wang 1998), which can be targeted with NPs. Specifically, lactoferrin-coated pegylated polymeric NPs have been used for delivery of neuroprotective molecules for the treatment of Alzheimer's disease in animal models (Liu et al. 2013). The intranasal administration route proved beneficial for bypassing the BBB, and NPs protected cargo against mucus and enzymes that compromise penetration and integrity in the nasal cavity. In mice studies, these intranasally administered lactoferrin-NPs loaded with a neuroprotective agent demonstrated a dose-dependent effect with an amelioration of cognitive defects (Liu et al. 2013). Notably, the lactoferrin-NPs were superior to the neuroprotective agent alone or untargeted NPs (Liu et al. 2013).

EV carriers for brain delivery have also been administered through the intranasal route (Sterzenbach et al. 2017). For example, human glioblastoma cells were transfected with a plasmid encoding Cre recombinase with a WW tag that promotes loading into EVs (Riling et al. 2015; Sterzenbach et al. 2017). EVs were then administered to transgenic mice carrying a stop cassette that inhibited expression of a gene encoding for a red fluorescent protein. Analysis of brain tissue demonstrated a high number of red fluorescent neurons (Sterzenbach et al. 2017), indicating that EV-based intranasal enzyme delivery was successful.

Delivery of NPs to the brain has also been enhanced utilizing RBCs (Brenner et al. 2018). Brenner et al. achieved improved NP-delivery to the brain after internal carotid artery injection of RH. Specifically, brain-specific uptake was 11.5% of the injected dose and the RH strategy displayed higher brain-to-blood ratios (27 fold) and brain to liver ratios (143 fold) compared to free NPs (Brenner et al. 2018). Notably, RBCs can efficiently shuttle NPs to several organs through administration of catheters placed into specific blood vessels that supply the target organ (Brenner et al. 2018).

5 Eye delivery

Macular degeneration is an age-related medical condition that promotes neovascularization of the subretinal space, leading to vision loss (Ambati et al. 2013). Presently, the only therapy available is in vitreous administration of anti-vascular endothelial growth factor (VEGF) molecules that block vascularization (Spaide et al. 2006). In the majority of patients, repeated intraocular injections result in retinal fibrosis or atrophy,
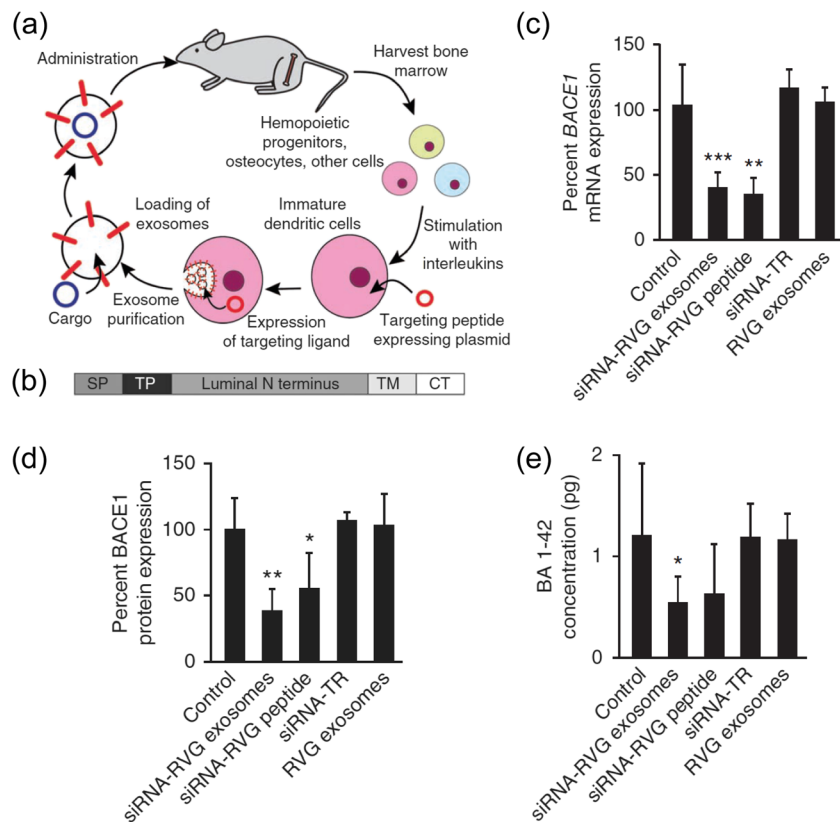
Fig. 3 Brain delivery of intravenously injected extracellular vesicles with small interfering RNA (siRNA) against beta-amyloid cleaving enzyme (BACE1) in a wild type mouse model. **a** Schematic of engineering, collection and administration of targeted extracellular vesicles for siRNA delivery. **b** Schematic representation on the fusion protein composed of: Lamp2b protein. SP, signal peptide; TP, targeting peptide; TM, transmembrane domain; CT, C terminus. **c** BACE1 mRNA levels in the brain cortical region (normalized to 18S RNA levels). **d** BACE1 protein levels in brain tissue. **e** Quantification of β-amyloid 1–42 (BA 1–42) in brain tissue samples. Data is presented as mean ± s.d. ($n = 5$). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared to untreated control. Reproduced from Alvarez-Erviti et al. 2011 with permission. RVG, rabies virus glycoprotein; TR, *in vivo* transfection reagent

consequently promoting eye damage. Intravenous injection of agents is also problematic, as the blood-retina barrier makes it challenging for therapeutic agents to reach the eye. To overcome these challenges, VEGF-targeting NPs have recently been developed (Luo et al. 2013). Intravenously administered arginylglycylaspartic acid (RGD)-coated PLGA NPs carrying a plasmid encoding for the VEGF intraceptor Flt23K were able to target the fundus of normal and degenerated eyes in three different induced choroid damage models: a gene-silenced and a laser-induced mouse model and a laser-induced monkey model (Luo et al. 2013). The plasmid encoding for Flt23K intraceptor efficiently targeted and seized VEGF into the endoplasmic reticulum, inhibiting paracrine and autocrine cell-stimulation, leading to decreased angiogenesis and fibrosis (Luo et al. 2013). Notably, in both mouse models, RGD-Flt23K-NPs localized preferentially to the extracellular matrix of lesion-containing eyes and were retained there for longer compared to Flt23K-NPs and Blank-NPs (Fig. 4a). Similarly, in the monkey model, the RGD-Flt23K-NPs were retained for one month after treatment, whereas the Flt23K-NPs and Blank-NPs were not detected (Fig. 4b) (Luo et al. 2013). Furthermore, RGD-Flt23K-NPs were more

effective at reducing the fibrotic lesion volume and choroidal neovascularization volume (CNV) (Fig. 4c,d) compared to Flt23K-NPs, Blank-NPs, and sham groups (Luo et al. 2013). Both mouse models noted a significant reduction in CNV lesion volume when treated with RGD-Flt23K-NPs compared to the other groups (Fig. 4e, f) (Luo et al. 2013). Moreover, the RGD-Flt23K-NPs were superior at reducing CNV lesion volume compared to intravitreal administration of a VEGF antibody (Fig. 4g). Furthermore, six weeks after treatment, more than the 85% of the RGD-Flt23K-NPs treated mice showed an improvement in visual acuity greater than 10%, whereas the other groups displayed no significant changes (Fig. 4h) (Luo et al. 2013).

6 Lymphatic delivery

The lymphatic system is part of both the circulatory and immune system and is composed by vessels, primary organs (thymus and bone marrow), and secondary organs (lymph nodes and spleen). The function of lymph nodes is to defend against pathogens. Lymph nodes are the
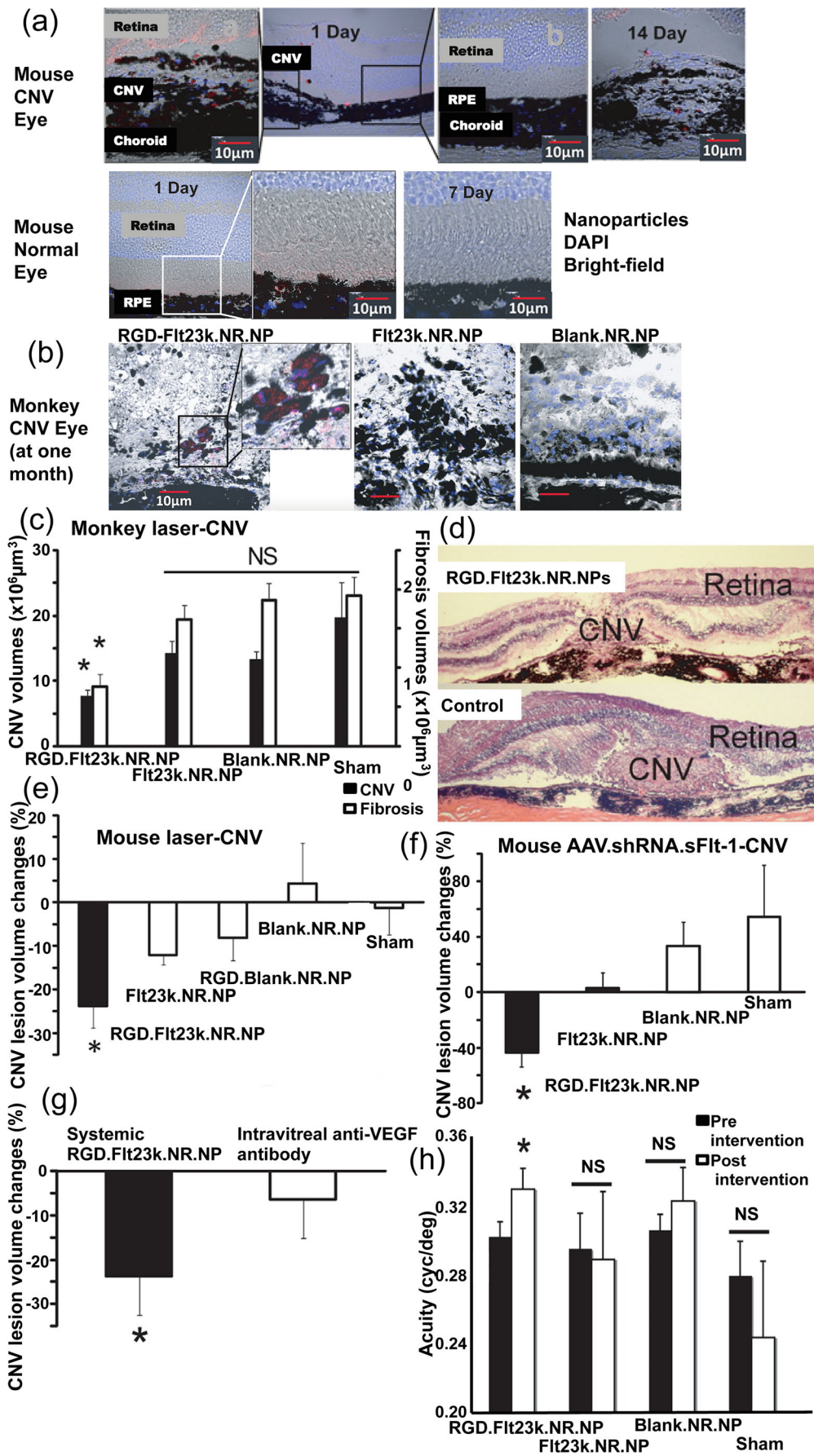
Fig. 4 Choroidal neovascularization (CNV) eye lesion delivery of intravenously injected PLGA NPs in mice and monkeys. **a** Confocal analysis of red-labeled arginylglycylaspartic acid (RGD) functionalized NPs carrying the recombinant vascular endothelial growth factor intraceptor (Flt23k) intraceptor and Nile red (RGD.Flt23k.NR.NPs) in mouse ocular tissue cryosections. **b** RGD.Flt23k.NR.NPs in CNV eye lesions in a laser-induced monkey model. **c** Volumes of CNV lesions and fibrosis in a laser-induced CNV monkey model after treatment with RGD.Flt23k.NR.NPs ($n = 14$), red-labeled bare NPs carrying Flt23k intraceptor (Flt23k.NR.NPs) ($n = 16$ and $n = 8$ for CNV volume and fibrosis respectively), red-labeled NPs (Blank.NR.NPs) ($n = 8$ and $n = 19$ for CNV volume and fibrosis respectively), and sham control ($n = 19$ and $n = 16$ for CNV volume and fibrosis respectively). **d** Representative images of CNV in a laser-induced CNV monkey model treated with RGD.Flt23k.NR.NP or sham control. **e** CNV lesion regression in a laser-induced mouse models ($n = 11$). **f** CNV lesion volumes in a soluble fms-like tyrosine kinase-1 (sFlt-1) knockdown mouse model treated with RGD.Flt23k.NR.NPs ($n = 9$), Flt23k.NR.NPs ($n = 10$), red-labeled blank NPs functionalized with RGD (RGD.Blank.NR.NPs) ($n = 10$), Blank.NR.NPs ($n = 10$) and sham group ($n = 10$). **g** CNV lesion reduction after RGD.Flt23k.NR.NP treatment and intravitreal injection of anti-VEGF antibody ($n = 10$, 2 weeks). **h** Visual acuity in a soluble fms-like tyrosine kinase-1 (sFlt-1) knockdown mouse model treated with RGD.Flt23k.NR.NP, Flt23k.NR.NPs, Blank.NR.NPs, or sham ($n = 11$, 4 weeks). *, $P < 0.05$ versus sham control (c, e, f and h) and intravitreal injection (g). Data are presented as mean ± s.e.m. Reproduced from Luo et al. 2013 with permission. AAV, adeno-associated virus; DAPI, 4′,6-diamidino-2-phenylindole; NS, non-significant; RPE, retinal pigment epithelium; shRNA, short hairpin RNA; VEGF, vascular endothelial growth factor

location of lymphocytes and antigen-presenting cells that in healthy conditions can start and lead an efficient immune response (Ji 2016). Lymph nodes are also the primary site of metastasis from adjacent tumors due to their location and structure (Ji 2016). For these reasons, the lymphatic system is considered a good target for therapy, and several NP-based approaches have recently been proposed. In mice models of lymph node metastasis, the delivery efficiency of direct injection of pegylated gold nanorods (PAuNRs) into the lymph node adjacent to the site of metastasis has been studied (Oladipo et al. 2017). The NPs drained into the metastasized lymph node and remained there for an extended period of time, making photothermal therapy possible (Oladipo et al. 2017). Near-infrared irradiation caused a rise in temperature that destroyed surrounding cells and prevented metastatic spread (Oladipo et al. 2017).

Stimulation of the immune system with ligands that bind to toll-like receptors (TLRs) can drive antitumor responses mediated by a subset of T cells (cytotoxic and natural killer). However, systemic administration of TLR ligands is often inefficient due to dose limitations. To improve lymphatic delivery, polymeric NPs encasing TLR7 ligands have been injected subcutaneously close to the inguinal space (Widmer et al. 2018). The NPs protected ligands and delivered them to the draining lymph nodes, particularly to monocytes, macrophages and dendritic cells (Widmer et al. 2018).

In addition to cancer treatment, the lymphatic system is a promising target for NP-based therapies for viral infections. Among other viruses, the human immunodeficiency virus (HIV) mainly proliferates inside macrophages and attacks CD4+ T cells in the lymphatic system (Fevrier et al. 2011; Vidya Vijayan et al. 2017). It is challenging for systemically administered anti-retroviral drugs to these reach reservoir sites, causing continuous viral proliferation with poor outcomes for patients (Kumar et al. 2018). Therefore, there is a need to develop nanocarriers that are able to deliver antiviral drugs to lymphatic organs. SLNs are stable drug delivery vehicles that can be orally administered, as they resist gastric acidity (Kumar et al. 2018) and are taken up by the intestinal lymphatic system that transports lipids and nanosized particle from the gut into systemic blood circulation (Singh et al. 2014). In rats, it has been shown that orally administered poorly water-soluble antiretroviral drugs encapsulated into SLNs accumulate in lymphatic organs (spleen and thymus). In conclusion, there are several promising NP strategies for lymphatic delivery, which will become increasingly important with the rising number of immunotherapies in development. However, transport mechanisms within the lymphatic system are still poorly understood and thought to be characterized by unique and complex processes, hampering the development of NPs that exploit the anatomy and physical parameters of lymph vessels. For this reason, computational models of mass transport in the lymphatic system could be valuable for the design of NPs (Kojic et al. 2017).

7 Bone delivery

Bone tissue is composed of osteocytes that regulate osteoblast and osteoclast activity; orchestrating a homeostatic process that finely controls bone matrix deposition and adsorption (Raggatt and Partridge 2010; Schaffler and Kennedy 2012). Pathological conditions such as fractures or chronic bone damage can impair this homeostasis, resulting in tissue damage. For this reason, anabolic strategies are explored as therapies to improve bone regeneration (Roberts and Ke 2018). Osteoblast-specific oligonucleotide aptamer-functionalized lipid NPs shielding osteogenic siRNA have been evaluated for bone regeneration (Liang et al. 2015). In a rat model, the aptamer-targeted NPs displayed increased accumulation in the bone (Fig. 5a), specifically in osteoblasts (Fig. 5b), and enhanced gene knockdown (Fig. 5c) compared to NPs with a random sequence aptamer (Liang et al. 2015). Gene knockdown was considerably higher in cells expressing alkaline phosphatase (typically osteoblasts) compared to cells that were positive for an osteoclast-associated receptor (Oscar) (Fig. 5d). After repeated injections, osteopenic rats treated

with aptamer-functionalized NPs displayed bone parameters comparable to healthy rats (C. Liang et al. 2015), indicating successful osteogenic treatment.

Bone marrow is a tissue encased by an inner bone structure responsible for the synthesis and secretion of functional circulating blood cells. Bone marrow can be affected by a spectrum of genetic disorders that cause medullary dysplasia or cytopenia, i.e. myelodysplastic syndromes (Wu et al. 2017).

Potential effective therapies consist on chemotherapeutic drugs, which also exhibit systemic toxicity resulting in multiple collateral effects. For this reason, Wu et al. proposed the use of biodegradable NPs loaded with decitabine and arsenic trioxide, chemotherapeutic agents that when combined induce a favorable response in patients affected by myelodysplastic syndromes (Wu et al. 2017). NPs were functionalized with alendronate-PEG-lipid molecules to enhance targeting
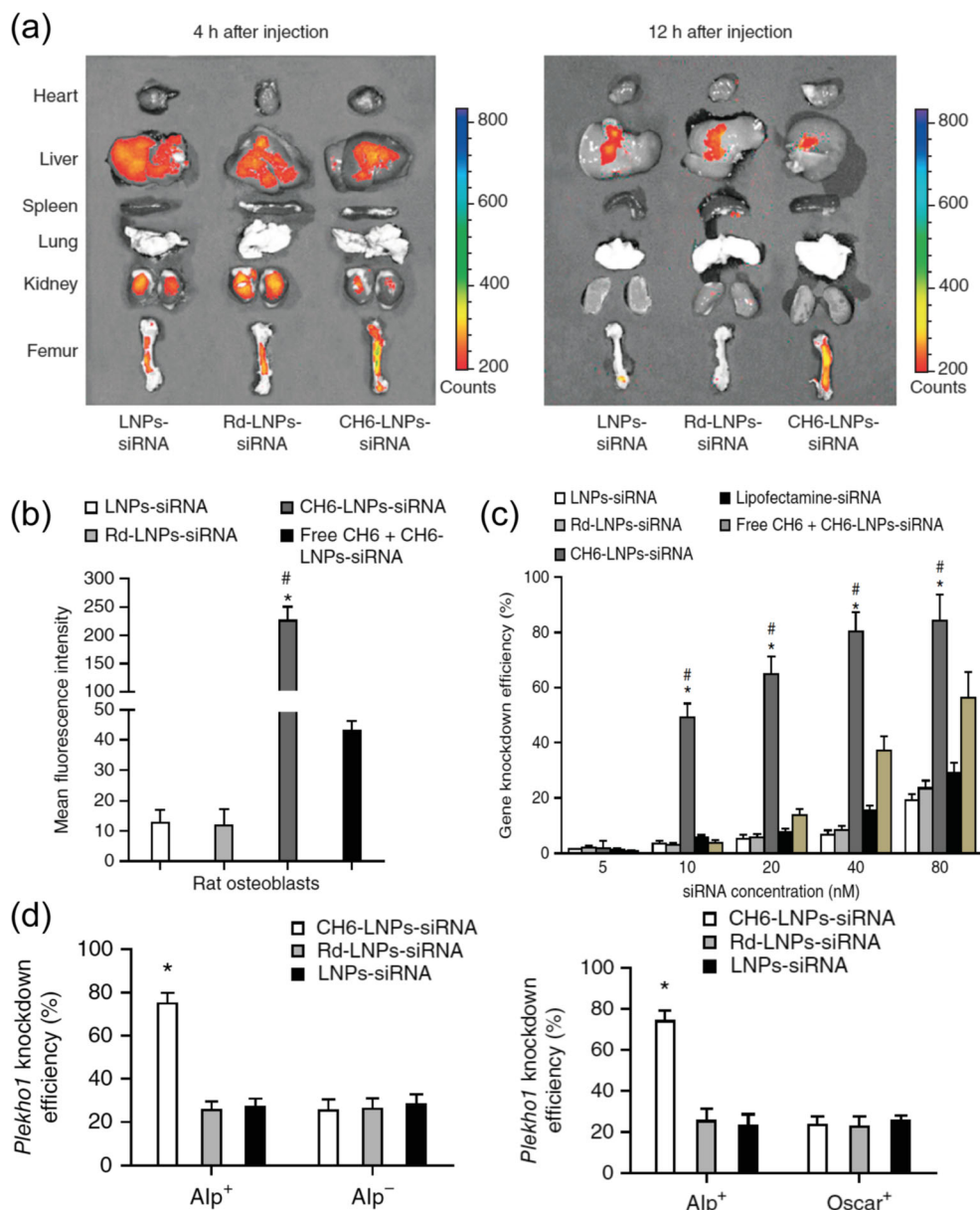

Fig. 5 Bone delivery of intravenously injected lipid-based nanoparticles (LNPs) with siRNA against pleckstrin homology domain containing O1 (Plekho1) in rats. a Fluorescent siRNA in organs of rats administered with LNPs-siRNA, random sequence (Rd) functionalized LNPs siRNA, or bone targeting aptamer (CH6) functionalized LNPs-siRNA. b siRNA uptake by primary osteoblasts. c Gene knockdown in primary rat osteoblasts. Data are presented as the mean ± s.d. ($n = 3$). d Plekho1 knockdown efficiency in alkaline phosphatase (Alp)+, Alp−, and osteoclast-associated receptor (Oscar) + cells following in vivo administration. Data are presented as means ± s.d. ($n = 6$). *, $P < 0.05$ for a comparison of CH6-LNPs-siRNA with LNPs-siRNA or Rd-LNPs-siRNA (b,c,d); #, $P < 0.05$ for a comparison of CH6-LNPs-siRNA with free CH6 + CH6- LNPs-siRNA (b,c). Reproduced from Liang et al. 2015 with permission

abilities. Alendronate is a bisphosphonate that mimics pyrophosphate and is used to treat pathological bone conditions, such as osteoporosis. In a mouse model, the bone-targeted NPs improved tissue-specific accumulation of the chemotherapeutic agents compared to untargeted NPs (up to 8-fold) (Wu et al. 2017). Four weeks after treatment, the white blood cell (WBC) and platelet counts were normal in 50% of the NP treated animals whereas the corresponding value was 25% in the free drug or untargeted NP groups. More than 200 days post-treatment mice that had received bone-targeted NPs displayed decreased mortality (~40%) compared to those that received the free drug (~70%), untargeted NPs (~75%) or phosphate buffered saline (~90%) (Wu et al. 2017). Ex vivo analysis of bone marrow cells demonstrated that bone-targeted NPs efficiently delivered chemotherapy to the target site, as evidenced by reduced transcription of target gene DNA methyltransferase (80% reduction) (Wu et al. 2017). Apoptosis was also increased in the bone marrow of mice injected with bone-targeted NPs (~70%) compared to free drugs (50%), untargeted NPs (~50%), or phosphate buffered saline (~10%) (Wu et al. 2017). On the contrary, liver cell apoptosis was less prevalent in the bone-targeted NP group compared to the other treatment groups (Wu et al. 2017). These results indicate that the bone-targeted NPs efficiently deliver chemotherapeutic agents to bone tissue, while reducing chemotherapy-mediated hepatic toxicity.

8 In utero delivery

Pediatric patients affected by hemoglobinopathies, particularly β-thalassemia, are frequently subjected to blood transfusions or bone marrow transplants, which are medical procedures that can lead to life-threatening complications (Modell and Darlison 2008). Despite increasingly precocious pre-natal diagnoses, there is a lack of efficient in utero gene therapy able to edit the mutated gene before birth (Fan et al. 2012). Peptide nucleic acids (PNAs) are synthetic polymers that differ from DNA and RNA structures in that the backbone of the molecule is formed by amino acids instead of carbohydrates (Nielsen et al. 1994). PNAs bind to DNA through a stable bond resulting in an enzyme-resistant carrier for genetic material (McNeer et al. 2013). PLGA-NPs with PNA coupled with donor DNA were injected via the intravitelline vein or into the amniotic cavity of fetal mice carrying the human β-thalassemia mutation (Ricciardi et al. 2018). The NPs accumulated preferentially in the fetal liver, where hematopoietic stem cells undergo rapid expansion and transfer to bone marrow (Fig. 6a). The treatment resulted in successful gene editing with consequent improved postnatal outcomes compared to controls (Fig. 6b, c) (Ricciardi et al. 2018). Treated mice also had an increased hemoglobin concentration (Fig. 6d) along with an improved red blood cell (RBC) phenotype

(Fig. 6e) and reduced reticulocyte count (Fig. 6f) (Ricciardi et al. 2018). After a single NP intravitelline injection, successful gene editing was detected in both fetal hematopoietic stem/progenitor cells and bone marrow cells of pups (Fig. 6g) (Ricciardi et al. 2018). The overall results confirm that in utero delivered NPs can mediate safe fetal gene editing resulting in normal fetal development and reduced post-natal β-thalassemia morbidity.

9 Protein corona

There has been extensive research into the modification of NPs following systemic administration to regulate organotropism, specifically the binding of biomolecules to the surface of NPs resulting in a protein-rich coating known as the protein corona. The protein corona significantly impacts the characteristics of NPs, causing variable changes in NP size, shape, charge, and aggregation capacity (Wolfram et al. 2014c). Consequently, these changes have implications in NP toxicity, targeting, biodistribution, and drug release, especially when transitioning from in vitro to in vivo environments (Wolfram et al. 2014c). With respect to biodistribution, the binding of certain plasma proteins to the protein corona can cause NPs to have affinities for specific tissues. A high concentration of opsonins, such as complement factors, fibrinogen, or immunoglobin G, in the protein corona promotes macrophage uptake of NPs (Nguyen and Lee 2017) and NP accumulation into the liver and spleen (Wolfram et al. 2014c). Enrichment with dysopsonins, including apolipoproteins (Apo) and albumin, was shown to increase NP circulation life and accumulation of NPs in organs other than the liver and spleen, such as the brain (Nguyen and Lee 2017; Wolfram et al. 2014c). Recent studies into the protein corona have explored methods to use this structure to improve therapeutic goals. One method involves the modification of NPs to create specific protein corona profiles from endogenous proteins. For instance, Schöttler et al. demonstrated that the stealth effect of pegylated NPs, referring to the reduction in non-specific cell uptake and increased blood circulation life of these nanoparticles, was due to the formation of protein coronas with high amounts of a clusterin, Apo J, from plasma (Schottler et al. 2016). Other nanomaterial compositions were shown to favorably bind to serum proteins that improved delivery to specific tissues, such as polysorbate-coated NPs binding to Apo E or Apo AI leading to an increased distribution in brain endothelium (Nguyen and Lee 2017). In regard to EVs, the characteristic and implications of the protein corona are largely unknown and are likely to be affected by the isolation method. Overall, to improve the efficiency of nanotherapeutics, studies must account for effects of the protein corona on NP functionality.
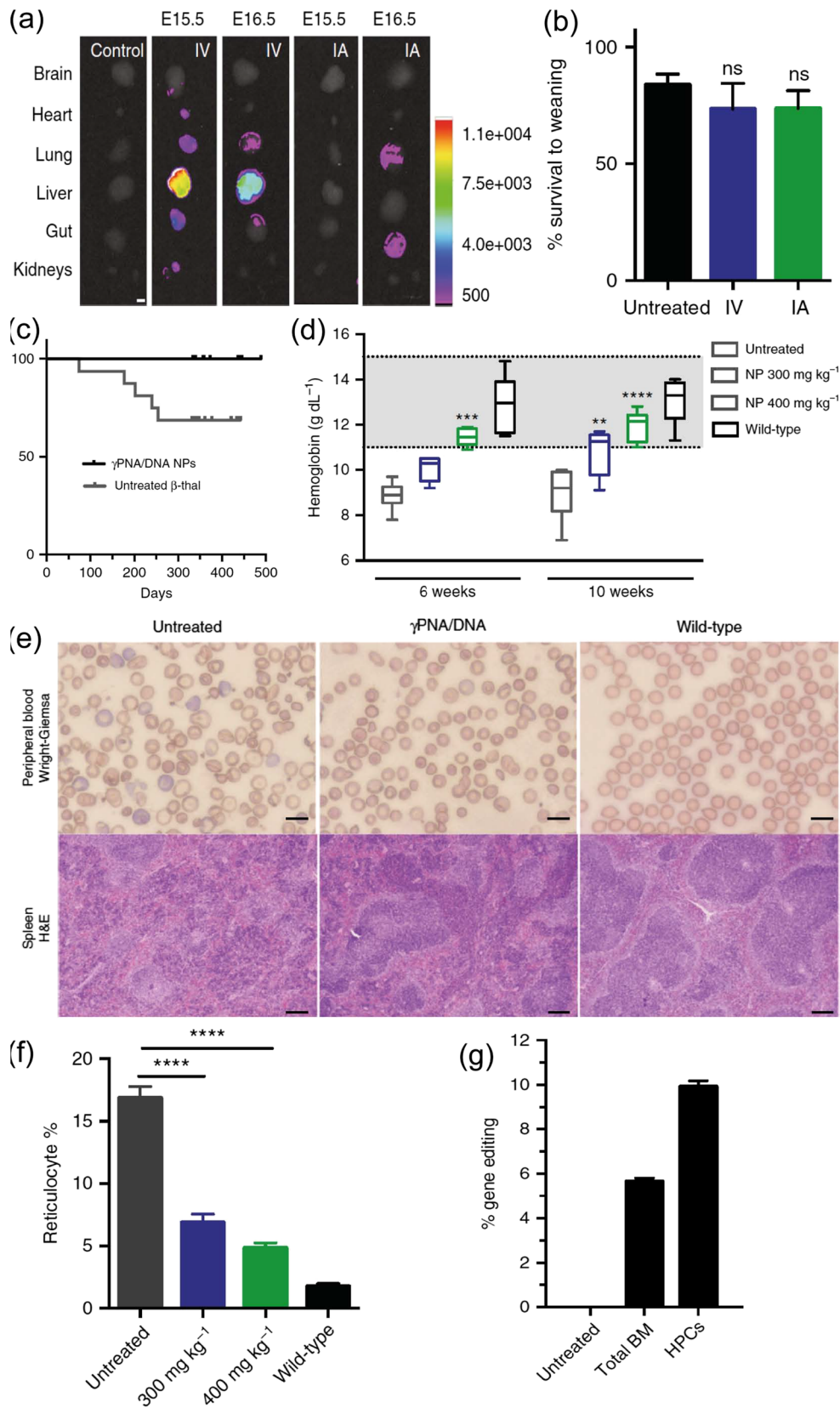
◄ **Fig. 6** Fetus delivery of intravenously (IV) and intra-amniotic (IA) injected PLGA NPs with γ tail-clamp peptide nucleic acids (γtcPNA) in a mouse model of human β-thalassemia. **a** Fetus organ biodistribution of NPs 3 h post-injection at indicated embryonic (E) stages ($n = 9$ for control, $n = 10$ for E15.5 IV, $n = 7$ for E16.5 IV and E15.5 IA, $n = 8$ for E16.5 IA). Scale bars, 2 mm. **b** Survival to weaning (21 days) ($n = 7$ for untreated, $n = 4$ for IV E15.5, $n = 7$ for IA E15.5). **c** Survival of fetus at E15.5 (IV) ($n = 16$). **d** Blood hemoglobin levels (E15.5 (IV) ($n = 6$), horizontal lines within the boxes indicate the median, the box indicates the first and third quartile, and the whiskers represent the range. **e** Blood cells (Wright–Giemsa staining) and spleen sections (hematoxylin and eosin staining) (E15.5 (IV)). Peripheral blood scale bars, 10 μm; spleen scale bars, 150 μm. **f** Reticulocyte count (E15.5 (IV)). Scale bar, 10 μm. **g** Gene edited total bone marrow and isolated hematopoietic progenitor cells (HPCs) (E15.5 (IV)) ($n = 3$). Data are presented as mean ± s.d. **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. Reproduced from Ricciardi et al. 2018 with permission

10 Conclusion

Many therapeutic agents that show promising results in cell culture fail *in vivo* due to biological barriers that hinder site-specific accumulation. These barriers consist of the immune system, various anatomical obstacles, and pressure gradients. NPs have been developed as tools to aid in the transport of therapeutic agents within and across compartments in the body. Although some barriers are inherent to all tissues, others are organ-specific, indicating that preconditioning strategies, administration route, and NP properties should be tailored based on the target organ. Despite the development of several targeted nanodelivery strategies, there is still a pressing need to further understand organ-specific macro and microscale obstacles that impede drug delivery. Knowledge of the structure and function of the target organ is necessary to design delivery strategies that lead to favorable drug distributions and therapeutic outcomes.

Besides successfully delivering therapeutic agents to target organs, NPs must also ensure delivery to the appropriate subcellular compartments, as the intracellular environment poses additional obstacles, such as the lysosome that rapidly degrades biomolecules. Multifunctional delivery strategies are necessary to finely orchestrate drug transport across a diverse set of extracellular and subcellular barriers. Both synthetic NPs and extracellular vesicles provide promising multi-pronged strategies for site-specific delivery.

Compliance with ethical standards

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