Review Article

Importance of integrating nanotechnology with pharmacology and physiology for innovative drug delivery and therapy – an illustration with firsthand examples

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Abstract
Nanotechnology has been applied extensively in drug delivery to improve the therapeutic outcomes of various diseases. Tremendous efforts have been focused on the development of novel nanoparticles and delineation of the physicochemical properties of nanoparticles in relation to their biological fate and functions. However, in the design and evaluation of these nanotechnology-based drug delivery systems, the pharmacology of delivered drugs and the (patho-)physiology of the host have received less attention. In this review, we discuss important pharmacological mechanisms, physiological characteristics, and pathological factors that have been integrated into the design of nanotechnology-enabled drug delivery systems and therapies. Firsthand examples are presented to illustrate the principles and advantages of such integrative design strategies for cancer treatment by exploiting 1) intracellular synergistic interactions of drug-drug and drug-nanomaterial combinations to overcome multidrug-resistant cancer, 2) the blood flow direction of the circulatory system to maximize drug delivery to the tumor neovasculature and cells overexpressing integrin receptors for lung metastases, 3) endogenous lipoproteins to decorate nanocarriers and transport them across the blood-brain barrier for brain metastases, and 4) distinct pathological factors in the tumor microenvironment to develop pH- and oxidative stress-responsive hybrid manganese dioxide nanoparticles for enhanced radiotherapy. Regarding the application in diabetes management, a nanotechnology-enabled closed-loop insulin delivery system was devised to provide dynamic insulin release at a physiologically relevant time scale and glucose levels. These examples, together with other research results, suggest that utilization of the interplay of pharmacology, (patho-)physiology and nanotechnology is a facile approach to develop innovative drug delivery systems and therapies with high efficiency and translational potential.

Keywords: nanotechnology; pharmacology; physiology; pathophysiology; drug delivery systems; drug synergism; blood-brain barrier; tumor metastasis; diabetes mellitus; anticancer therapy

Introduction

The application of nanotechnology in drug delivery has gained increasing interest over the past few decades[1, 2]. A myriad of nanoparticles (NPs) have been constructed using various polymers, lipids, inorganic materials or their combinations with the desired physicochemical properties and biological functions for the treatment of various diseases[1, 3, 4], such as cancer[5-7], diabetes[8, 9], and central nervous system (CNS) disorders[10, 11]. In general, NP formulations are capable of enhancing drug efficacy and reducing normal tissue toxicity in preclinical studies; however, their conversion to approved clinical uses has been limited[12, 13]. In addition to financial, ethical and regulatory hurdles to drug development[13, 14], insufficient attention to the correlations between individual patient biology and NPs and between the pharmacology of delivered drugs and the NP design may also contribute to the low rate of translation from the bench to bedside[13, 15, 16].

The majority of studies in the field of nanomedicine have

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focused on the development of new NP systems and delineation of their physicochemical properties in relation to their biological fate and functions, particularly in cancer diagnosis and therapy[17, 18]. Typically, NP physicochemical parameters such as particle size, shape, surface charge, surface ligand modification and chemistry are optimized to overcome one or more aspects of macroscopic (whole body) and microscopic biological barriers via the solid tumor enhanced permeability and retention (EPR) effect[19, 20] or active targeting by ligand-receptor interactions (Figure 1)[3, 17]. Compared to free drugs in solution form, drugs encapsulated in NPs exhibit different pharmacokinetics (eg, extended half-life time) and improved tumor targeting, leading to higher tumor accumulation of the nanocarriers[12, 21, 22]. However, to be biologically effective, the NPs must bring the drug concentration to the therapeutic level at the drug target sites (eg, nuclear DNA, mitochondria) (Figure 1, right panel). The local bioavailability of NP-delivered drugs largely depends on whether the NPs can gain entry to the cancer cells and release their cargo at the site of drug action at a therapeutically effective rate[3, 23, 24]. These characteristics of NPs, while critical for their therapeutic effect, have not received adequate attention in the conventional design of nanomedicine. For example, the clinically used liposomal formulation (ie, Doxil®) is known to reduce the cardiotoxicity that is commonly associated with free doxorubicin (DOX) solution, but it does not efficiently enter cancer cells and release the loaded DOX intracellularly[25, 26]. As a result, it does not demonstrate improved efficacy compared to free DOX in solution despite its increased half-life and tumor accumulation and reduced cardiotoxicity[27, 28]. Therefore, in the application of nanotechnology to drug delivery, the design of nanocarriers must be placed in the context of the pharmacology of the delivered drugs, that is, to deliver the drugs to the *right* site at the *right* time and the *right* levels (*3Rs*).

The ultimate goal of the clinical use of drug delivery systems is to enhance drug efficacy while reducing unwanted normal tissue side effects. To achieve this goal, the *3Rs* are essential and are applicable not only to injectable nanomedicine for cancer therapy but also to other nanotechnology-based devices or carriers that release an active pharmaceutical ingredient (API) in response to disease state-associated stimuli (*eg*, elevated glucose concentrations in diabetes mellitus or the tumor microenvironment (TME) in cancer)[9, 24, 29, 30]. In spite of advances in nanotechnology, the *in vivo* performance of drug delivery systems often does not meet the medical need. One solution to this inadequacy is to thoroughly understand drug
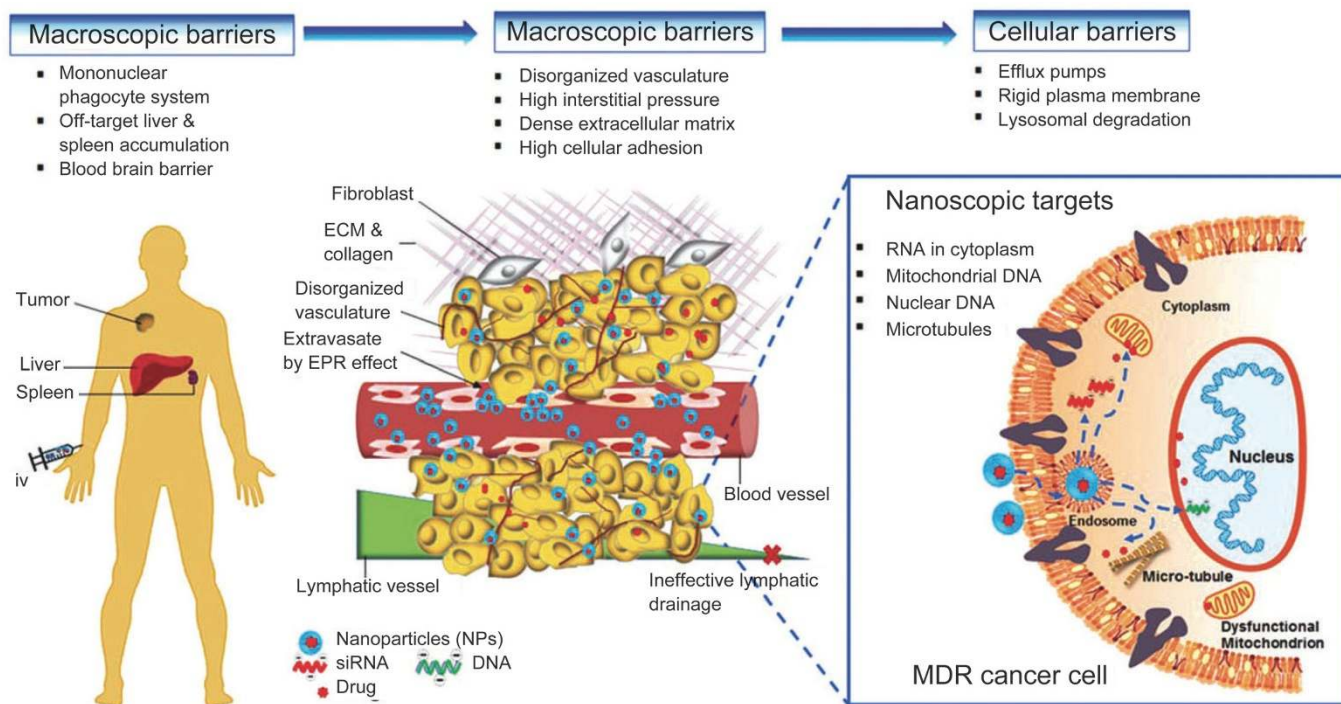
Figure 1. Illustration of multiple biological barriers to NPs and its delivered drugs before reaching nanoscopic targets of diseased cells. Left panel: at the macroscopic level (non-cellular), iv administered NPs are sequestered, accumulated and eventually eliminated by mononuclear phagocytic system (MPS) consisting mainly of the liver and spleen. In addition, the blood-brain barrier (BBB) is highly selective for many foreign substances, thus, preventing the entry of drug containing NPs into the brain. Middle panel: at the microscopic level (non-cellular), the complex tumor microenvironment (TME), *eg*, poor neo-vasculatures, dense extracellular matrix (ECM), limits the tumor permeation and retention of NPs, leading to low drug accumulation at the tumor necrotic region. Right panel: at the cellular level, the nucleus and mitochondrion are the primary organelles of genetic mutation responsible for complicated MDR phenotypes, such as overexpression of efflux transporters (*eg*, P-gp), membrane rigidity, lysosomal sequestration, apoptosis evasion, drug detoxification and altered drug target. All of the MDR mechanisms can occur simultaneously to confer drug resistance, leading to sub-optimal therapies. The figure is reproduced from Zhang *et al*[3] with permission.

pharmacology, human physiology and disease pathology and integrate each aspect into the design and evaluation of drug delivery systems for specific diseases.

In this review, we address an important question in the field of nanotechnology-based drug delivery: how can we harness the interplay between biology and nanotechnology to develop clinically relevant drug delivery systems at low cost and a high success rate? Important pharmacological and physiological mechanisms and pathological features of diseases are described for exploitation in the design of nanotechnology-enabled drug delivery systems and therapies. Firsthand examples from our research of improving therapies of cancer and diabetes are presented to illustrate the principles and importance of integration of nanotechnology with pharmacology and (patho-)physiology. The rational design of various target- and disease-driven nanotechnology-based systems are explored by (1) elucidating the molecular mechanisms of synergistic drug combinations; (2) exploiting circulatory system physiology; (3) harnessing the brain transport pathway of naturally occurring lipoproteins; (4) tackling hypoxia in the TME; and (5) mimicking physiologically relevant insulin release. It is hoped that the insights gained from these research explorations on drug delivery systems can build a bridge between the fields of nanomaterials and biological systems and provide a new perspective for the design and development of clinically useful nanomedicines.

Integration of nanotechnology with pharmacology for enhanced chemotherapy in multidrug-resistant cancer

Multidrug resistance (MDR) in cancer is a common clinical problem. It involves complex cellular and non-cellular mechanisms (eg, the aberrant TME), which can collectively contribute to reduced drug concentrations, activities and efficacy at tumor sites and within cancer cells, leading to ineffective chemotherapy[31-33]. To overcome the multifactorial MDR mechanisms for improved chemotherapy, various approaches have been investigated, such as the development of new anticancer drugs that are poor substrates for efflux transporters (eg, P-glycoprotein (P-gp)), MDR modulators or chemo-sensitizers (eg, inhibitors of P-gp); downregulation of MDR-associated enzymes using RNA interference; synergistic drug combination with different mechanisms of drug actions and multifunctional NPs[6, 34, 35]. In our laboratory, solid polymer-lipid hybrid NPs (PLNs) have been designed to bypass efflux transporters and synergistic drug combinations or drug-nanomaterial combinations in NPs have been explored[3, 4, 6, 21, 32, 36-42].

In the clinic, to counteract MDR in cancer, drug combination therapy is commonly used as the standard of care for a broad range of cancers in which single drug therapies are ineffective[43, 44]. Often, drugs in combination are selected based on their different mechanisms of action, the lack of cross-drug resistance and the generation of synergistic (ie, more-than-additive) effects[6, 45]. Ideally, a rationally selected synergistic drug combination should provide new therapeutic possibilities via different mechanisms of drug action while simultane-

ously minimizing normal tissue side effects due to the reduced dose of each drug. However, the clinical outcome of combination therapy is often confounded by severe adverse normal tissue effects with little improvement in efficacy[45]. In many cases, drug combinations show strong cytotoxicity in vitro; however, once administered intravenously (iv) into the body, the drug combination non-specifically distributes to normal organs before reaching the tumor due to the different pharmacokinetic profiles of each drug, resulting in increased adverse effects[6]. In addition, even if the drugs arrive at the tumor site, the synergistic ratio of the drug combination may not be maintained.

Application of nanotechnology in drug delivery makes it possible to deliver synergistic drug combinations ratiometrically and spatial-temporally to the sites of drug action[3, 6, 21, 36-38, 46-52]. With deepened understanding of the molecular mechanisms involved in specific drug combinations and the use of nanosized drug carriers, several nanoparticle formulations of synergistic drug combination have been moved to clinical trials[46-50, 53] or have gained approval by the United States (US) Food and Drug Administration (FDA) or other regulatory agencies[54, 55]. In the following sections, the importance of intracellular drug-drug and drug-nanomaterial interactions in the rational design of synergistic combination nanomedicines is explained in more detail.

a) Harnessing intracellular drug-drug interactions to design synergistic nanomedicine

The initiative of combining two clinically used anticancer drugs, DOX and mitomycin C (MMC), stemmed from their differences in mechanism of action and activation under hypoxia. It is known that under the hypoxic conditions of the TME, the cytotoxicity of DOX is attenuated, as its activity partially relies on the presence of oxygen (O_2) to generate free radicals (ie, superoxide) that can cause DNA damage[33, 56]. In contrast, MMC can be activated under hypoxia through a sequential one electron reduction pathway that enhances its cytotoxicity and can generate free radicals under normoxia (ie, in the presence of oxygen)[57, 58]. In addition, DOX and MMC damage DNA by different mechanisms: DOX causes DNA double-strand breaks by poisoning topoisomerase II_α (TII_α), an enzyme responsible for catalyzing the unwinding of DNA for transcription and replication[59, 60], whereas MMC induces DNA intra-strand and inter-strand crosslinks to disrupt cell maintenance and replication[61]. However, the clinical combination of DOX and MMC (DOX-MMC) was halted owing to its severe cardiotoxicity to cancer patients[62, 63]. We perceived that this off-target adverse effect could be reduced with optimal scheduling and dosing of the two drugs by rationally designing a drug nanocarrier system[64-68].

With knowledge of the mechanistic actions of DOX and MMC individually in cancer cells, we first investigated whether combining DOX and MMC could generate synergistic cytotoxicity in multiple murine and human breast cancer cell lines. Detailed in vitro pharmacological studies on the

DOX-MMC combination showed that the MMC bio-reductive metabolism, leading to the formation of activated MMC and formaldehyde, played an important role in the synergistic interaction between DOX and MMC (Figure 2A)[66]. The resultant formaldehyde depletion of glutathione (GSH), an important antioxidant that can decrease intracellular DOX concentration and reactive oxygen species (ROS)-associated cellular damage, causes the higher cytotoxicity of DOX. In addition, formaldehyde reacted with DOX to produce an active form of DOX, doxoform, that can crosslink DNA. The significantly increased DNA crosslinking adducts during the DOX-MMC treatment triggered the activation of the DNA repair enzyme system by DOX, which in turn promoted their collision with stalled TII$_\alpha$, leading to enhanced DNA double-strand breaks in cancer cells and subsequent cell apoptosis[66]. This proposed interaction mechanism suggested a critical window for treatment exposure of the DOX-MMC combination to achieve anticancer synergism because cancer cells are able to recover from MMC-induced metabolic disruption (*eg*, reduced GSH) and DNA lesions within a short time interval (<4 h)[69]. The synergistic DOX-MMC combination could be used against cancer cells that have sufficient bio-reductive enzymes to activate MMC.

To determine whether co-delivery of the DOX-MMC combination by the same nanocarrier is important for their synergism, the schedule and sequence of drug administration were studied[67, 68]. Screening the effects of various exposure schedules and doses of the DOX-MMC combination on the survival of breast cancer cells revealed that the synergism occurred only when MMC was administered either simultaneously with or after DOX exposure at an optimum DOX-to-MMC molar ratio of 2:1 but not when MMC was given prior to DOX[67, 68]. Based on these findings, a PLN formulation co-loaded with DOX-MMC (DMPLN) was designed with a key capability of synchronizing co-delivery of locally bioavailable DOX-MMC combination at synergistic ratios to cancer cells (Figure 2B (i)&(ii))[36, 65]. As a result, DMPLN provided superior advantages over free DOX-MMC solution and liposomal DOX (DOXIL®) with enhanced tumor cell apoptosis and minimum cardiotoxicity (Figure 2B (iii)&(iv)), as well as prolonged host-survival in both human and murine MDR tumor models[25, 37, 38].

b) Exploiting intracellular drug-nanomaterial interactions to design in situ bio-activated nanomedicine

In general, NPs can circumvent efflux pumps in MDR cancer cells via endocytosis pathways[32, 41, 70]. However, in some MDR cells, in addition to overexpressing drug efflux transporters (*ie*, P-gp), the rigid and thickened cellular membrane restricts the drug influx and NP uptake as a result of increased cholesterol and sphingolipid content in the cancer cell membrane compared to non-resistant ones[71-74]. Imbalance of cholesterol homeostasis is also associated with obesity and breast cancer[75-77]. To overcome such multifactorial MDR in cancer cells, we have rationally designed a sequential two-step targeting PLN system containing the ROS-generating anticancer drug MMC and the polyunsaturated fatty acid docosahexaenoic acid (DHA)[78]. DHA is capable of improving cell membrane fluidity due to the presence of six carbon-carbon double bonds within its chemical structure[79]. Thus, as a liquid lipid nanomaterial of the PLN, the DHA component, as the "first punch", softened the rigid membrane of MDR cancer cells, facilitating cellular entry of the NPs. Subsequently, *in situ* enzymatic bio-activation of MMC near or inside the mitochondria produced free radicals that initiated an intracellular chain reaction of lipid peroxidation of DHA and endogenous lipids (Figure 3A)[78, 80]. The entire course of the intracellular anticancer synergistic interaction of MMC and DHA was accomplished by the nanocarrier PLN co-loaded with MMC-DHA at a fixed ratio (MMC-DHA-PLN). The nanotechnology-enabled system acted as a targeted "nano-reactor" that coordinated and maximized the synergistic reactions between MMC and DHA at intracellular targets (*eg*, mitochondria). Compared to the non-co-encapsulated free MMC-DHA combination, MMC-DHA-PLN ensured the proximate interactions between MMC and DHA at each subsequent delivery step from the reduction in membrane rigidity to the enhancement in mitochondrion-targeted toxicity (Figure 3B)[78]. As anticipated, MMC-DHA-PLN had markedly enhanced anticancer cytotoxicity against MDR cells *in vitro* and inhibited tumor growth with no adverse effects detected in an orthotopic MDR breast tumor model[78].

Integration of nanotechnology with physiological transport for targeting cancer metastases in the lungs and brain

Cancer metastasis from the primary tumor to distant organs, such as the lungs, liver and brain, is a devastating medical condition that contributes to the majority of cancer-related deaths[81, 82]. To treat metastases, conventional chemotherapy and radiotherapy and newly emerging targeted therapy (*eg*, neutralizing antibody to growth factors) are the main treatment modalities[81, 82]. Nonetheless, the current clinical treatments for tumor metastases are far from optimal due to their severe adverse effects and limited efficacy (*eg*, drug resistance)[83, 84]. In primary solid tumors, where the EPR effect is pronounced, NPs typically can minimize normal organ toxicity and enhance therapeutic efficacy via passive or active targeted drug delivery[19, 85]. However, the EPR effect may not occur in metastatic tumors, especially when they are at an early stage and present as micron-sized lesions[86, 87]. Moreover, owing to the highly selective biological barriers presented in Figure 1, including the predominant hepatic removal of foreign particles and the blood brain barrier (BBB), off-target accumulation of NPs in unintended organs occurs frequently[88]. Therefore, it is challenging to transport NPs to metastatic tumors by simple passive and/or active targeting approaches. In the following examples, we present strategies developed from our research work to mitigate these challenges by exploiting the natural systemic circulation and brain transport pathways.
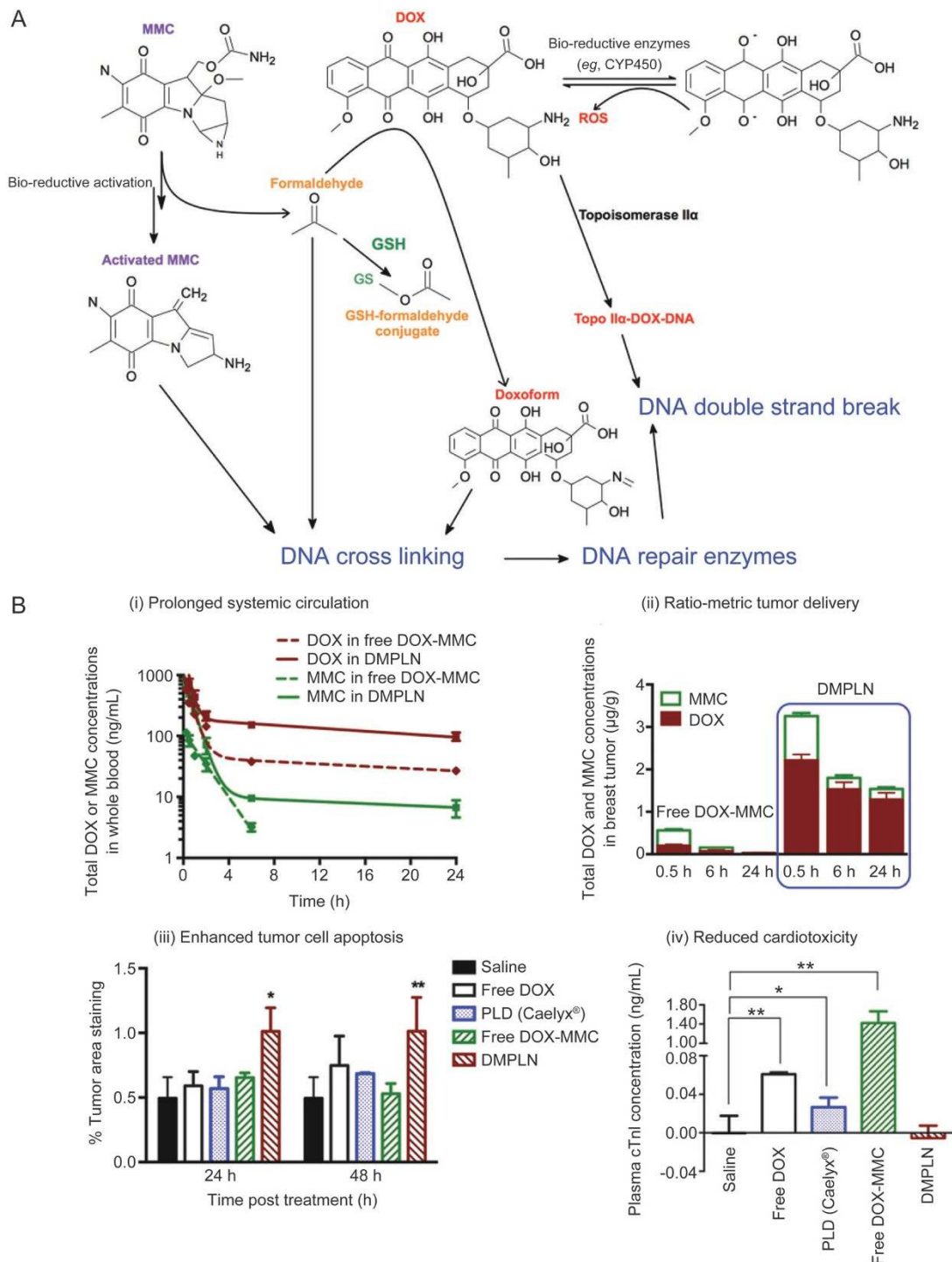
Figure 2. Biochemical interactions between anticancer drugs, DOX and MMC, and their ratio-metric co-delivery using DMPLN. (A) Synergistic interaction of DOX-MMC combination. The bio-reductive metabolism of anticancer pro-drug MMC plays a key role in enhancing DNA crosslinking, including bio-activated MMC, the formaldehyde during MMC bio-reductive metabolism, and the doxoform from the reaction of the formaldehyde and DOX. The formaldehyde also depletes GSH by the formation of inactive GSH-formaldehyde conjugates, leading to increased intracellular DOX concentration. DOX enters the nucleus to inhibit the progression of TII_α by forming the TII_α-DOX-DNA complex. The significant increased DNA crosslinking adducts during the DOX-MMC treatment triggers the activation of DNA repair system, which in turn promotes their collision with stalled TII_α, leading to enhanced DNA double strand breaks. (B) Pharmacokinetics and bio-distribution of DMPLN in orthotopic EMT6 murine breast tumor model. (i) DOX and MMC concentrations in whole blood as a function of time; (ii) Comparison of total DOX and MMC tumor accumulation between the free DOX-MMC combination and DMPLN; (iii) Percent of positive caspase-3 staining area in the breast tumor area 24 h and 48 h post-injection. * and ** indicates statistically significant difference between the DMPLN group and the other groups with $P<0.05$, $P<0.01$ or $P<0.005$, respectively. All data are mean±standard error of the mean (SEM) ($n=3$). Figure 2A is adapted from Shuhendler *et al*[66] and Figure 2B is reproduced from Zhang *et al*[36] with permission.
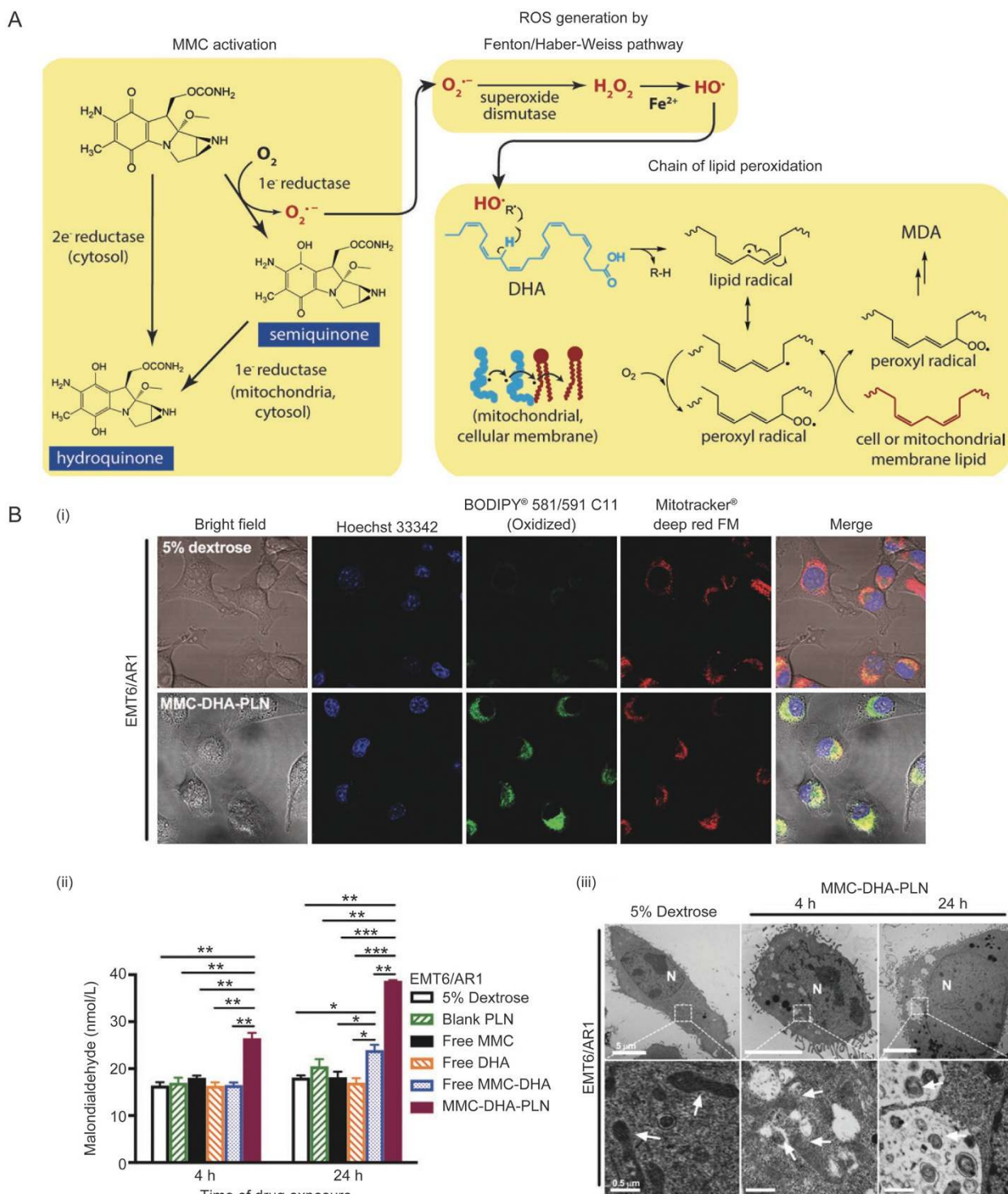
Figure 3. Mitochondrial enzymes triggered *in situ* lipid peroxidation of synergistic MMC-DHA combination and their sequential targeting capability using MMC-DHA-PLN. (A) Reaction schemes showing the cascade of lipid peroxidation produced by MMC-DHA combination that elevates intracellular oxidative stress. Activation of MMC through one-electron bio-reduction by mitochondrial enzymes generates the free radical, superoxide ($O_2^{\cdot-}$), which in turn is converted to the hydroxyl radical ($HO^{\cdot}$) to attack the methylene bridge between C=C double bonds of DHA, leading to a chain of lipid peroxidation. (B) MMC-DHA-PLN targeted mitochondrial lipid peroxidation and induced irreversible ultra-structural damages to mitochondria in MDR EMT6/AR1 murine breast cancer cells with high expression of P-gp and rigid cell membrane. (i) Laser confocal microscope images of lipid peroxidation localization (green fluorescence) in mitochondria (red fluorescence) of cells treated with 5% dextrose and MMC-DHA-PLN; (ii) The level of MDA before and after various free MMC and/or DHA formulations, blank PLN or MMC-DHA-PLN for 4 h or 24 h; (iii) Transmission electron microscopy images of drug-resistant cells treated with 5% dextrose and MMC-DHA-PLN for 4 h and 24 h. All data are means±SEM (*n*=3–4) with *$P<0.05$, **$P<0.01$, and ***$P<0.001$. The figures are adapted and reproduced from Zhang *et al*[78] with permission.

a) Exploiting systemic circulation to design lung metastasis-targeted RGD nanoparticles

Lung metastasis is common in several types of advanced cancers, including breast cancer, and is often associated with low patient survival[89, 90]. In particular, for metastatic triple-negative breast cancer (TNBC), which lacks target receptors of estrogen, progesterone and human epidermal growth factor, the prognosis is poor, as hormonal and targeted therapies are not available and chemotherapy is ineffective in the late stages of the disease despite an initial response[90-92]. In light of the overexpression of the integrin $\alpha_v\beta_3$ receptors on the tumor neovasculature and many types of cancer cells, including TNBC cells[93, 94], the natural targeting ligand sequence *Arg-Gly-Asp* (RGD) peptide was grafted on the surface of nanocarriers for targeted delivery of anticancer drugs to the tumor[95]. However, our early attempt to increase the accumulation of RGD-conjugated solid lipid nanoparticles (SLNs) in primary TNBC revealed significantly reduced tumor penetration and increased liver uptake compared to non-targeted NPs despite prolonged SLN retention time in the tumor[21]. Optimization of RGD density on the surface of NPs only modestly improved the NP accumulation in the solid tumor[96]. This observation is similar to other group's studies showing that introduction of RGD as a targeting moiety may increase the host immunogenicity and protein adsorption (*eg*, opsonin), leading to unwanted adverse effects and pronounced hepatic uptake[29, 97].

Unlike the cases of primary breast tumor where intravenous (iv) administered RGD-conjugated NPs and the payload therein are first removed by the liver and the spleen before reaching the breast, NP transport to the lungs occurs before the passage through the liver and spleen via naturally endowed systemic circulation. Following the direction of blood circulation, the iv administered NPs first enter the tumor-bearing lungs before the "first-pass effect" of liver clearance (Figure 4A). This is particularly advantageous for improving the delivery of the ligand-conjugated NPs as chemotherapeutics to the diseased sites in the lungs[98]. Based on the physiological characteristics of both systemic and pulmonary circulation, we have optimized an RGD-conjugated PLN system (RGD-PLN) so that it can highly accumulate in TNBC metastasis-bearing lungs[98]. Upon arrival at the highly vascularized networks of the lungs, the RGD-PLN bind to the $\alpha_v\beta_3$ integrin receptors overexpressed on both the tumor cells and the angiogenic blood vessels near metastatic tumor modules (Figure 4B). This design concept was proven viable by microscopic examination of the tumor area, which depicted much higher accumulation of RGD-PLN than the non-targeted PLN in both the tumor vasculature and metastatic tumor cells (Figure 4C). Consequently, RGD-PLN co-loaded with DOX and MMC (RGD-DMPLN) significantly reduced the metastatic burden and extended the median survival time in a lung metastasis model compared to a non-targeted DMPLN (Figure 4D&E). It is worth noting that off-target binding of RGD-PLN could occur during circulation due to the abundant expression of integrin receptors such as $\alpha_v\beta_3$ receptors on the macrophage and human endothelial cells[99-101]. Thus, exploitation of the systemic circulation direction can potentially minimize such off-target effects, as *iv*-administered RGD-PLN reaches the disease site at the lungs first and binds to the tumor neovasculature and tumor cells that overexpress integrin $\alpha_v\beta_3$ receptors.

b) Harnessing naturally occurring lipoprotein to design self-decorating nanoparticles for drug delivery to the brain

Nearly 15%–30% of patients with advanced breast cancer develop brain metastases during progression of the disease[102, 103]. Brain metastases are associated not only with a poor prognosis but also with severe neurological impairment[102, 103]. The treatment of brain metastases is challenging due to the inability of most therapeutic agents to penetrate the BBB, leading to subtherapeutic drug accumulation in the CNS[102-104]. The BBB consists of a continuous layer of tight junction-expressing endothelial cells and presents a highly selective biological barrier. The drug efflux transporters (*eg*, P-gp, multidrug resistance-associated proteins (MRPs), and breast cancer resistance protein (BCRP)) found on brain endothelial cell membranes further expel substrate molecules, including many effective anticancer drugs (*eg*, DOX), from the brain[105-107]. As a result, it restricts passive entry of nearly 98% of all drugs to the brain at the blood-brain interface[105-107]. Therefore, development of effective strategies for shuttling drugs across the BBB has been a long-standing goal in the drug delivery field.

Various non-invasive strategies have been investigated for delivering therapeutic agents into the brain[106, 108-110]. The examples include utilization of ligands for overexpressed receptors on the BBB (*eg*, transferrin, insulin, low-density lipoprotein (LDL), and Angiopep-2), of essential nutritive compounds required for cell metabolism and survival of the CNS (*eg*, glutathione, choline, glucose), or of cationic peptides that electrostatically interact with the negatively charged brain microvessel endothelial cells[106, 108-112]. Among these strategies, the receptor-mediated transport appears to be the most favorable due to the specificity of ligand-receptor interactions. Monoclonal and single-domain antibodies have been developed for various endogenous BBB transcytosis receptors (*eg*, receptors for insulin and transferrin)[111, 113-117]. However, the antibody approach suffers from high cost and the potential host immunogenic response[118].

Of the various receptor-mediated brain-penetrating systems, NPs present more advantages than antibody-drug conjugates (ADC), bispecific antibodies, and fusion proteins because of their easy manufacture, low cost, high loading capacity, flexibility of encapsulating various cargos, and controllable drug release kinetics[36, 107, 119-121]. Nanoparticle carriers can be engineered by surface decoration of targeting antibodies or ligands via covalent bonds or physical coating[10, 119]. Compared to pre-engineered nanocarrier systems, NPs that are able to adsorb receptor-binding moieties from the blood during circulationare more attractive owing to their low immunogenicity and cost effectiveness.

We have designed a platform nanoparticle system based on a terpolymer consisting of a starch backbone and grafted
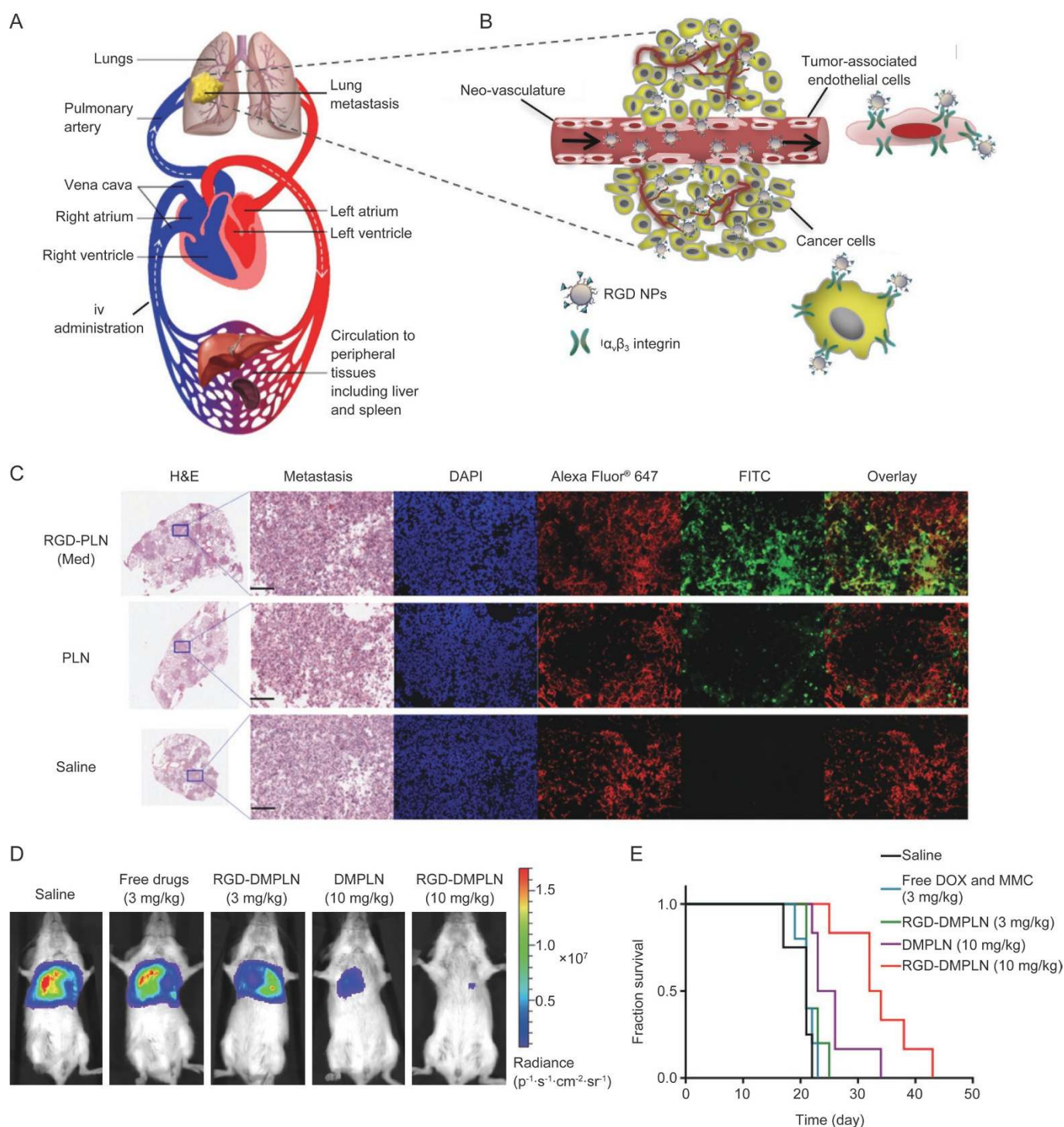
Figure 4. Exploiting the physiology of circulatory system to design lung metastasis-targeted RGD-NPs. (A) The flow direction of the blood circulation. The human cardiovascular system consists of pulmonary and systemic circulation components. The iv administrated NPs enter the veinous circulation and then follow the direction of blood flow (white arrows) to reach the lungs before arriving at clearance organs, such as the liver and spleen. (B) Once reaching the lungs, RGD-PLN targeted both tumor neo-vasculatures and cancer cells that overexpress $\alpha_v\beta_3$ integrin receptors. (C) Confocal fluorescence microscopic images of the metastasis-bearing lungs inoculated with MDA-MB-231-*luc*-D3H2LN human TNBC cells in SCID mice 4 h post iv injection of optimized RGD-PLN, non-targeted PLN or saline. Transverse sections of the lung were made and metastatic tumor nodules appear purple under H&E staining. The zoomed tumor area was stained with DAPI for nuclei (blue) and Alexa Fluor® 647 labelled CD-31 antibody for blood vessels (red) and FITC for FITC-labeled NPs (green). Scale bar: 200 μm for all zoomed images. (D) *In vivo* bio-luminescent images of mice treated with free DOX-MMC solution, DMPLN and RGD-DMPLN at two dose regimens on the day 28th after the initial tumor inoculation. (E) Kaplan-Meier plot of the tumor-bearing mice for all groups. RGD-DMPLN improved the survival of lung metastasis-bearing mice compared to free DOX-MMC in solution and non-targeted DMPLN. Figure 4C, 4D and 4E are reproduced by Zhang *et al*[98] with permission.

poly(methacrylic acid) and polysorbate 80 (PS 80)[122-124]. An *in vitro* binding assay showed good binding affinity of the terpolymer NPs to recombinant human ApoE protein. It has been demonstrated that the PS 80 chains protruding on the nanoparticle surface can recruit apolipoproteins (*eg*, Apolipoprotein E (ApoE)) in the plasma upon iv administration and then be transcytosed via low density lipoprotein receptor (LDLR) recognition to enter the brain parenchymal side (Figure 5A)[125-127]. The abundant hydroxyl and carboxylic acid groups present on starch and methacrylic acid enable easy chemical modification and the loading of a wide variety of BBB-impermeable agents with different physicochemical properties, including a magnetic resonance imaging (MRI) contrast agent[124], fluorescence dyes[128, 129], the hydrophilic anti-cancer drug DOX[128, 129], the hydrophobic drug docetaxel (DTX)[130] and the therapeutic antibody trastuzumab (TRA)[131].

DOX-encapsulated terpolymer NPs accumulated in the brain metastasis area via transcytosis and the EPR effect (Figure 5B) and inhibited tumor growth (Figure 5C)[128]. The terpolymer was also modified and combined with a lipid to form a nanoparticle formulation of DTX (DTX-NP). Compared to clinically used solution form of DTX (Taxotere®), DTX-NP delivered a larger amount of DTX to the brain (Figure 5D) and exerted a greater therapeutic effect in a brain metastasis model (Figure 5E)[130]. Delivery of a therapeutic antibody to the brain requires particular considerations differing from the delivery of small molecules. To deliver therapeutic antibody TRA to human epidermal growth factor receptor-2 (HER2)-positive cancer cells in the brain metastasis area, we developed an intracellularly eroded solid-lipid polymer matrix containing a TRA-conjugated terpolymer. The nanoconstructs were able to shield TRA in the circulatory system and deliver it to brain tumors via a two-step targeting process[131]. The constructs remained intact during systemic circulation, crossed the BBB (*ie*, the first step in targeting the brain), and then n dissociated and exposed TRA, enabling the second-step targeting of TRA to the cancer cells within the brain and thus generating a therapeutic effect[131]. Such a self-decorating platform nanosystem exhibits versatile drug delivery capabilities and a tremendous potential for the rational design of non-invasive nanotechnology-based treatments of various brain disorders.

Integration of nanotechnology with pathophysiological features for disease-responsive drug delivery and enhanced radiation therapy

Pathophysiological characteristics, such as hypoxia in the TME and unregulated hyperglycemia in diabetes, are distinct from normal physiological conditions. In this section, we describe the design and first application of clinically relevant, bio-reactive hybrid manganese dioxide (MnO_2) NPs to modulate multiple pathological factors in the TME for improving radiation therapy. We also present a nanotechnology-enabled closed-loop insulin delivery system that utilizes a stimuli-responsive nanohydrogel and MnO_2 NPs (MDNPs) to mimic normal endocrinology for regulating insulin release in response to pathophysiological conditions of hyperglycemia

in a timely self-regulated manner.

a) Tackling pathological factors of the tumor microenvironment using bio-reactive hybrid MDNPs to sensitize cancer cells to radiation therapy

The abnormal TME manifests itself by several major hallmarks, including hypoxia, overexpression of hypoxia-regulated genes, acidosis, and ROS generation[132-135], among which hypoxia is a poor prognostic factor implicated in tumor resistance to mainline treatments, *eg*, radiotherapy (RT)[136, 137] and chemotherapy[33]. For RT, the presence of O_2 enhances the production of DNA double-strand breaks in cancer cells and permanently "fixes" DNA damage caused by direct ionization or water radicals into a more difficult - to repair state[137, 138]. However, the hypoxia found in many solid tumors directly hinders this mechanism, resulting in the reduced efficacy of RT (*eg*, decreased DNA double-strand breaks)[137, 139]. Moreover, hypoxia and ROS lead to overexpression of hypoxia-inducible factor-1α (HIF-1α), a master regulator of many downstream genes and proteins[140-144], such as vascular endothelial growth factor (VEGF)[145, 146] and carbonic anhydrase IX (CAIX)[132, 147]. All of these pathological factors contribute to locally increased oxidative stress[148, 149], acidosis[150, 151], angiogenesis[142], cancer cell proliferation and metastasis[134], leading to poor patient prognosis (Figure 6A (i)).

Various nanotechnology-based strategies have been developed to modulate TME, including 1) nanoparticle formulations of angiogenesis inhibitors or small interfering RNA (siRNA) against vascular endothelial growth factor receptor-2 (VEGFR-2) to remodel the tumor vasculature, 2) compounds for reducing/degrading collagen content in the tumor extracellular matrix, and 3) cytokines to modulate the phenotype of tumor-associated macrophages (TAMs)[134]. Given the multiple pathological factors of the TME and their pleiotropic effects on poor therapeutic outcomes, our laboratory has developed clinically suitable bio-reactive MDNP formulations and applied them for the first time to attenuate several negative features of the TME simultaneously *ie*, hypoxia, acidosis, and ROS generation (*eg*, excessive hydrogen peroxide (H_2O_2)) (Figure 6A(i))[152-154]. Through a simple catalytic chemical reaction at the pathological pH (~6.8) of the TME, the MDNPs can sustainably convert excess H_2O_2 and protons (H^+) into O_2, leading to reduction in hypoxia, oxidative stress and acidity in the TME[152, 154]. The pH- and H_2O_2 concentration-dependent reactivity endows the MDNPs with a tumor-selective function due to the higher physiological pH (~7.4) and lower ROS level in the systemic circulation and in normal tissue[152, 154]. The *in situ* oxygenation by MDNPs mitigates the hypoxia of TME, which in turn attenuates tumor-promoting factors and overcomes hypoxia-induced radio-resistance, thereby enhancing the efficacy of RT (Figure 6A(ii)).

A new iv injectable formulation of MDNPs was developed by incorporating MnO_2 into a lipid matrix with a polymer corona to improve biocompatibility and bio-distribution, thereby helping protect the MDNPs during their systemic circulation and enabling their passive accumulation in the solid
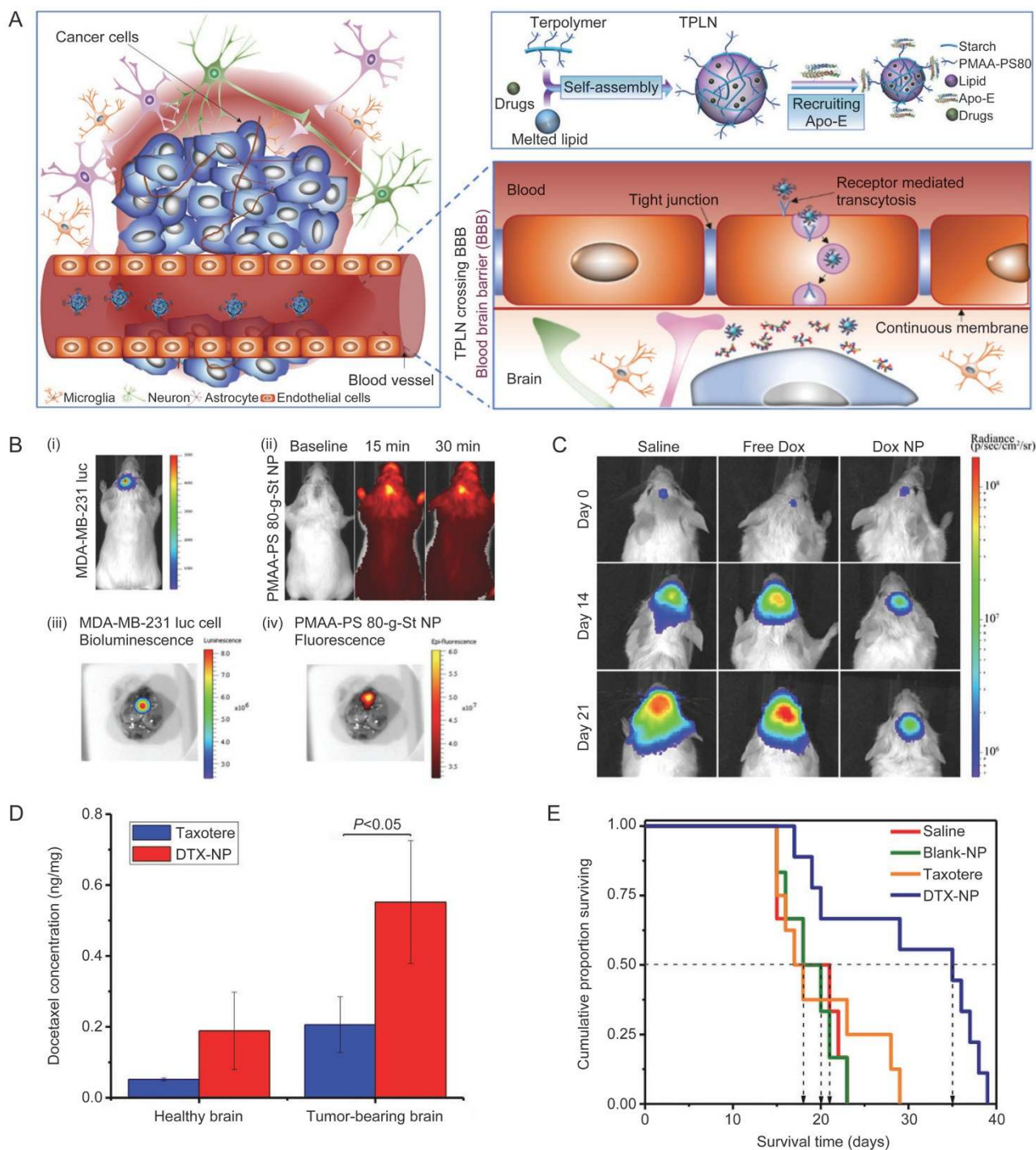
Figure 5. Harnessing the physiology of naturally occurring lipoprotein to design self-decorating terpolymer NPs or terpolymer-lipid hybrid NPs (TPLN) for drug delivery to the brain. (A) Schematic of the receptor-mediated transcytosis mechanism of PMAA-PS 80 (terpolymer)-containing polymeric NPs penetration through the BBB to deliver encapsulated drug to brain cancer cells. (B) *In vivo* bioluminescent images of brain tumors and optical whole-body and ex vivo images of brain accumulation of NP. The brain metastases of MDA-MB-231-*luc*-D3H2LN cells in NRG-SCID mice were established by intracranial injection: (i) Bioluminescence of luciferase expressing tumor cells in the inoculated brain tumors 10 min following *ip* injection of luciferin solution; (ii) PMAA-PS 80-g-St NPs were labeled with a near infrared dye (HiLyte Fluor 750) and imaged after iv tail vein injection; (iii) Bioluminescent image of brain tumor; (iv) Fluorescence image of nanoparticles in an excised mouse brain. (C) *In vivo* images of brain tumor bioluminescence showing tumor growth inhibition in NRG-SCID mice. (D) Docetaxel (DTX) concentration in tumor-bearing brains at 15 min after iv injection of DTX-NP compared to Taxotere® (free DTX in solution). The concentration of DTX in the perfused brains of mice was quantified using LC-MS/MS following iv injection of DTX-NP or Taxotere® at the dose of 20 mg/kg DTX. All data are mean±standard deviation (SD) (*n*=3). (E) Kaplan–Meier survival curve of tumor-bearing mice following the treatment of saline, blank NP, Taxotere® (20 mg/kg DTX) or DTX-NP (20 mg/kg DTX). All data are mean±standard deviation (SD) (*n*=9). Figure 4B, 4C and Figure 4D, 4E are reproduced from Li *et al*[128] and He *et al*[130], respectively with permission.
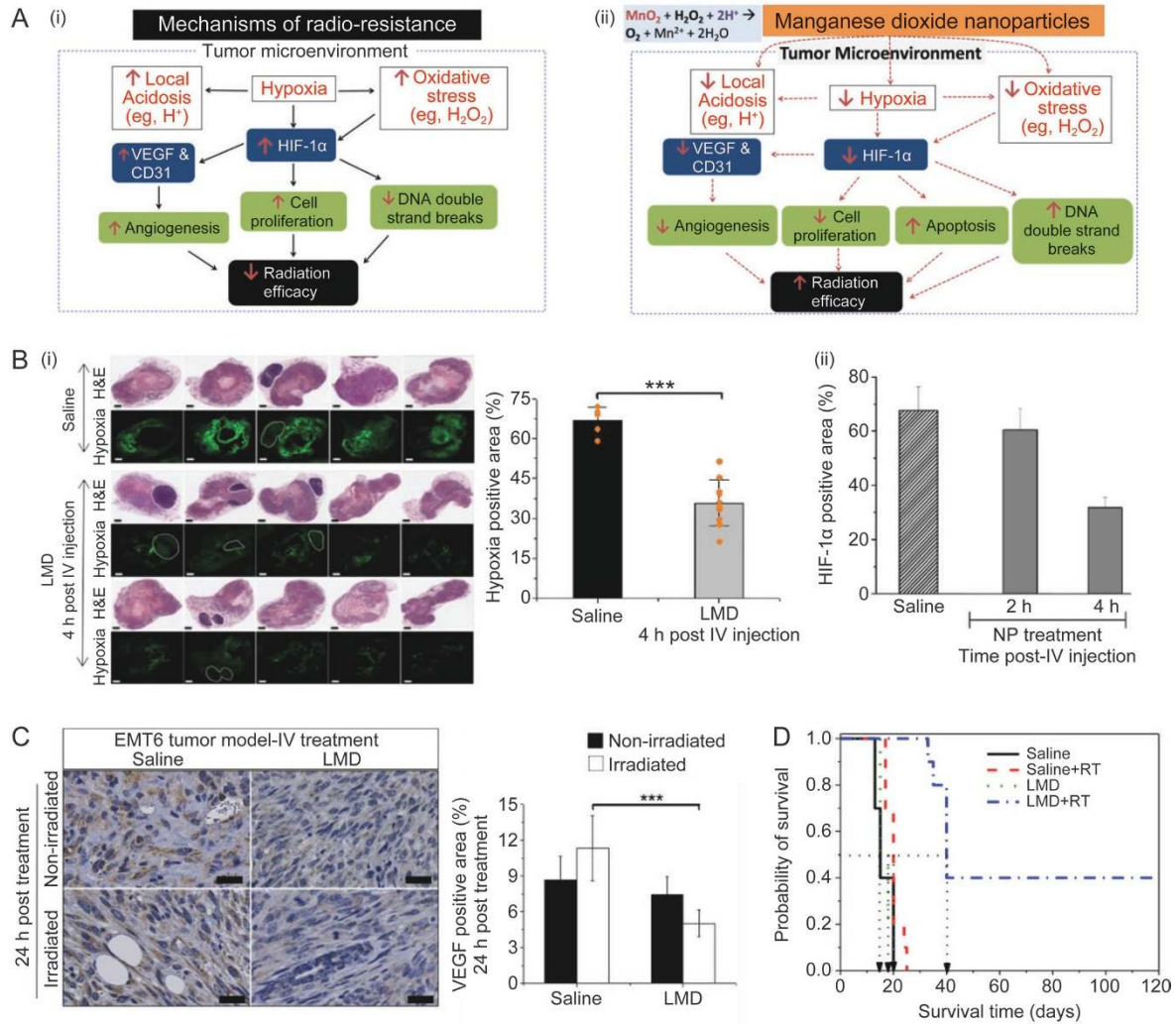
Figure 6. (A) (i) Scheme of TME-associated radiation resistance; (ii) Modulation of the TME by MnO$_2$ NPs to enhance RT efficacy. Hypoxia characterized by low O$_2$ levels in the TME contributes to up-regulation of HIF-1α, an important transcription factor that regulates the metabolism and proliferation of cancer cells and angiogenesis. Such low O$_2$ levels reduce the mechanism of O$_2$ enhanced cell killing by radiation therapy. MnO$_2$ NPs are able to decompose H$_2$O$_2$ (reduced oxidative stress) and scavenges H$^+$ (decreased local acidosis) to create a more favorite TME for cell killing by radiation therapy. (B) (i) Effect of iv treatment with LMD NPs on tumor hypoxia determined by pimonidazole analysis at 4 h after treatment; (ii) the hypoxia level in tumors for LMD or saline-treated animals at 2 h and 4 h. The scattered points (orange dots) represent tumor hypoxia level of individual animal to identify the heterogeneity in tumor hypoxia among animals following LMD treatment. Scale bar, 1 mm. Data show mean±SD (*n*=5 for saline- and *n*=10 for LMD-treated group). (C) Effect of LMD NPs treatment alone or with radiation on tumor VEFG expression in breast tumor. Representative IHC staining for VEFG and corresponding quantification at 24 h after iv treatment. Scale bars: 25 μm and data show mean SD (n=3/group). (D) Kaplan-Meier survival curves for all treatment groups. Horizontal dotted line is 50% survival level. *** indicates statistically significant difference with *P*<0.001. All *in vivo* experiments were performed in female BALB/c mice orthotopic implanted with EMT6 murine breast cancer cells. Figure 5B, 5C, 5D are reproduced from Abbasi *et al*[155] with permission.

tumor via the EPR effect[3, 152, 155]. Preclinical studies using orthotopic breast tumor models demonstrated good tumor accumulation and sustained effects of the lipid-polymer-based MDNPS (LMD NPs) on modulating TME factors, *ie*, hypoxia and HIF-1a and VEGF expression (Figure 6B&C)[152, 155]. The optimal time interval post iv injection of the LMD NPs was identified as 4 h, at which point intra-tumoral oxygenation reached a maximum (Figure 6B)[152, 155]. This time interval was then selected for the application of ionizing radiation to treat breast tumors following iv injection of the LDMN NPs. The

in situ effect of the LMD NPs was so powerful that a single pretreatment with LMD NPs followed by 10 Gy local irradiation of the tumor led to profound improvement in response to RT. Compared to RT alone, which essentially exhibited no inhibitory effect on tumor growth in this hypoxic breast tumor model, the combination of LMD NPs with RT strikingly prolonged the median survival time by 500% and resulted in a 40% cure rate (Figure 6D)[152, 154, 155]. Our recent studies on combination therapy with MDNPs and chemotherapy (*ie*, DOX) demonstrated that pretreatment with MDNPs also signifi-

cantly enhanced the efficacy of chemotherapy and antitumor immunity[156]. These findings suggest potential broader clinical applications for the modification of TME factors to enhance the effectiveness of cancer treatments.

b) Mimicking dynamic insulin release from human pancreatic β-cells by utilizing a fast responsive nanogel and self-O_2 replenishing MDNPs for tight glucose control

Type 1 diabetes mellitus (T1D) is a metabolic disorder affecting more than 347 million people globally[157]. In T1D patients, insulin-producing pancreatic β-cells are damaged, and these patients rely on multiple administrations daily of exogenous insulin to sustain their lives[158, 159]. Insulin is a key hormone regulator of glucose metabolism that decreases blood glucose levels to maintain them below 5.6–6 mmol/L (~100 mg/dL) (Figure 7A)[160]. In healthy individuals, insulin secretion by pancreatic β-cells is synchronized with glucose level via a quick pulsatile surge when the glucose level rises after a meal and brings it down to euglycemia within 30–60 min[161, 162] (Figure 7A).

Mimicking this dynamic insulin release pattern is critical to avoid extended tissue exposure to hyperglycemia and the risk of hypoglycemia. However, even with frequent self-monitoring of glucose level and administration of anticipated amounts of insulin, conventional insulin therapy is far from the ideal physiological response[163-165]. Therefore, closed-loop insulin delivery systems have been developed to automate these steps with the use of continuous blood glucose monitors and insulin pumps to better emulate physiological conditions and minimize user's intervention (Figure 7B). However, the cost of the pumps and accessories and the short usage duration (3–7 d) of the accessories remain to be resolved[166, 167].

Recent advances in nanomaterials offer new opportunities for developing next-generation closed-loop insulin delivery systems that better mimic the endocrinological secretion of insulin[9]. Our laboratory introduced pH-responsive hydrogel NPs to replicate the rapid and dynamic insulin release at physiologically relevant time scales[168-174]. The hydrogel NPs, embedded in a pH-insensitive polymer membrane (Figure 7C), undergo a volume-phase transition from a swollen state at physiological pH to a collapsed state at acidic pH below their pK_a[175, 176]. When combined with glucose oxidase (GOx), the pH-responsive nanogels shrink in response to decreased local pH due to the production of gluconic acid from the catalyzed glucose oxidation. The glucose-responsive volume change of the nanogels regulates the permeability of the composite membrane and thus controls the rate of insulin release from the reservoirs (Figure 7D)[168-174].

The nanometer-sized hydrogel is a key component imparting a short response time to the composite membranes. Reduction in the dimensions of a material from millimeters to nanometers dramatically decreases analyte diffusion distance (Figure 7C). Consequently, it will take a much shorter time for nanogel to reach volume change equilibrium, as diffusion time scales with the square of diffusion distance $t = x^2/qD$ where x is the mean diffusion distance, D is the diffusion coefficient, t

is the diffusion time, and q is a dimensionality constant)[177, 178]. For example, for a solvent molecule (*eg*, H_2O) with $D = 10^{-6}$ cm^2/s, diffusion over a 1 mm distance will take 10 000 s (2.8 h), while in a nanogel with characteristic dimensions of 100 nm, it will take 10^{-4} s (Figure 7C). In this way, the pH-responsive NPs (150–300 nm) embedded in the membrane would change their volume in response to a glucose-converted pH signal within a fraction of second. As a result, the alteration of insulin release rate across the membrane can occur within seconds after a step change in environmental glucose concentration *in vitro* (Figure 7D)[168, 171].

A common and long-standing problem in biosensors that utilize a glucose oxidation-produced signal to detect glucose level is the insufficient O_2 supply *in vivo*. In a small confined subcutaneous tissue where a sensor is placed, O_2 level is not proportional to glucose concentration, especially in the state of hyperglycemia. Although catalase is incorporated in some devices to recover consumed O_2, it can only convert half of the byproduct H_2O_2 back to O_2. To solve this problem, we introduced MDNPs in the composite membrane to continuously catalyze the breakdown of H_2O_2 to molecular O_2 and water at a one-to-one molar ratio ($H_2O_2 + 2H^+ \xrightarrow{MnO2} O_2 + 2 H_2O$) (Figure 7D)[169, 172, 173]. In this way, the incorporated MDNPs not only reduced the enzyme-damaging H_2O_2, a by-product of glucose oxidation, preserving the enzymatic activity of GOx and catalase, but also raised the microdomain pH, avoiding over-acidification at low glucose concentration[172, 179]. As a result, the engineered MDNP-containing GOx-based closed-loop insulin delivery system was able to provide rapid stepwise pulsatile insulin release at pathophysiologically relevant changes in glucose concentration (*eg*, from 100 to 200, to 400 mg/dL) and long-term GOx stability compared to systems without MDNPs[172, 179]. After implantation in T1D rats, this nanotechnology-enabled insulin delivery system was able to reproducibly bring elevated blood glucose levels down to normal levels within 30 min after induced hyperglycemia, which mimics the endogenous insulin response time of healthy rats (Figure 7E) and resembles the pattern in healthy humans (Figure 7A)[169, 171, 179]. For human application, the prototype device needs to be scaled up according to the body size ratio and required insulin dose and be refillable with insulin solution for repeated administration. Nevertheless, these results suggest that the design and testing of nanotechnology-based drug delivery systems should be guided tightly by the disease-associated pathophysiology. In other words, the medical application of a drug delivery system should be understood thoroughly before investing time and finance in engineering tasks.

Conclusions and future perspectives

The ultimate goal of nanotechnology-enabled drug delivery systems in the clinic is to improve patient survival and quality of life. This review has emphasized the convergence of nanotechnology, pharmacology and physiology in the rational design of effective drug delivery systems. To design clinically translatable nanomedicine for cancer therapy, it is important to
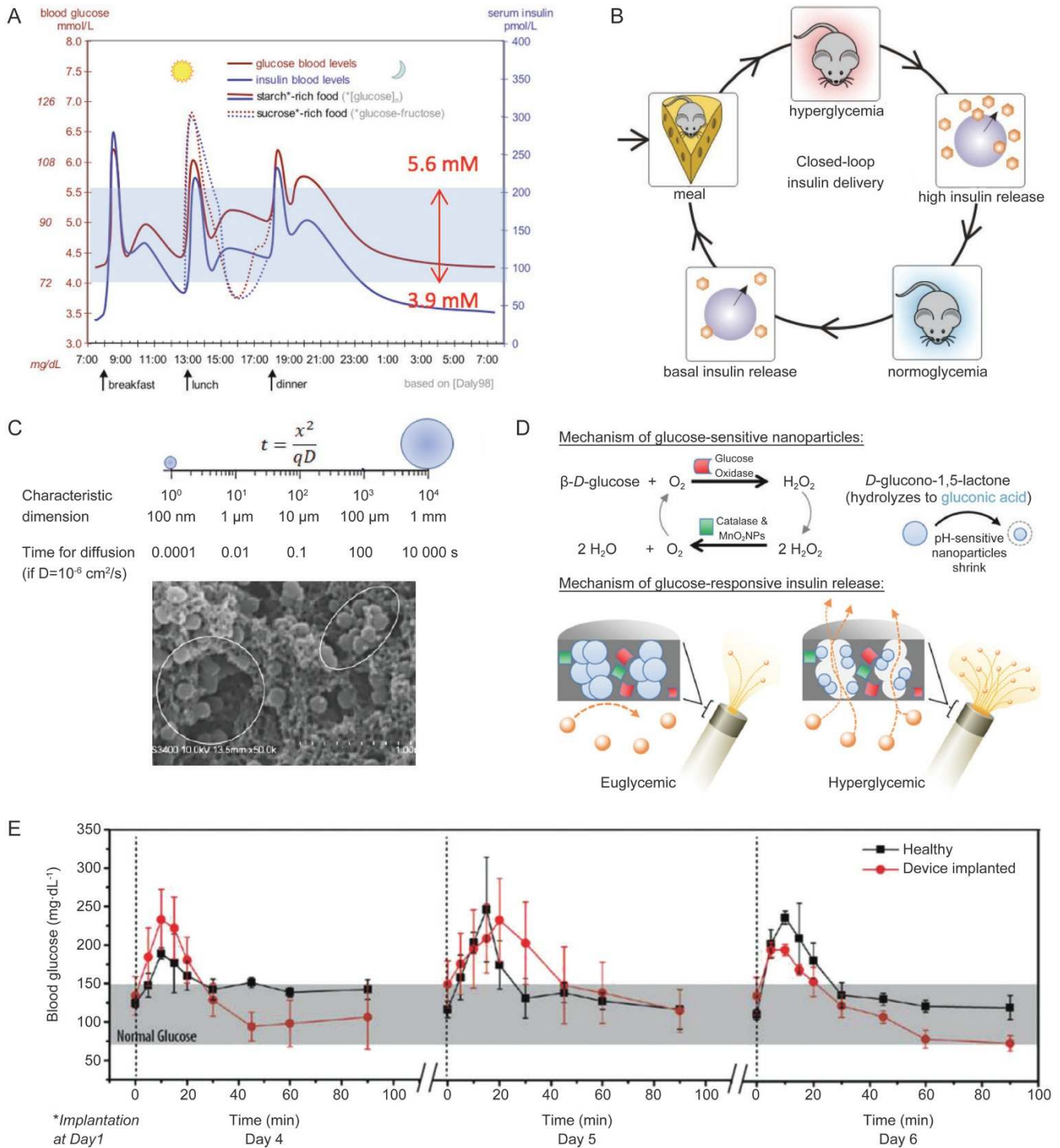
Figure 7. (A) Profile of blood glucose and insulin concentration with high-starch and high-sucrose diets. (B) Schematic illustrating the concept of closed-loop insulin delivery for the management of blood glucose levels in Type 1 diabetes patients. (C) Comparison of the time for a molecule with assumed $D=1\times10^{-6}$ cm^2/s to diffuse a distance within a material with milliliter to nanometer sizes (top) and a scanning electron microscopic image of nanohydrogel particles in a composite membrane (bottom). (D) Schematic of a chemically-driven closed-loop insulin delivery device based on glucose oxidase, pH-responsive nanogel, catalase and MnO$_2$ NPs. Glucose oxidase catalyzes the oxidation of environmental glucose to gluconic acid, which induces shrinkage of embedded pH-sensitive nanogels, leading to increased membrane porosity and faster diffusive release of insulin from the device. Catalase and MnO$_2$ nanoparticles remove by-product H$_2$O$_2$ and regenerate O$_2$, which is required for glucose oxidation. (E) Blood glucose levels of healthy (square) and diabetic rats implanted with the closed-loop insulin delivery device (circle) over a 90 min period following a bolus injection of glucose. Error bars represent SD (n=3). The implanted devices enabled blood glucose levels to return to a normal range within ~30 min after glucose injection, thus mimicking the physiological insulin response of healthy rats. The figures are reproduced from Daly et al[160], Gordijo et al[172], Li et al[169] and Chu et al[171] with permission.

have an in-depth understanding of the pharmacological mechanisms of synergistic drug combinations, biological pathways of TME and cancer cells, and physiological characteristics of normal and disease states, in addition to the physicochemical properties of NPs (*eg*, size, surface charge and shapes). Initial efforts to acquire knowledge about relevant pharmacology, physiology and pathophysiology are essential for providing critical criteria for designing and evaluating nanotechnology-based drug delivery systems with desirable functionalities for the treatment of various diseases.

The importance of integrating these biological factors into the design of nanomedicine is illustrated by our firsthand preclinical examples presented herein and demonstrated by several nanomedicine products successfully developed for clinical use. The first successful example of nanomedicine translation is the design of *Stealth* Doxil® (*Pegylated* liposomal DOX) with surface *PEGylation* to minimize blood protein adsorption and liver uptake[27, 180]. Both preclinical and clinical studies of *Pegylated* liposomal DOX revealed similar pharmacokinetic and toxicity profiles, including extended blood circulation of DOX and selective tumor uptake via the leaky microvasculature and reduced DOX-associated cardiotoxicity among various species and tumor types[27, 180]. The biomimetic design of Abraxane® (albumin-bound paclitaxel) is another successful example that utilizes the natural binding of a hydrophobic drug to the serum protein albumin to deliver water-insoluble paclitaxel to the tumor[181]. This formulation improves the pharmacokinetics of the drug compared to the castor oil-based paclitaxel (Cremophor®EL) by two delivery mechanisms – glycoprotein 60 receptor-mediated endothelial cell transcytosis and the EPR effect[181]. A recently approved liposomal formulation (Vyxeos™) for treating acute myeloid leukemia (AML)[182] is a perfect example of the rational design of synergistic drug combination thorough pharmacological evaluation and preclinical studies followed by successful translation to human patients. The daunorubicin-cytarabine molar ratio fixed at 5:1 in the formulation was identified *in vitro* against leukemia cells, and the capability of the liposomes to deliver the synergistic drug ratio and provide enhanced efficacy and reduced toxicity was demonstrated in rodent tumor models[182]. These benefits were then translated successfully to human patients.

These aforementioned examples of bench-to-bed translation suggest that integration of nanotechnology with biology can boost the success rate of nanomedicine development. Nevertheless, extrapolation of animal data to human application is challenging. Most laboratory animals (*eg*, rodents, canines, pigs) have different anatomical sizes than humans, which could directly influence physiological parameters (*eg*, the amount of enzymes and blood flow) of their internal organs. For example, a drug administered to laboratory mice is more rapidly cleared due to the relatively higher amount of hepatic enzymes and faster blood flow than those in human beings[183]. In addition, biochemical parameters such as protein binding and enzyme activity can be distinctive among species. Although the amino acid sequences of many proteins are conserved evolutionarily, small changes in the primary amino acid residues could result in profound differences in the substrate specificity of metabolizing enzymes (*eg*, albumin and cytochrome P450). This can be particularly critical in the design of drug combination nanomedicine that relies on metabolic activities for synergistic interactions[183]. Thus, it is difficult to predict all the parameters in drug metabolism, pharmacokinetics and toxicity from *in vitro* and preclinical experiments[183]. Currently, in addition to established physiologically relevant mathematical models, organ-on-a-chip systems that recapitulate the complex organ functions at the microscale level provide an alternative preclinical-to-clinical model to robustly evaluate drug delivery systems for reduced discrepancies between their performance in preclinical studies and clinical trials[184].

Meanwhile, continuous investigations on intricate interactions between nanomaterials and biological systems will uncover underlying mechanisms of "black boxes" after the bio-distribution of NPs administered to the body, leading to new strategies for manipulating physiological conditions to maximize the impact of nanotherapeutics on disease treatments[185-188]. Moreover, understanding molecular interactions among NP components (*ie*, nanomaterials, loaded drugs) within the nanostructure can shed light on valuable mechanisms leading to the rational design of drug delivery systems with desirable physicochemical properties for precisely controlled drug loading and release[51, 173, 189-191]. Fine-tuned nanocarriers and nanoconstructs will complement desired pharmacological aspects of the drug (*eg*, site of the drug action, ratiometric delivery) for optimal *in vivo* performance. We expect that the integration of nanotechnology with pharmacology and physiology will expedite the successful development and application of clinical nanotherapeutics, which in turn will make the clinical goal – improved patient survival and quality of life – become a reality in the near future.

Acknowledgements

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