Materials Today Bio 20 (2023) 100658

Contents lists available at ScienceDirect

Materials Today Bio

journal homepage: www.journals.elsevier.com/materials-today-bio

Toward nanotechnology-enabled application of bilirubin in the treatment and diagnosis of various civilization diseases

Yaqi Cui, Cuiping Wu, Linpeng Li, Haibo shi, ChunYan Li *, Shankai Yin **

Shanghai Key Laboratory of Sleep Disordered Breathing, Department of Otolaryngology-Head and Neck Surgery, Otolaryngology Institute of Shanghai JiaoTong University, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, 200233, China

ARTICLE INFO

Keywords:
Bilirubin-derived nanoparticles
Reactive oxygen species
Immunity modulation
Metabolic regulation
Antiproliferative

ABSTRACT

Bilirubin, an open chain tetrapyrrole, has powerful antioxidant, anti-inflammatory, immuno-suppressive, metabolic-modulating and anti-proliferative activities. Bilirubin is a natural molecule that is produced and metabolized within the human body, making it highly biocompatible and well suited for clinical use. However, the use of bilirubin has been hampered by its poor water solubility and instability. With advanced construction strategies, bilirubin-derived nanoparticles (BRNPs) have not only overcome the disadvantages of bilirubin but also enhanced its therapeutic effects by targeting damaged tissues, passing through physiological barriers, and ensuring controlled sustained release. We review the mechanisms underlying the biological activities of bilirubin, BRNP preparation strategies and BRNP applications in various disease models. Based on their superior performance, BRNPs require further exploration of their efficacy, biodistribution and long-term biosafety in nonhuman primate models that recapitulate human disease to promote their clinical translation.

1. Introduction

Bilirubin is a common yellowish pigment derived from hemoglobin that is insoluble in water and soluble in alkali, benzene, chloroform, and other organic solvents. Bilirubin ($C_{33}H_{36}N_4O_6$), an open chain tetrapyrrole, has a curling structure, an extended conjugated double bond system, and an active hydrogen atom. The former configuration increases the hydrophobicity of bilirubin and facilitates its access to the inside of lipid molecules, while the latter two characteristics constitute the structural basis of its antioxidant property. The human body possesses a complete bilirubin metabolite pathway, including transportation to the liver for glucuronic acid modification and the secretion of resultant conjugated bilirubin (CB) by the biliary tract. Unconjugated bilirubin (UCB), the form of bilirubin that is not processed in the liver, cannot be excreted from the body. UCB can act as a potent antioxidant, anti-inflammatory, immunomodulatory, metabolic-modulating and antiproliferative activities agent. The *in vivo* UCB concentration is negatively associated with the prevalence of a number of diseases, such as stroke [1,2], peripheral arterial disease [3,4], obesity [5,6], metabolic syndrome [4], type 2 diabetes [6], Crohn's disease [7], and chronic kidney disease (CKD) [8]. In individuals with Gilbert's syndrome (GS), who have congenital nonhemolytic jaundice, elevated UCB appears to prevent cardiovascular mortality [9]. Steadily elevated total bilirubin protects against disorders such as hypertension, complications of diabetes, and chronic obstructive pulmonary illnesses [9,10]. These results provide evidence for the great potential of bilirubin in medical applications.

The medical application of bilirubin has been hampered by two key issues. First, most UCB, which is conveyed through albumin transporters, does not undergo cellular uptake. Second, bilirubin is susceptible to oxidation, which may cause instability *in vivo*. Nanomedicine preparation strategies have developed rapidly in recent years. Fortunately, nanotechnology offers a fresh solution to overcoming the obstacles to the biological application of bilirubin. Specifically, designed bilirubin-derived nanoparticles (BRNPs) can achieve high hydrosolubleity, stability, and efficacy by covalently attaching bilirubin to hydrophilic molecules or loading it in nanocarriers. Moreover, BRNPs can deliver bilirubin to target organs or cells. It can even pass physiological barriers such as the blood–brain barrier [11]. Some BRNPs facilitate the controlled sustained release of bilirubin, prolong the circulation time of bilirubin rather than eliminating it, and improve bilirubin cell penetration.

In recent years, increasing numbers of advanced BNRPs have emerged

* Corresponding author.
** Corresponding author.
E-mail addresses: 7250012693@shsmu.edu.cn (C. Li), yinshankai@china.com (S. Yin).

https://doi.org/10.1016/j.mtbio.2023.100658
Received 1 February 2023; Received in revised form 28 April 2023; Accepted 3 May 2023
Available online 4 May 2023

that have shown promising treatment and diagnosis outcomes in many disease models. In this review, we systematically discuss the antioxidant, anti-inflammatory, immunoregulatory, metabolic-modulating and anti-proliferative properties of bilirubin (and the underlying mechanisms); the structural design principles of BRNPs; and their use in various diseases.

2. Antioxidant property of bilirubin

2.1. Bilirubin possesses antioxidant properties

Bilirubin scavenges oxidizing substances with high efficiency. In homogeneous solution or in liposomes mimicking the cellular environment, bilirubin at micromolar concentrations reacts with lipid peroxyl radicals (LOO·), producing a nonradical product or oxygen [12]. Reactive oxygen species (ROS) such as superoxide anion (O_2^-·) and hydroperoxyl radicals (HO_2^-·) [13] and reactive nitrogen species (RNS) such as peroxynitrite ($ONOO^-$) in the presence of NO and superoxide radical (O_2^-·) also react with bilirubin [14]. In superoxide anion decomposition and peroxyl radical-trapping assays, bilirubin has a lower IC_{50} and competes for more peroxyl radicals in unsaturated fatty acid salt solution than the water-soluble vitamin E analog Trolox, a strong endogenous antioxidant [13].

Based on its scavenging of ROS and RNS, bilirubin has been used to protect cells from oxidative stress. Dore et al. [15] exposed primary hippocampal neurons to H_2O_2 and measured the neuron survival rate to mimic oxidative stress damage. Bilirubin at 25–50 nM increased neuron survival against H_2O_2-induced toxicity, suggesting a neuroprotective effect [15]. Basiglio et al. [16] applied bilirubin in a tert-butylhydroperoxide (tBuOOH)-induced cholestasis model and found that bilirubin abolished ROS production and membrane-lipid oxidation in isolated rat hepatocytes. Bilirubin (17.1 μM) inhibited oxidized glutathione biliary excretion increases and bile flow decreases in isolated perfused rat livers, implying prevention of oxidative stress-induced biliary secretory failure [16].

Stocker et al. reported that the antioxidant activity of bilirubin is considerably enhanced at a 2% oxygen concentration compared to a 20% (indoor air) oxygen concentration [12]. At 2% oxygen, bilirubin inhibits oxidation in liposomes more strongly than a-tocopherol, the best antioxidant for lipid peroxidation [12]. Therefore, the antioxidant activity of bilirubin might be useful in hypoxia-related diseases, such as hypoxic brain injury, respiratory failure, and sleep apnea.

Hyperbilirubinemia exerts a significant protective effect in free radical-producing diseases, such as asphyxia [17], sepsis [17], stroke [1,2], cardiovascular disease [3,18,19], obesity [5] and cancer [20]. In cross-sectional epidemiological studies, patients with free radical-producing diseases exhibited considerably smaller increases in serum bilirubin, suggesting a benefit of excess bilirubin [5,17]. Retrospective cohort studies have shown negative correlations of bilirubin levels with disease prevalence and progression [2,18,19]. However, interventional clinical studies to establish the effect of bilirubin in free radical-related diseases are needed.

2.2. Mechanism underlying the antioxidant activity of bilirubin

The antioxidant activity of bilirubin is linked to its structure, the bilirubin-biliverdin cycle, and intranuclear regulation (Fig. 1). Stocker et al. [12] showed that after bilirubin oxidation, there was a decrease in absorbance at 450 nm and the appearance of a new peak at wavelengths >550 nm. This outcome indicates that bilirubin scavenges ROS either by donating a hydrogen atom attached to the C-10 bridge of the tetrapyrrole molecule (Fig. 1) to form a carbon-centered radical (BR·) with resonance stabilization [12], or by some other path.

The bilirubin-biliverdin cycle strongly amplifies the antioxidant effect of bilirubin [21]. In the bilirubin-biliverdin cycle, bilirubin first reacts with oxyradicals (such as alkylperoxyl radicals 2,2′-azobis hydrochloride (AAPH) [21]) to form biliverdin, which is converted into bilirubin by biliverdin reductase (BVRA) (Fig. 1). This process might explain why 10 nM bilirubin prevents the neurotoxicity elicited by a 10,000-fold higher concentration of hydrogen peroxide [21]. Huang et al. [22] reported that preconditioning with bilirubin and biliverdin protected lens epithelial cells (LECs) against H_2O_2 damage, an effect reversed by knockdown of BVRA.

Finally, bilirubin activates the transcription factor nuclear factor erythroid-derived 2-like 2 (Nrf2) [22,23] (Fig. 1), which induces a number of antioxidant defense mechanisms to reduce oxidative damage and maintain cellular redox homeostasis [24]. Activation of Nrf2 triggers the synthesis of heme oxygenase-1 (HMOX1 or HO-1), leading to the

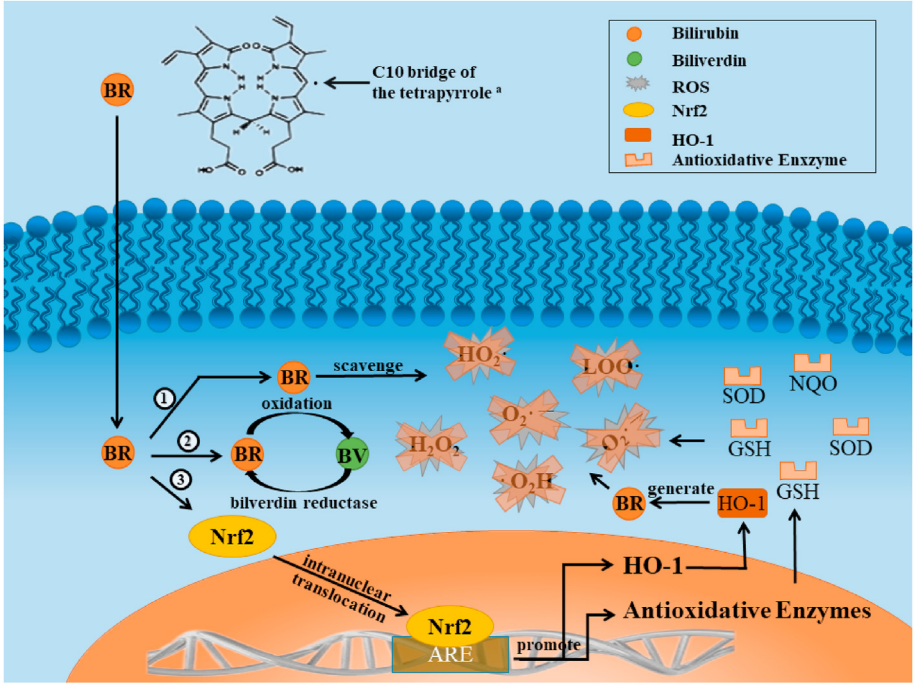

Fig. 1. Mechanism underlying the intracellular antioxidant activity of bilirubin. (1) When bilirubin enters the cytoplasm, the C-10 bridge of its tetrapyrrole structure donates a hydrogen atom to scavenge ROS, including lipid peroxyl radical (LOO·), superoxide anion (O_2^-·), hydrogen peroxide (H_2O_2), and hydroperoxyl radical (HO2-·). [a] represents the structure of a carbon-centered radical BR· (2) Bilirubin is converted into biliverdin by oxidation, which in turn is transformed into bilirubin by biliverdin reductase. The bilirubin-biliverdin cycle greatly amplifies the antioxidant activity of bilirubin. (3) Bilirubin activates the intranuclear translocation of Nrf2 and promotes the expression of heme oxygenase-1 (HO-1) and antioxidants, such as glutathione (GSH), superoxide dismutase (SOD), and NADPH-quinone oxidoreductase (NQO). The bilirubin regenerated by Nrf2/HO-1 and the antioxidant enzymes decreases ROS levels synergistically.

generation of biliverdin and bilirubin [24]. The intranuclear regulation of bilirubin warrants further investigation.

3. Anti-inflammatory and immunomodulatory properties of bilirubin

3.1. Bilirubin inhibits inflammation and modulates immune imbalance

Elevated serum bilirubin levels are beneficial in excessive inflammation and hypernormal immune responses. High serum bilirubin levels have been linked to a lower incidence of inflammatory disorders, such as asthma [25], Crohn's disease [7], and psoriasis [26], as well as other diseases linked to an excessive immune response, such as ischemic cerebral infarction [1,2], cardiovascular diseases [3], and chronic obstructive pulmonary disease [25].

Bilirubin has anti-inflammatory and immunomodulatory properties *in vivo*. For example, after application of 30 mg kg^{-1} bilirubin in a sepsis-induced organ failure model (prepared intraperitoneally (i.p.) by a single dose of 5 mg kg^{-1} lipopolysaccharide (LPS), followed by cecal ligation and puncture (CLP) to promote and worsen subacute infectious peritonitis), the expression levels of pro- and anti-inflammatory cytokines were decreased and increased, respectively [27]. Bilirubin also decreased the activation and activities of T cells, improving tissue injury in sepsis and increasing the survival rate of the two-hit sepsis model [27]. Using the papain-induced acute type 2 airway inflammation mouse model, He et al. [28] showed that bilirubin significantly reduces the level of Group 2 innate lymphoid cells (ILC2s), which are important in allergic airway inflammation. However, the direct application of bilirubin is limited by its hydrophobicity and the use of an alkaline or organic solution to attain the desired concentration of bilirubin could be harmful *in vivo*.

3.2. Mechanisms underlying the anti-inflammatory and immunoregulatory properties of bilirubin

Bilirubin prevents inflammation damage directly by inhibiting the NF-κB pathway of macrophages and promoting apoptosis of effector T cells (Fig. 2). Physiological concentrations of bilirubin (0.1–1.2 mg/dL in serum) inhibit the nuclear factor kappa-B (NF-κB) pathway by restraining the phosphorylation of the inhibitor of NF-κB (IκB-α) [29]. This process blocks IκB-α degradation and the intracytoplasmic release of the NF-κB dimer into the nucleus [30]. Bilirubin thus reduces the expression of NLRP3 inflammasomes, IL-1, TNF-α, and IL-6 by blocking the NF-κB pathway [30], thereby protecting cells from inflammation-induced damage [31]. Additionally, bilirubin induces lymphocyte apoptosis by activating exogenous death receptor apoptotic pathways, such as CD95 and p38-MAPK, and promoting the endogenous mitochondrial apoptotic pathways associated with Bax, caspase-8, and caspase-3 [32,33].

Bilirubin modulates immunity indirectly by inhibiting the migration of monocytes, modulating the expression of immune cell receptors, and impeding the cytotoxic effect of complement (Fig. 2). Vogel et al. [34] found that bilirubin blocks ROS generation in vascular endothelial cells, which is induced by vascular cell adhesion molecule 1 (VCAM-1) or intercellular adhesion molecule 1 (ICAM-1) signaling, thus impeding the disruption of endothelial tight junctions and preventing monocytes from entering the artery intima. By disrupting ICAM-1- and VCAM-1-induced signaling, bilirubin suppresses immune-cell recruitment and the inflammatory response.

Bilirubin modulates macrophage-associated receptor expression, inhibits MHC-II expression in antigen-presenting cells (APCs), and suppresses costimulatory molecule expression in T cells. The bilirubin concentration was positively and independently associated with CD163 expression in macrophages [35], a marker of anti-inflammatory M2 macrophage polarization. Zhao et al. reported that bilirubin derivatives increase macrophage M2 marker (CD206) expression and decrease macrophage M1 marker (CD86) expression in the pancreas [36], suggesting promotion of the conversion of macrophages from the proinflammatory M1 to the anti-inflammatory M2 phenotype.

Bilirubin disrupts the Jak/Stat-1 pathway to suppress MHC-II expression [37], thus reducing the production of IL-12 and IL-23 and suppressing the differentiation of naive $CD4^+$ T cells into inflammatory T helper 17 (Th17) cells [38,39]. Bilirubin also modulates Th17 cells by limiting glycolysis and downregulating glycolysis-related genes (phosphoglycerate-kinase-1 [PGK1], aldolase A [ALDOA]) in Th17 cells, thus exerting an immunosuppressive effect [40] (Fig. 2). In addition to suppressing the activation and functions of T-cells, bilirubin can also promote the accumulation of myeloid-derived suppressor cells, such as T-regulatory (Treg) cells [27] (Fig. 2). In an autoimmune

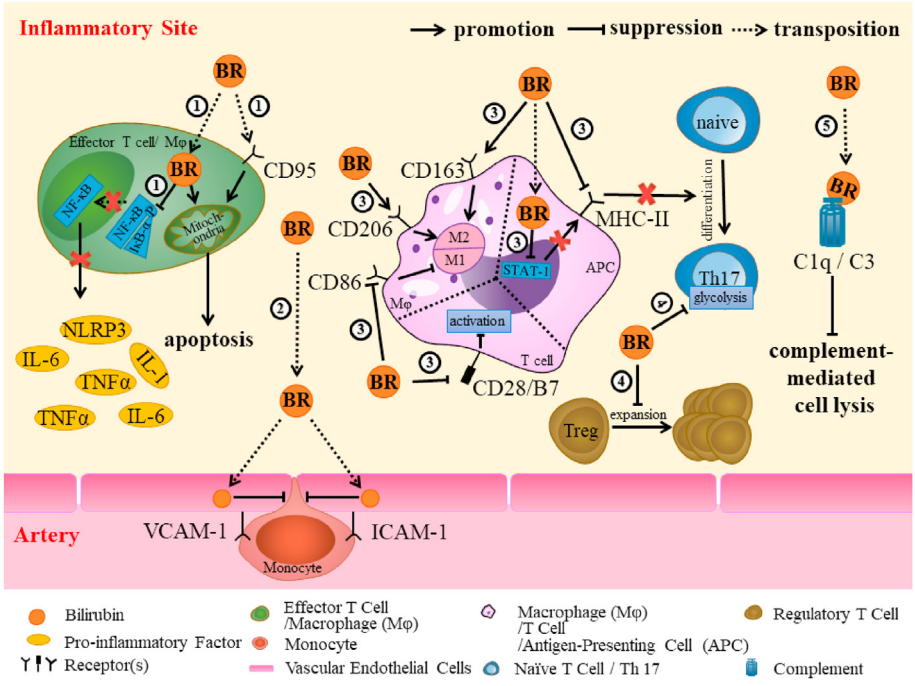

Fig. 2. Mechanisms underlying the anti-inflammatory and immunoregulatory properties of bilirubin: (1) In the inflammatory site, bilirubin suppresses the expression of proinflammatory factors, such as NLRP3 inflammasomes, IL-1, IL-6, and TNF-α, by inhibiting the NF-κB pathway of macrophages (Mφs), and induces the apoptosis of effector T cells by activating exogenous death receptor apoptotic pathways, such as CD95 signaling, and promoting endogenous mitochondrial apoptotic pathways. (2) Bilirubin disrupts ICAM-1- and VCAM-1-induced signaling, thus inhibiting monocyte migration. (3) Bilirubin modulates the expression of subtype-specific receptors (including CD163, CD206, and CD86), MHC-II, and costimulatory molecules (including CD28 and B7) in macrophages (Mφs), antigen-presenting cells (APCs), and T cells, respectively. This process promotes the conversion of macrophages from the proinflammatory M1 to the anti-inflammatory M2 phenotype, hampers the differentiation of Th17 cells, and suppresses the activation of T cells. (4) Bilirubin modulates inflammatory Th17 cells by suppressing glycolysis and promoting the accumulation of myeloid-derived suppressor cells, such as T-regulatory (Treg) cells. (5) Bilirubin suppresses the C1q or C3 stage of complement-mediated cell lysis.

encephalomyelitis (EAE) model, Liu et al. [32] showed that bilirubin reduced the expression of CD28, B7-1, and B7-2 costimulatory molecules in T cells, thus inhibiting T-cell activation and regulating immunity. Additionally, bilirubin interferes with the binding of C1q and C3 to purified immunoglobulin or immune complexes (Fig. 2), thereby inhibiting complement-mediated cell lysis [41].

4. Other physiological properties of bilirubin

4.1. The metabolic effects of bilirubin

Several studies have found a negative correlation between UCB concentration and metabolic disorders, including age-related weight gain, dyslipidemia, insulin resistance and diabetes [5,6,42]. Bilirubin can alleviate insulin resistance by improving the dysregulation of adipocytokine expression in adipose tissues [43]. Moreover, bilirubin has been proven to directly influence metabolic processes, such as glucogenesis, lipogenesis, and energy production, by activating various nuclear and cytoplasmic receptors, including aryl hydrocarbon receptor (AhR), and constitutive androstane receptor (CAR), peroxisome proliferator-activated receptors α and γ (PPARs) [44–46].

4.2. The anti-proliferative property of bilirubin

Bilirubin has been shown to inhibit the proliferation of cancer cells and vascular smooth muscle cells (VSMCs). Activation of AhR by bilirubin has been associated with this effect, which involves the inhibition of phosphorylation of cyclin D1 and retinoblastoma tumor suppressor protein (Rb), thereby leading to the arrest of the cancer cell cycle [47,48]. In addition, studies have reported that bilirubin can impede the cell cycle progression of VSMCs at the G1 phase by suppressing the mitogen-activated protein kinase (MAPK) signaling pathway [49–51]. Moreover, bilirubin has demonstrated its potency as a ligand for the apolipoprotein D (ApoD) molecule [52], which selectively modulates the proliferative response of VSMCs through a mechanism related to nuclear translocation of extracellular signal-regulated kinase (ERK) 1/2 [53]. Therefore, it is reasonable to postulate that bilirubin exerts some of its antiproliferative effects via the ApoD molecule.

5. BRNP construction strategies

Given its beneficial physiological properties and easily metabolizable nature, endogenous bilirubin has great potential for the treatment of diseases associated with oxidative stress, inflammation or excessive

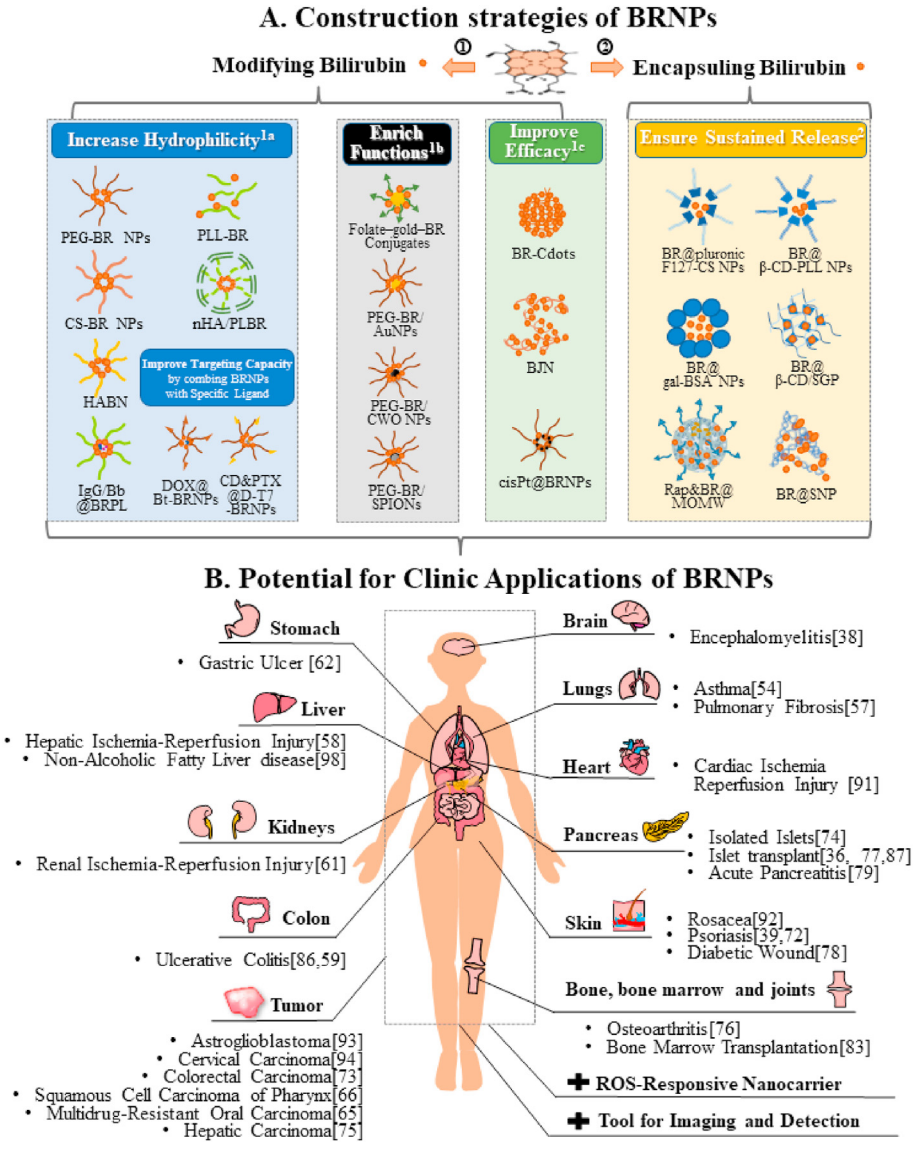

Fig. 3. BRNP construction strategies and potential applications. ***(A) BRNP construction involves modifying and encapsulating bilirubin.*** **(1a)** Bilirubin is modified with hydrophilic substances to increase its hydrophilicity, which is the key obstacle to exploring the protective effects of bilirubin. The combination of bilirubin with different agents (nHA/PLBR, CS-BR NPs, etc.) affects the surface charges, size and spatial configuration, which could alleviate cytotoxicity, promote adhesion, and extend circulation time. In addition, combining bilirubin with specific ligands (DOX@bt-BRNPs, CD&PTX@D-T7-BRNPs etc.) can enhance targeted delivery and facilitate transport across physiological barriers. **(1b)** The use of bilirubin in conjunction with metal nanoparticles offers a promising avenue for the development of effective therapeutic applications and innovative imaging in medicine. FGB nanoconjugates and PEG-BR@CWO NPs have presented a satisfactory anticancer effect in experiments. In addition, bilirubin in PEG-BR/AuNPs and PEG-BR/SPIONs retained enzyme and ROS- responsivity for controlled imaging of biological and pathological processes. **(1c)** The characteristics of bilirubin are amplified through nanotechnology. BR-CDots present satisfactory near-infrared (NIR) imaging and pH biomonitoring. BJN and cisPt@BRNPs improve the therapeutic efficacy of bilirubin. **(2)** Loading bilirubin into nanocarriers benefits its controlled and sustained release. ***(B) BRNPs have been used in disease models and for other applications (bottom).*** BRNPs protect against ROS, excessive inflammation, dysregulated immunity, dysregulated metabolism, and excessive proliferation, suggesting clinical utility. Bilirubin can also be used as an ROS-responsive nanocarrier and imaging tool.

immune responses, metabolic dysregulation, and excessive proliferation. However, the application of bilirubin has been hampered by its low hydrosolubleity and instability. BRNPs have been developed to overcome these issues. Below, we classify BRNPs based on their design principles. Briefly, the construction of BRNPs involves modifying and encapsulating bilirubin (Fig. 3).

5.1. Constructing BRNPs by modifying bilirubin

5.1.1. Covalently binding bilirubin with diverse hydrophilic substances

BRNPs can be generated by covalently binding bilirubin to another hydrophilic molecule. For example, PEGylated bilirubin (PEG-BR) is the monomer of PEG-BR monolayer-based nanoparticles, which are called PEGylated bilirubin micelles [54]. The carboxyl group of bilirubin is relatively active and can be activated by agents such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS) and carbonyldiimidazole (CDI). PEG-BR conjugates are synthesized by coupling the activated carboxyl group of bilirubin with the amines of PEG, called the carboxylammonia condensation reaction. Subsequently, uniform-sized PEGylated bilirubin micelles are formed by dissolving PEG-BR conjugates, evaporating the solvent, hydrating the film layer, and sonicating enough time. The hydrophobic bilirubin terminal aggregates within the closed structure and the hydrophilic PEG terminal forms the outer layer, namely self-assembly. PEGylation is a process whereby the physical and chemical properties of biological molecules are altered to improve drug solubility, decrease immunogenicity, enhance drug stability, and prolong retention time in the blood while reducing proteolysis and renal excretion [55]. In this way, PEGylated bilirubin micelles increase the bilirubin concentration, stability, and bioavailability [56–58].

Bilirubin can be modified by different hydrophilic substances and ligands via crosslinking reactions. In addition to PEG-BR, bilirubin has also been linked to hyaluronic acid (HA-BR) [59], polylysine (PLL-BR) [36,60,61], and chitosan (CS-BR) [62] via the carboxylammonia condensation reaction. These designs of BRNPs provide various therapeutic advantages (Table 1). Firstly, the interaction of HA with CD44—an adhesion molecule overexpressed in inflammation and involved in angiogenesis, lymphocyte activation, and cell migration—promotes bilirubin targeting to inflammation-damaged cells and proinflammatory macrophages [59,61]. Second, the positive surface charge of PLL or CS promotes the adhesion of BRNPs to cells by attracting negatively charged cell membranes. Third, HA coating significantly decreases the albumin absorption ratio of PLL-BR (HA/PLL-BR), leading to extended circulation of BRNPs. The reduction in the albumin affinity of PLL-BR and HA/PLL-BR might due to surface charge reversal (from 19.5 ± 0.4 to -23.9 ± 0.2 mV) [61]. This work offers a new perspective to predict and adjust protein absorption ratios for BRNPs. At the same time, self-assembled BRNPs can be covalently combined with the ligands biotin [63] (for biotin transporter [overexpressed in malignant tumors]) and T7 [64] (for transferrin receptor [overexpressed in glioma cells and the BBB]), enhancing BRNP targeting.

Table 1
Design of BRNPs constructed by modifying bilirubin.

Nanocomponents	Therapeutic Advantages
PEG-BR [38,54]	increases the bilirubin concentration, stability, and bioavailability
BR-HA [59]	targets inflamed tissues
PLL-BR [36,60]/CS-BR [62]	promote adhesions; alleviate cytotoxicity
HA/PLL-BR [61]	targets inflamed tissues, extends circulation time
Folate-Gold-Bilirubin [65]	visualizes tumors; targets tumors
PEG-BR/AuNPs [67]	images CT tissues; possesses GSH-responsibility
PEG-BR/CWO NPs [66]	activate PDT within deep tissues
PEG-BR/SPIONs [68]	Images MRI and near-infrared tissues; possesses ROS-responsibility
BR-CDots [71]	images near-infrared tissues; possesses PH-responsibility and enzyme-responsibility
Bilirubin-JPH203 Hydrogel [72]	suppresses excessive immune response; promotes skin permeation; prolongs the retention time
cisPt@BRNPs [73]	visualizes tumors; promotes PTT

5.1.2. Conjugating bilirubin with metal nanoparticles

The conjugation of bilirubin with metal nanoparticles represents a commonly employed strategy for modifying the functions of BRNPs. Current examples include the folate-gold-bilirubin (FGB) nanoconjugate [65], PEGylated bilirubin-coated CaWO4 nanoparticles (PEG-BR/CWO NPs) [66], PEG-BR-coated gold nanoparticles (PEG-BR/AuNPs) [67], and PEGylated bilirubin-coated iron superparamagnetic oxide nanoparticles (PEG-BR/SPIONs) [68] (Table 1). Bilirubin-conjugated-metal nanoparticles show increased stability in saline environments across a wider range of pH levels, and improved biocompatibility compared to simple metal nanoparticles [46,47], resulting from the surface binding of bilirubin to metallic ions. The conjugation of bilirubin with metal nanoparticles is has furthermore been shown to expand therapeutic potential [65,66] and augment diagnostic functionality [67,68].

Folate-gold-bilirubin visualizes tumors while the modification of folate improves the specificity of the antitumor effect of bilirubin by targeting folate receptor (FR)-positive tumors [65]. Pizzuti et al. [66] utilized bilirubin as a photosensitive agent in combination with X-ray-activated CaWO4 (CWO) to form PEG-BR/CWO NPs. Their hypothesis was that exposure of CWO cores to X-rays would generate ultraviolet (UV) and visible light, which would then excite bilirubin and prompt the generation of $^1O_2$ [69]. Therapy utilizing photosensitizers to induce ROS production to kill cancer cells in target tissue by activation of X-rays and specific light exposure is called radiation-induced photodynamic therapy (RT-PDT). Dynamic light scattering (DLS) and gel permeation chromatography (GPC) indicated that both UV and X-rays caused an increase in NP size and the presence of secondary peaks of polymer residues, suggesting that PEG-BR degraded back into its PEG and BR precursors and that bare CWO nanoparticles agglomerated. PEG-BR/CWO nanoparticles showed higher levels of $^1O_2$ production when exposed to X-rays compared to PBS and CWO NPs, suggesting the efficiency and necessity of BR for photodynamic $^1O_2$ formation. PEG-BR/CWO NPs exhibited a great therapeutic effect in a murine cancer model, suggesting bilirubin as a photosensitive agent for tumor therapy. However, further investigation is needed to compare its role in PDT with other photosensitizers. Based on this research, bilirubin conjugated with metal nanoparticles could be a promising treatment option for advanced cancer therapy.

PEG-modified gold nanoparticles (PEG-S/AuNPs) cannot detect glutathione (GSH), an indicator of healthy hepatocytes and hepatic detoxification function [70], so they cannot differentiate between normal and diseased liver tissue. Yoo et al. [67] utilized BR-PEG/AuNPs, which possess coordination bonds that are weaker than the S–Au bonds of GSH, to trigger degradation of the PEG-BR shell and subsequent aggregation of residual AuNP cores in response to elevated GSH levels. The resulting BRNPs enhance CT signals in the normal liver with no enhancement in GSH-deficient livers, allowing for identification of various hepatic function-related disorders and liver-metastasized malignancies. Lee et al. [68] explored the use of bilirubin-based nanoparticles in combination with superparamagnetic iron oxide nanoparticles (SPIONs) for the direct detection of reactive oxygen species (ROS) in whole blood samples. The PEGylated bilirubin (PEG-BR) shell of PEG-BR/SPIONs undergoes oxidation and peels off the SPION core, leading to SPION aggregation and a notable signal change in T2 magnetic resonance imaging (MRI) in response to ROS. Additionally, upon loading the hydrophobic dye cypate within PEG-BR/SPIONs, the near-infrared (NIR) dye is released from the nanoparticle shell after reaction with ROS, resulting in a 'turn-on' fluorescence signal [68]. In light of the findings, it could be concluded that the conjugation of bilirubin with metal nanoparticles might have promising applications in the fields of imaging and biomonitoring.

5.1.3. Additional strategies of bilirubin modification

Other methods can be used to modify bilirubin and design nanostructures, such as BR-CDots, bilirubin-derived nanoconjugates and cisplatin-chelating bilirubin nanoparticles. Fathi et al. [71] synthesized bilirubin-derived carbon dots (BR-CDots) through solvothermal synthesis. The resulting TEM images showed lattice fringes corresponding to accepted d-spacing values, indicating a graphitic core structure. The C 1s peak from X-ray photoelectron spectroscopy (XPS) analysis revealed the presence of surface functional groups, such as C–C/C═C, C–N/C–O, and COOH configurations. BR-CDots could utilize the surface-state emission of the surface functional groups to detect changes in pH that distinguish between cancerous and healthy cells. The emitted fluorescence from both the carbon core and surface functional groups of BR-CDots might create a noninvasive means for imaging biological structures in living organisms.

By nanoprecipitating, Jiang et al. [72] combined bilirubin with an inhibitor (JPH203) of mammalian target of rapamycin (mTOR) activation in the expansion of Th17 cells. Bilirubin-JPH203—integrated by two stable hydrogen bonds—combines two drugs into one to enhance therapeutic efficacy, while bilirubin-JPH203 is encapsulated in the hydrogel nanoplatform to promote drug permeation into the skin and prolong the retention time [72]. Moreover, self-assembled PEG-BR conjugates can chelate cisplatin (cisplatin-chelating bilirubin nanoparticles, cisPt@BRNPs) by coordinating the PtII center with the lone-pair electrons of bilirubin. However, the strong bond of bilirubin and PtII hinders the sufficient release of cisplatin. The BRNP finally achieved anticancer effectiveness as well as tumor visibility by combining photothermal therapy (PTT, section 6.4) of cancers with photoacoustic imaging (PAI, section 6.9.2).

5.2. Constructing BRNPs by encapsulating bilirubin in nanocarriers

Bilirubin can be loaded into nanocarriers, which increase the bilirubin concentration, and facilitate controlled bilirubin release. Pluronic F127 and chitosan, which are amphiphilic and self-assemble into micelles, are used to nanoencapsulate bilirubin [74]. Although the encapsulation efficiency of pluronic F127-chitosan nanoparticles was 10.1 ± 0.2%, nanoencapsulated bilirubin improved the survivability and functionality of isolated murine islets after exposure to hypoxic stress in comparison to unencapsulated bilirubin [74]. Bovine serum albumin (BSA) has several binding sites for bilirubin and can be further modified by galactose as a targeting agent. Yang et al. [75] developed a dual functional galactose-BSA (gal-BSA) nanoparticle that not only transported hydrophobic bilirubin to treat tumors, but also utilized the affinity of BSA for bilirubin to adsorb bilirubin around tumors and reduce its accumulation in the surrounding tissue. Gal-BSA-NPs are a stable carrier that remain a homogeneous particle size after 1000-fold dilution and for two weeks. Its glutathione-sensitive S–S bond also facilitates targeted treatment for liver cancer. Xue et al. [76] designed mutifunctional MOMW nanocarriers in which rapamycin (Rap) and bilirubin are carried by the mesopores and shell, respectively, in an anterior cruciate ligament transection (ACLT) rat model. MOMW is composed of a decorated mesoporous polydopamine (MO) -metal organic framework (M) modified with WYRGRL (W), a collagen II-targeting peptide, respectively enabling NIR-responsive controlled bilirubin release, MR/IVIS imaging, and targeting of collagen [76]. Although the encapsulation efficiency of MOWM bilirubin is 8.9%, it shows a relatively sustained and temperature-controlled bilirubin release (in the first 24 h–38.74% [45 °C] vs. 8.01% [37 °C]; in the first 72 h–46.38% [45 °C] vs. 11.38% [37 °C]), and it preferentially targets chondrocytes and cartilage tissues. Similarly, self-assembled PLCD (β-CD- PLL) nanoparticles [77], β -CD/bioadhesive hydrogel matrix (thiolated γ-polyglutamic acid based-poloxamer hydrogel, SGP) nanoparticles [78], and silk fibrin nanoparticles (SNPs) [79] can carry bilirubin, increasing its solubility and enabling stable and sustained release. Currently, bilirubin-loaded nanocarriers are limited, requiring further research to improve their design and encapsulation efficiency. Similarly, controlled release and targeted delivery of bilirubin using nanocarriers require further exploration. Improvements in bilirubin delivery systems could enhance disease treatment efficacy and overcome the limitations of conventional therapies.

To conclude, nanomedical approaches could offer a promising avenue for facilitating the biological application of multifunctional bilirubin by enhancing its hydrosolubleity, increasing its stability, targeting damaged tissues, passing through physiological barriers, and ensuring controlled or sustained release. Currently, clinical studies have proven the safety of the parenteral administration of bilirubin in an albumin-bound formulation [80]. Bilirubin pharmacokinetics were described with a distribution volume of 9.9 (±2.0) L and a total plasma clearance rate of 36 (±16) mL min^{-1} [80]. Compared with albumin-bound bilirubin, BRNPs have great potential to further elevate the circulation concentration and prolong the circulation time of bilirubin in clinical settings based on the multiple application advantages of nanomedical approaches. To modify bilirubin, versatile options for binding molecules provide a broad range of applications for bilirubin. However, it is necessary to undertake further design efforts to ensure the stability of bilirubin and achieve its efficient delivery. For loading bilirubin in nanocarriers, the encapsulation of bilirubin enhances its stability by providing added protection against oxidation, light exposure, and enzymatic degradation. The ample surface area of nanocarriers also allows for flexible modification design options to target specific receptors or cell types [81]. Nevertheless, it is important to note that nanocarriers can induce additional toxicity and immunogenicity, so they require careful attention [82].

6. *In vivo* applications of BRNPs in multiple pathological models

BRNPs have been applied in diseases linked to oxidative stress, excessive inflammation, abnormal autoimmune responses, metabolic dysregulation or excessive proliferation (Fig. 3). BRNPs accumulate at the target site and scavenge ROS, inhibit proinflammatory cytokines, suppress the maturation of $CD4^+$ T cells, ameliorate metabolic disorders, or halt the uncontrolled growth of cancer cells.

Compared with traditional antioxidant nonnanodrugs, BRNPs have four advantages: (1) bilirubin has strong antioxidant and ROS scavenging abilities, as well as anti-inflammatory, immunomodulatory, metabolic-modulating, and antiproliferative properties; (2) endogenous bilirubin possesses greater biocompatibility than other exogenous agents; (3) BRNPs prefer to aggregate in damaged organs that are rich in ROS or immune cells [59,83], reducing the likelihood of off-target effects; and (4) BRNPs can carry multiple drugs [72,76], facilitate imaging [68,76], and enable sustained release [77,78]. To better describe the current applications of BRNPs, we divided them based on their pathological types and special application scenarios (Table 2).

6.1. Ischemia–reperfusion injury

Ischemia–reperfusion injury (IRI) is associated with oxidative stress and inflammation. Recanalization of an ischemic artery causes intracellular overproduction of ROS, release of inflammatory factors, and inflammatory cell attack, thereby damaging cells and causing organ dysfunction. IRI is a significant issue in individuals with coronary artery disease, hepatic transplant or resection, and acute kidney injury.

BRNPs have been applied in cardiac, hepatic, and renal IRI models, inducing temporary ischemia through blockage of arteries. For cardiac IRI, bilirubin (10 mg kg^{-1}; via intraperitoneal route (i.p.); 1 h before occlusion) was found to decrease the rat coronary infarct area [84]. Bilirubin ditaurate (50 μM), a synthetic water-soluble analog of bilirubin, improved postischemia functional results and ameliorated myocardial oxidative damage *ex vivo* [85]. In recent years, nanotechnology has been increasingly combined to explore the physiological role of bilirubin.

The PEGylated bilirubin micelles are a common type of BRNP for increasing the water solubility of bilirubin. Their average diameter is approximately 80–100 nm, and their zeta potentials (ZPs) are

Table 2
Applications of BRNPs.

Construction strategy of bilirubin-derived nanoparticles	Dose and mode of administration	Disease model (additional function)
PEGylated bilirubin micelles	*Dose*: 10 and 30 mg kg^{-1} *Mode*: intraperitoneal injection at 5 min before and at 24 h after reperfusion.	Cardiac IRI [91]
PEGylated bilirubin micelles	*Dose*: 10 mg kg^{-1} *Mode*: intravenous injection 30 min before model induction	Hepatic IRI [58]
nHA/PLBR	*Dose*: 5 mg kg^{-1} *Mode*: intravenous injection once after model establishment	Renal IRI [61]
Bilirubin@pluronic127-chitosan nanoparticle	*Dose*: 0, 5, 10, or 20 mM *Mode*: incubated isolated murine islets exposed to hypoxic stress with bilirubin or BRNPs	Isolated Islets [74]
PLL-BR conjugate	*Dose*: 20 μM *Mode*: incubated islets with bilirubin or PLL-BR for 24 h before islet transplantation	Islet transplant [36]
Bilirubin@PLCD	*Dose*: equivalent to 20 μM bilirubin *Mode*: transplanted into the body along with the islets	Islet transplant [77]
PEGylated bilirubin micelles	*Dose*: 10 mg kg^{-1} *Mode*: intraperitoneal injection at days −1, 0, and 1 of transplantation and then once every two days	Islet transplant [87]
PEGylated bilirubin micelles	*Dose*: 10 mg kg^{-1} *Mode*: 5 daily intravenous injections after transplantation	Bone marrow transplantation [83]
CS-BR nanoparticles	*Dose*: 10 mg kg^{-1} *Mode*: 8 daily oral administrations before model induction	Gastric Ulcers [62]
PEGylated bilirubin micelles	*Dose*: 125 mg kg^{-1} *Mode*: intravenous injection at 1 day after establishment of murine model	Ulcerative Colitis [86]
Hyaluronic Acid-Bilirubin Nanoparticles	*Dose*: 30 mg kg^{-1} of HABN (10 kDa, 100 kDa or 700 kDa) *Mode*: 7 daily oral administrations after model establishment	Ulcerative Colitis [59]
Pegylated Bilirubin Micelles	*Dose*: 2, 10, 50 mg kg^{-1} *Mode*: intravenous injections at days 12, 14, and 16	Asthma [54]
RAP&BR@MOMW	*Dose*: 50 μg mL^{-1} BRNPs 20 μL *Mode*: intraarticular injection every four days for six weeks after establishment of rat model	Osteoarthritis [76]
BR@SNPS	*Dose*: 1 mg mL^{-1}, volume unclear *Mode*: intravenous injection twice at 4 h and 10 h after the establishment of murine model	Acute Pancreatitis [79]
PEGylated Bilirubin Micelles	*Dose*: 20, 40, 60 mg kg^{-1} *Mode*: an intravenous injection 2 h before each inductive injection of LL-37 4 times	Rosacea [92]
Gox&TPZ@HMBRN	*Dose*: 200 μL of 7.5 mg mL^{-1} *Mode*: an intravenous injection every other day 3 times	HUVEC, 293 T, U87MG, and MDA-MB-231 cells, and U87MG (astroglioblastoma cells) tumor -bearing mice [93]
TH-302@BR-Chitosan NPs	*Dose*: 25 mg kg^{-1} (equivalent to 3.75 mg kg^{-1} TH-302) *Mode*: an intravenous injection, time unclear	HeLa cells, and HeLa (cervical carcinoma cells) tumor-bearing mice [94]
cisPt@BRNPS	*Dose*: equivalent to 15 mg kg^{-1} PEG-BR, 1 mg kg^{-1} cisplatin *Mode*: an intravenous injection, time unclear	HT-29 cells and HT-29 (colorectal carcinoma cells) xenograft tumor model [73]
PEG-BR/CWO NPs	*Dose*: 10 mg mL^{-1} *Mode*: two intra-tumoral injections on days 6 and 7 after HN31 inoculation	HN31 cells, HN31 (squamous cell carcinoma of the pharynx cells) xenograft murine [66]
Folate–gold–bilirubin (FGB) nanoconjugate	*Dose*: 0.5 mg kg^{-1} *Mode*: Intraperitoneal injection every other day	P-expressing KB-Ch(R)-8-5 cells, and KB-Ch(R)-8-5 (multidrug-resistant oral carcinoma cells) xenograft mouse [65]
BR@gal-BSA-NPs	*Dose*: equivalent to 20 mg kg^{-1} BR *Mode*: an intravenous injection every three days 6 times	HepG2 cells, and HepG2 (hepatic carcinoma cells) tumor-bearing mice [75]
PEGylated Bilirubin Micelles	*Dose*: 50 mg mL^{-1} (equivalent to 11.6 mg bilirubin kg^{-1}) *Mode*: 5 daily intravenous injections beginning on day 3 postimmunization.	Autoimmune Encephalitis [38]
PEGylated Bilirubin Micelles	*Dose*: 0.1, 0.5, 2.5 mg mL^{-1} *Mode*: twice topical administrations for 6 consecutive days	Psoriasis [39]
Bilirubin-JPH203 Nanoparticles	*Dose*: 1 mM (bilirubin in hydrogel) *Mode*: 6 daily topical administrations	Psoriasis [72]
PEGylated Bilirubin Micelles	*Dose*: 5, 20, 40 mg kg^{-1} *Mode*: an intravenous injection every other day for 14 days	Pulmonary Fibrosis [57]
PEGylated Bilirubin Micelles	*Dose*: 30 mg kg^{-1} *Mode*: an intraperitoneal injection every other day for 4 weeks	Non-Alcoholic Fatty Liver Disease [98]
Bilirubin@β-CD/SGP	*Dose*: equivalent to 1 mg BR mL^{-1} *Mode*: topical administration post-wound surgery	Diabetic wound [78]
DOX@bt-BRNPs	*Dose*: equivalent to 4 mg kg^{-1} DOX. 40 mg kg^{-1} BRNPs, 10% mole percentage of bt-PEG-BR *Mode*: an intravenous injection every 3 days for 5 times since day 0 of the model establishment	Tumor (ROS-responsive nanocarrier) [63]
CD&PTX@D-T7-BRNPs	*Dose*: equivalent to 3.6 mg kg^{-1} CD, 1.7 mg kg^{-1} PTX *Mode*: an intravenous injection every other day 6 times beginning on day 10 of the induction of the model	Tumor (ROS-responsive nanocarrier) [64]
ACUPA-SN38@BRNPs		Tumor (ROS-responsive nanocarrier) [100]

(*continued on next page*)

Table 2 (*continued*)

Construction strategy of bilirubin-derived nanoparticles	Dose and mode of administration	Disease model (additional function)
	Dose equivalent to 5 mg kg^{-1} SN38 *Mode*: an intravenous injection every 3 days 5 times beginning on day 0 of the model establishment	
DOX@BRNPs	Dose:20 mg kg^{-1}, (equivalent to 2 mg kg^{-1} DOX) *Mode*: an intravenous injection every 3 days for 5 times beginning on day 0 of the model establishment	Tumor (ROS-responsive nanocarrier) [101]
PTX & IND @ PEG-BR/ce6-FFVLK NPs@ macrophage membrane	*Dose*: unclear *Mode*: an intravenous injections every 4 days 3 times beginning on day 7 of in situ tumor plantation	Tumor (ROS-responsive nanocarrier) [102]
Ce6&Dc@morpholine-BNPs	*Dose*: equivalent to 3 mg kg^{-1} Ce6, 10.5 mg kg^{-1} Dc *Mode*: an intravenous injection every 3 days for 4 times after 8 days of the induction of the model	Tumor (ROS-responsive nanocarrier) [103]
Losartan@CS-BR NPs	*Dose*: 33.3 mg kg^{-1} (140 μg losartan and 0.25 μg BR per mice, respectively) *Mode*: an intravenous injection every 3 days 3 times at 12 weeks after the induction of the model	Hepatic Fibrosis (ROS-responsive nanocarrier) [104]
CLT@BRNP	*Dose*: equivalent to 1 mg kg^{-1} CLT *Mode*: an intravenous injection every other day 5 times beginning on day 14 of the induction of the model	Rheumatoid Arthritis (ROS-responsive nanocarrier) [105]
IgG/Bb@BRPL	*Dose*: 100 μL; equivalent to 120 μM Bb *Mode*: 6 intra-articular injection on day 35, 40, 45, 50, 55, and 60	Osteoarthritis (ROS-responsive nanocarrier) [60]
BA@HABN	*Dose*: equivalent to 100 μg kg^{-1} BA *Mode*: an intravenous injection 6 h after the model establishment	Acute Kidney Injury (ROS-responsive nanocarrier) [99]
PEG-BR/AuNPs	*Dose*: 100 mg kg^{-1} *Mode*: 1 h after intravenous injection for CT images	Liver Diseases (CT Imaging) [67]
PEG-BR/SPIONs	*Dose*: 200 μL; equivalent to 1.5 mM Fe *Mode*:12 h after incubation of blood sample for T2 relaxation time; 6 h after intraperitoneal injection for *ex vivo* fluorescence images	Sepsis (MRI imaging) [68]
BR-CDots	*Dose*: 100 μL; unclear concentration *Mode*: a subcutaneous injection	Near-Infrared Fluorescence Imaging [71]

approximately −30 mV [54,86,87]. The former parameter indicates that PEGylated bilirubin micelles are likely to enter cells through clathrin-mediated endocytosis [88], while the latter parameter suggests a sufficient suitable force for PEGylated bilirubin micelles to avoid aggregation and attain physical stability [89,90]. The pharmacokinetic profile of PEGylated bilirubin micelles was assessed in mice at a dosage of 50 mg kg^{-1} via either intravenous (i.v.) or intraperitoneal (i.p.) routes, while administration of UCB at a dosage of 11.6 mg kg^{-1} via the i. p. Route was assessed [54]. BRNPs displayed a longer half-life (9.39 h, 4.18 h, 5.07 h), lower clearance (0.790 mL h^{-1}, 0.596 mL h^{-1}, 5.80 mL h^{-1}), and a larger area under the concentration-time curve (553 (mg mL^{-1}) h, 364 (mg mL^{-1}) h, 39 (mg mL^{-1}) h) than UCB. In another study, intravenous injection of 150 mg kg^{-1} PEGylated bilirubin was found to induce rare behavior changes and little weight loss, as well as unchanged blood test parameters and no histological evidence of damage to major organs [86], indicating little apparent acute toxicity in rodents. These findings offer valuable insight into the safety and efficacy of PEGylated bilirubin micelle treatments for various medical conditions.

In a cardiac IRI animal model, PEGylated bilirubin micelles (10 and 30 mg kg^{-1}; i. p.) both pre- and postreperfusion decreased the levels of ROS and proinflammatory factors, cardiac apoptosis, and myocardial infarct size [91]. Unfortunately, BRNPs were not compared with bilirubin alone in terms of targeting, slow release, and therapeutic efficacy. In a hepatic IRI model, PEGylated bilirubin micelles (10 mg kg^{-1}; i. v.) exerted a significant protective effect against hepatocellular injury by lowering oxidative stress, the generation of proinflammatory cytokines, and neutrophil recruitment [58]. For renal IRI, HA-coated ε-polylysine-bilirubin nanoparticles (nHA/PLBR) (5 mg kg^{-1}; i. v.), which target CD44-overexpressing damaged kidney tissues, accumulated in the injured kidney. They exhibited better stability and biocompatibility, as well as greater antioxidant and anti-apoptotic activities than either ε-polylysine-bilirubin (PLBR) nanoparticles or bilirubin [61]. The average diameter of nHA/PLBR is 226.9 ± 4.5 nm, which indicates that its uptake probably depends on clathrin-mediated endocytosis [88]. The ZP is −23.9 ± 0.2 mV, suggesting a relatively stable status in suspension [89].

6.2. Organ transplant

Organ transplantation is the final curative method for patients with organ failure. However, organ IRI, host-versus-graft reaction (HVGR), and graft-versus-host disease (GVHD) hamper organ transplantation.

BRNPs have been applied locally to isolated islets and systematically injected into experimental animals to scavenge ROS in IRI and inhibit excessive immune responses in HVGR. Encapsulation of isolated murine islets using either 5–20 μM BR@pluronic127-chitosan nanoparticles [74] or 20 μM PLL-BR conjugate [36] was found to exert cytoprotective effects against oxidative and inflammatory damage *ex vivo*. For BR@pluronic127-chitosan nanoparticle, the average diameter is 27.1 ± 1.4 nm, indicating that they are likely endocytosed in a clathrin/caveolin independent manner. The *in vitro* release test shows an initial burst of release in 8 h and a steadier release up to 48 h, suggesting that the nanocarrier could ensure a sustained release *in vivo*. For the PLL-BR conjugate, the grafting ratio reaches 61.36% ± 2.45%, indicating that bilirubin accounts for approximately 61.3% of the PLL-BR mass. The relative solubility of PLL-BR is up to 18 times that of free BR at a pH of 7.4, proving the improvement of hydrophobicity through modification of bilirubin. BR@PLCD nanoparticles (the amount of bilirubin loaded in BRNPs is 20 μM; delivered via incubation of the transplanted islets) significantly increased the stable blood glucose time and accelerated glucose clearance compared to free BR in diabetic mice [77]. Similarly, the relative solubility of BR@PLCD is approximately 17 times that of free BR at a pH of 7.4. In addition, PEGylated bilirubin micelles (10 mg kg^{-1}, i. p.) also promoted bilirubin accumulation in islet grafts. Systemic administration of PEGylated bilirubin micelles increased the survival rate of islet transplantation to a level greater than that of free bilirubin at the same dose [87].

BRNPs have also been used for bone marrow transplantation to

ameliorate GVHD by reducing systemic and local inflammation [83]. PEGylated bilirubin micelles (10 mg kg^{-1}; i. v.; one time daily for 5 days (qd. X 5)) for treatment after murine bone marrow transplantation accumulated in GVHD organs and enhanced overall survival, reduced circulating inflammatory cytokine levels, and decreased T cell activation but maintained T cell tolerance [83].

6.3. Inflammatory diseases

BRNPs have been applied in inflammatory disease models including gastric ulcer, colitis, asthma, osteoarthritis, acute pancreatitis and rosacea to alleviate excessive inflammation and oxidative stress [30,54,59,62,76,79,86]. In the acute ethanol-induced gastric ulcer model, chitosan-bilirubin nanoparticles (nCS-BR) (10 mg kg^{-1}; via the oral route (or.); qd. X 7) accumulated in the stomach and exerted powerful antioxidant and anti-inflammatory effects [62]. The grafting ratio of nCS-BR reached 61.36% ± 2.45%.

PEGylated bilirubin micelles (125 mg kg^{-1}; i. v.) in a dextran sodium sulfate (DSS)-induced mouse model of inflammatory bowel disease preferentially accumulated in the ulcerative colon and significantly inhibited disease [86]. Moreover, hyaluronic acid-bilirubin (HA-BR) micelles (30 mg kg^{-1}; or.; qd. X 8) made full use of the interaction of HA with CD44 to target the inflamed colon [59]. Hyaluronic acid-bilirubin nanomedicine (HABN) accumulates in the inflamed colonic epithelium, regulates innate immune responses, suppresses the levels of proinflammatory cytokines, promotes body weight recovery, and inhibits the decrease of colon length [59]. The anti-inflammatory effect of HABN was significantly superior to PBS, HA, HACN (hyaluronic acid-cholesterol nanoparticles), PEG-BN, and HA + BR [59], suggesting that bilirubin treatment could have therapeutic potential in ulcerative colitis. The grafting ratio of HABN is about 4 molecules of bilirubin per 100-kDa HA molecule, suggesting a small mass percentage of bilirubin.

Kim et al. [54] showed that PEGylated bilirubin micelles (20 and 50 mg kg^{-1}; i. v.; 3 times) ameliorated Th2-mediated lung inflammation by reducing antibody-stimulated cytokine secretion of $CD4^+$ T cells and decreasing Th2 populations, thus suppressing symptoms of experimental allergic asthma. For osteoarthritis, Rap&Bilirubin@MOMW (50 μg mL^{-1} BRNPs 20 μL; intra-articular (i.a.); every four days for six weeks) codelivered rapamycin (Rap; autophagy) and bilirubin. In the anterior cruciate ligament transection (ACLT) rat model, Rap&Bilirubin@MOMW delayed cartilage degeneration by scavenging ROS, promoting autophagy, and inhibiting the NF-κB pathway [76]. In acute pancreatitis (AP), bilirubin@silk fibrin nanoparticles (BR@SNPs) (1 mg mL^{-1}; i. v.; 2 times) exerted a significant *anti*-AP therapeutic effect by reducing oxidative stress, downregulating the expression of proinflammatory cytokines, suppressing neutrophil and macrophage migration, inhibiting the NF-κB pathway, and activating the Nrf2/HO-1 pathway [79]. The pancreatic enzyme-degradable property and the package protection property of SNP facilitate precise drug delivery and decrease the albumin adsorption to 10%, extending the circulation of BRNPs. The encapsulation efficiency of BR@SNPs reached 82% ± 2.4%, presenting high efficiency to deliver bilirubin. For rosacea, PEGylated bilirubin (20, 40 and 60 mg kg^{-1}; i. v.; 4 times) micelles preferentially localized at inflammatory skin lesions in a LL-37-induced rosacea mouse model. BRNPs alleviate inflammation and angiogenesis by decreasing ROS levels, suppressing vascular endothelial growth factor (VEGF) expression and reducing the expression of proinflammatory cytokines and chemokines [92].

6.4. Tumors

Bilirubin at high concentrations is supposed to defend against cancer by inhibiting the excessive proliferation of cancer cells [45,46] (section 4.2). Rathinaraj et al. [65] designed a folate–gold–bilirubin (FGB) nanoconjugate (0.5 mg kg^{-1}; i. p.; every other day (qod)), which suppressed the growth of drug-resistant tumor cells and oral carcinoma xenografts in mice. Yang et al. [75] reported that BR-loaded gal-BSA-NPs (equivalent to 20 mg kg^{-1} BR; i. v.; every 3 days for 6 times (q3d x 6)) released bilirubin continuously and exerted strong antitumor effects *in vitro* and *in vivo* [75].

In addition, bilirubin was employed to augment the potency of chemotherapy drugs, such as glucose oxidase (Gox), tirapazamine (TPZ) and TH-302 [93,94]. Glucose oxidase (Gox) can deplete glucose, block the energy supply, and cause accumulation of H_2O2, suppressing tumor growth and even killing cancer cells [95]. However, Gox-mediated chemotherapy also causes oxidative damage to normal tissues. Shan et al. [93] converted bilirubin into organosilica-based hollow mesoporous bilirubin nanocarriers (HMBRNs), which were loaded with Gox and tirapazamine (TPZ, a bio-reductive prodrug). The studies *ex vivo* confirmed that the antioxidant activities of bilirubin protected normal cells from oxidative damage induced by Gox. The removal of H_2O_2 by bilirubin also enhanced the effects of Gox on glucose decomposition in cancer cells. Gox&TPZ@HMBRN (7.5 mg mL^{-1} BRNPs 200 μL; i. v.) thus exhibited decreased systemic toxicity and increased antitumor effects in the chemotherapy [93]. TH-302 is a hypoxia-activated prodrug (HAP) that completes its nontoxic-to-toxic transformation specifically in hypoxic tumors [96]. However, HAP-based chemotherapy cannot work in normoxic tumor regions. Chen et al. [94] designed TH-302@BR-Chitosan, which utilizes the antioxidant property of bilirubin to deplete oxygen in well-perfused peripheral tumor regions and to enhance chemical susceptibility for TH-302.

BRNPs are also used in photothermal therapy (PTT) [73,94] and radiation-induced photodynamic therapy (RT-PDT) [66]. Chen et al. [94] discovered that TH-302@BR-Chitosan (25 mg kg^{-1}; i. v.) with irradiation at a wavelength of 680 nm induced a quick increase to ~58 °C at the tumor regions of mice, exhibiting an optimal inhibitory impact on tumor development and recurrence. Similarly, in response to laser irradiation at 808 nm (1 W cm^{-2}), cisPt@BRNPs produced a significant amount of heat, which could increase the solution's temperature by more than 60 °C under 3 min. In the HT-29 (human colorectal cancer) xenograft tumor model, cisPt@BRNPs (equivalent to 15 mg mL^{-1} PEG-BR; i. p.) with subsequent laser irradiation contributed to near-complete tumor regression, while cisPt@BRNPs without irradiation failed to produce appreciable antitumor effects [94]. Despite the impressive antitumor effects, the underlying mechanisms of PTT for BRNPs remain to be elucidated. Pizzuti et al. [66] combined bilirubin with X-ray-activated and PDT-inducible CWO (in section 4.1), and PEG-BR/CWO NPs (10 mg mL^{-1}; intratumoral (i.t.); 2 times) exhibited a greater therapeutic effect than X-ray therapy in a murine xenograft model of head and neck cancer. Compared to CWO NPs, PEG-BR/CWO NPs efficiently produce 1O2 during X-ray irradiation and lead to enhanced necrosis and mitotic arrest within treated tumors. Combined radio/photodynamic therapy (RT-PDT) overcame the limited tissue penetration of light in PDT, enabling treatment for cancers of the deep organs.

6.5. $CD4^+$ T helper (Th) cell-mediated autoimmune diseases

Encephalomyelitis (EAE) and psoriasis are characterized by $CD4^+$ T helper (Th) cell-mediated autoimmune infiltration in the central nervous system and the dermis. Kim et al. [38] found that multiple intravenous injections of PEGylated bilirubin micelles (50 mg mL^{-1}; i. v.; 5 times) significantly delayed the onset of EAE and suppressed its incidence, severity, and progression without causing systemic immunosuppression. APC maturation is inhibited by scavenging ROS in dendritic cells (DCs) and macrophages (Møs) and negatively regulating the differentiation of naive $CD4^+$ T cells into Th17 cells [38].

Keum et al. [39] showed that topical administration of PEGylated bilirubin micelles (0.1, 0.5 and 2.5 mg mL^{-1}; via the topical route; two times per day for 12 times (bid. X 12)) attenuated the upregulation of ROS levels in keratinocytes, suppressed the secretion of inflammatory cytokines, the recruitment of immune cells, and the differentiation of naive $CD4^+$ T cells into Th17 cells, ameliorating the symptoms of psoriasis. Moreover, by integrating bilirubin with JPH203, an inhibitor of

mTOR activation, BJN (equivalent to 1 mM bilirubin; via topical route; qd. X 6) scavenged ROS, accumulated in irritated keratinocytes, suppressed Th17 differentiation, and reduced IL-17 A secretion [72]. Overall, BRNPs have therapeutic potential for $CD4^+$ T helper (Th) cell-mediated autoimmune diseases [27,40].

6.6. Pulmonary fibrosis

Pulmonary fibrosis, as a sequela of severe coronavirus pneumonia [97], has attracted increasing attention because of the coronavirus disease 2019 (COVID-19) pandemic. It is accompanied by a significant elevation of ROS, hyperactivation of macrophages, and terminal differentiation of resident epithelial fibroblasts into myofibroblasts [57]. In a bleomycin-induced pulmonary fibrosis mouse model, PEGylated bilirubin micelles (5, 20, 40 mg kg^{-1}; i. v.; qod x 14) were taken up by lung epithelial cells, protected against oxidative stress, inhibited neutrophil recruitment and macrophage activation, and alleviated pulmonary fibrosis [57].

6.7. Nonalcoholic fatty liver disease (NAFLD)

It has been discovered that bilirubin has the ability to modulate lipid metabolic dysregulation by regulating adipocytokine expression in adipose tissues [43] and activating various nuclear and cytoplasmic receptors to influence the process of lipid metabolism, lipid transport, and lipogenesis [44–46] (section 4.1). In light of the metabolic-regulatory function of bilirubin, Hinds et al. [98] treated diet-induced obese mice with NAFLD PEGylated bilirubin micelles (30 mg kg^{-1}; i. p.; qod. X 14) and showed that they had significantly reduced liver enzyme activities, hepatic fat accumulation, and de novo lipogenesis.

6.8. Diabetic wounds

Advanced glycation end-products (AGEs) and ROS induced by hyperglycemia inhibit the healing of diabetic wounds and cause chronic and persistent inflammation [78]. BR@β-CD/SGP (equivalent to 1 mg mL^{-1} BR via the topical route) promoted wound healing and tissue remodeling in streptozotocin (STZ)-induced diabetic mice and nondiabetic mice, suggesting its potential for use in wound dressing [78].

6.9. Other applications

6.9.1. BRNPs as ROS-responsive nanocarriers

Upon reacting with ROS, bilirubin is oxidized into hydrosoluble products, with the result that van der Waals forces cannot maintain micellar stability. Because disruption of bilirubin micelles could cause release of loaded drug(s), BRNPs have been evaluated as ROS-responsive nanocarriers for targeted drug delivery to tissues rich in ROS. Combined with the strong ROS scavenger functions of BRNPs [99], ROS-responsive bilirubin-derived nanocarriers have been applied in tumors [63,64,100–103], hepatic fibrosis [104], rheumatoid arthritis [105] osteoarthritis [60], and acute kidney injury [99].

6.9.2. BRNPs for imaging

As described in section 5.3, BR-CDots could emit near-infrared fluorescence from both the carbon core and surface functional groups, rendering them suitable for imaging biological structures *in vivo*. The intensity of BR-CDot fluorescence changed in response to environmental pH, providing a potential imaging method for distinguishing between cancerous and healthy cells [71]. Bilirubin also had the potential to convert into PEG-BR-coated metal NPs utilized in imaging (section 5.2). The PEG-BR shell offers excellent stability to the metal NPs and plays a critical role in the detection of ROS (PEG-BR/SPIONs) [68] and the GSH-responsive enhancement of CT signals (PEG-BR/AuNPs) [67]. Alterations in GSH levels in hepatocytes have been linked to liver disease, making GSH imaging a promising tool for the diagnosis and monitoring of liver function [70]. BRNPs were also applied in photothermal imaging [73,94] and photoacoustic imaging (PAI) [73]. By demonstrating the heat generated by cisPt@BRNPs and TH302@BR-Chitosan NPs in PTT (section 6.4), photothermal imaging could visualize the range of PTT treatment. Additionally, periodic light irradiation caused the thermal expansion and tiny vibrations (acoustic signals) of cisPt@BRNPs at the same modulation frequency as the light, which is known as PAI. PAI has the potential to supplement whole-body imaging methods by providing more localized, clearer, and faster imaging, particularly when the targeted lesion is located within the light dissipation limit [106]. Further research is warranted to explore the potential of these novel BRNPs in medical diagnostics.

In conclusion, BRNPs offer a promising approach for the treatment and diagnosis of various diseases due to their anti-inflammatory, antioxidant, metabolic-regulatory and anticancer properties. Different administration routes of BRNPs have been reported, mainly including intravenous (i.v.), intraperitoneal (i.p.), oral, transdermal, intra-articular (i.a.), and intratumoral routes (i.t.). Among these BRNPs, PEGylated bilirubin micelles are the most extensively studied type [54]. However, further studies are necessary to ascertain the pharmacokinetics of other BRNPs and administration routes. Nonetheless, BRNPs represent a potential therapeutic and diagnostic option, and their application merits continued investigation.

7. Discussion

Current research indicates that bilirubin has the potential to protect cells from oxidative stress, suppress excessive inflammation, and modulate immune responses, by scavenging ROS and affecting immune cells. While the underlying mechanism of bilirubin's antioxidant activities is well-established, additional research is necessary to comprehend the molecular process by which bilirubin modulates a variety of immune cells. Additionally, bilirubin has metabolic regulatory and antiproliferative properties by activating a range of nuclear and cytoplasmic receptors, rendering bilirubin a promising alternative for treating metabolic disorders and cancer. Due to its numerous beneficial effects, bilirubin shows great promise as a potential candidate in curing multiple disorders.

Bilirubin shows promise for use in diseases of the central nervous system (CNS), such as stroke [1] and glioma [64]. Neurotoxicity related to the use of bilirubin is a rare occurrence in the context of localized peripheral application. The threshold of neurotoxicity of bilirubin is low in CNS (22 nmol L^{-1} per kilogram of birth weight for newborns less than 3 kg in weight; up to 66 nmol L^{-1} for newborns heavier than 3 kg), making it crucial to consider the possibility of neurotoxicity in this condition [107,108]. We also want to emphasize that, although some reports of the toxicity exist for BRNPs, there are only limited comprehensive clinical studies available. The accumulation of small nanoparticles in tissues and organs can cause acute toxic effects and chronic adverse effects by mechanisms including modulation of cell signaling pathways and immunogenicity [82,109–111]. Published reports focusing on cell culture and rodent animal models do not fully recapitulate nanoparticle potential toxicity responses in humans and are therefore limited in their predictive power of possible hazards to humans [112]. Therefore, it might be beneficial to consider conducting additional studies, such as nonhuman primate trials, to facilitate the translation of BRNPs from the laboratory to clinical application.

The preparation and therapeutic efficacy of BRNPs can be further improved. BRNPs should target key cells in pathogenesis (*e.g.*, macrophages, vascular endothelial cells, and tumor cells). Immune-privileged organs, such as the brain, testis, and inner ear, have specialized physiological barriers that protect against foreign substances and limit the immune response. However, these barriers pose a challenge to effective bilirubin delivery and treatment of related disorders. To overcome these barriers, novel drug delivery strategies, including modifications of T7 for BRNPs [64], have been developed. With the discovery of specific

targeting mechanisms [113], we anticipate further progress in overcoming these barriers and enhancing bilirubin transport in the future.

Oxidative stress, immune imbalance, metabolic dysregulation, and excessive proliferation are implicated in a wide range of diseases. Although multiple small molecules proposed to have antioxidant, immunomodulating, metabolic-modulating or antiproliferative effects have exhibited therapeutic potential in preclinical studies, clinical trial results have been disappointing. Thus, the efficacy of BRNPs must be validated in animal models that recapitulate human diseases. Additionally, the metabolic and long-term adverse effects *in vivo* should be evaluated, and the repeatability and reproducibility of large-scale production of BRNPs are prerequisites for their clinical application [114,115].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgement

The authors acknowledge the financial support from Natural Science Foundation of China (82071042), International Cooperation and Exchange of the National Natural Science Foundation of China (Grant No. 81720108010), Natural Science Foundation of Shanghai (20ZR1442300).

References

[1] M. Thakkar, J. Edelenbos, S. Dore, Bilirubin and ischemic stroke: rendering the current paradigm to better understand the protective effects of bilirubin, Mol. Neurobiol. 56 (8) (2019) 5483–5496, https://doi.org/10.1007/s12035-018-1440-y.

[2] Y. Choi, S.J. Lee, W. Spiller, K.J. Jung, J.Y. Lee, H. Kimm, J.H. Back, S. Lee, S.H. Jee, Causal associations between serum bilirubin levels and decreased stroke risk: a two-sample mendelian randomization study, Arterioscler. Thromb. Vasc. Biol. 40 (2) (2020) 437–445, https://doi.org/10.1161/ATVBAHA.119.313055.

[3] M. Ozeki, H. Morita, M. Miyamura, T. Fujisaka, S.I. Fujita, T. Ito, K. Shibata, S. Tanaka, K. Sohmiya, M. Hoshiga, N. Ishizaka, High serum bilirubin is associated with lower prevalence of peripheral arterial disease among cardiac patients, Clin. Chim. Acta 476 (2018) 60–66, https://doi.org/10.1016/j.cca.2017.11.013.

[4] P. Novak, A.O. Jackson, G.J. Zhao, K. Yin, Bilirubin in metabolic syndrome and associated inflammatory diseases: new perspectives, Life Sci. (2020) 257 118032, https://doi.org/10.1016/j.lfs.2020.118032.

[5] N. Seyed Khoei, A. Grindel, M. Wallner, C. Molzer, D. Doberer, R. Marculescu, A. Bulmer, K.H. Wagner, Mild hyperbilirubinaemia as an endogenous mitigator of overweight and obesity: implications for improved metabolic health, Atherosclerosis 269 (2018) 306–311, https://doi.org/10.1016/j.atherosclerosis.2017.12.021.

[6] Y. Wei, C. Liu, F. Lai, S. Dong, H. Chen, L. Chen, L. Shi, F. Zhu, C. Zhang, X. Lv, S. Peng, G. Hao, Associations between serum total bilirubin, obesity and type 2 diabetes, Diabetol. Metab. Syndrome 13 (1) (2021) 143, https://doi.org/10.1186/s13098-021-00762-0.

[7] Q. Su, X. Li, W. Mo, Z. Yang, Low serum bilirubin, albumin, and uric acid levels in patients with Crohn's disease, Medicine (Baltim.) 98 (19) (2019), e15664, https://doi.org/10.1097/MD.0000000000015664.

[8] J. Wang, B. Wang, M. Liang, G. Wang, J. Li, Y. Zhang, Y. Huo, Y. Cui, X. Xu, X. Qin, Independent and combined effect of bilirubin and smoking on the progression of chronic kidney disease, Clin. Epidemiol. 10 (2018) 121–132, https://doi.org/10.2147/CLEP.S150687.

[9] A.C. Bulmer, B. Bakrania, E.F. Du Toit, A.C. Boon, P.J. Clark, L.W. Powell, K.H. Wagner, J.P. Headrick, Bilirubin acts as a multipotent guardian of cardiovascular integrity: more than just a radical idea, Am. J. Physiol. Heart Circ. Physiol. 315 (3) (2018) H429–H447, https://doi.org/10.1152/ajpheart.00417.2017.

[10] T. Inoguchi, S. Sasaki, K. Kobayashi, R. Takayanagi, T. Yamada, Relationship between Gilbert syndrome and prevalence of vascular complications in patients with diabetes, JAMA 298 (12) (2007) 1398–1400, https://doi.org/10.1001/jama.298.12.1398-b.

[11] S. Parvez, M. Kaushik, M. Ali, M.M. Alam, J. Ali, H. Tabassum, P. Kaushik, Dodging blood brain barrier with "nano" warriors: novel strategy against ischemic stroke, Theranostics 12 (2) (2022) 689–719, https://doi.org/10.7150/thno.64806.

[12] R. Stocker, Y. Yamamoto, A.F. McDonagh, A.N. Glazer, B.N. Ames, Bilirubin is an antioxidant of possible physiological importance, Science 235 (4792) (1987) 1043–1046, https://doi.org/10.1126/science.3029864.

[13] J.A. Farrera, A. Jauma, J.M. Ribo, M.A. Peire, P.P. Parellada, S. Roques-Choua, E. Bienvenue, P. Seta, The antioxidant role of bile pigments evaluated by chemical tests, Bioorg. Med. Chem. 2 (3) (1994) 181–185, https://doi.org/10.1016/s0968-0896(00)82013-1.

[14] C. Mancuso, A. Bonsignore, E. Di Stasio, A. Mordente, R. Motterlini, Bilirubin and S-nitrosothiols interaction: evidence for a possible role of bilirubin as a scavenger of nitric oxide, Biochem. Pharmacol. 66 (12) (2003) 2355–2363, https://doi.org/10.1016/j.bcp.2003.08.022.

[15] S. Dore, S.H. Snyder, Neuroprotective action of bilirubin against oxidative stress in primary hippocampal cultures, Ann. N. Y. Acad. Sci. 890 (1999) 167–172, https://doi.org/10.1111/j.1749-6632.1999.tb07991.x.

[16] C.L. Basiglio, F.D. Toledo, A.C. Boaglio, S.M. Arriaga, J.E. Ochoa, E.J. Sanchez Pozzi, A.D. Mottino, M.G. Roma, Physiological concentrations of unconjugated bilirubin prevent oxidative stress-induced hepatocanalicular dysfunction and cholestasis, Arch. Toxicol. 88 (2) (2014) 501–514, https://doi.org/10.1007/s00204-013-1143-0.

[17] D.A. Benaron, F.W. Bowen, Variation of initial serum bilirubin rise in newborn infants with type of illness, Lancet (N. Am. Ed.) 338 (8759) (1991) 78–81, https://doi.org/10.1016/0140-6736(91)90074-y.

[18] E.H. Temme, J. Zhang, E.G. Schouten, H. Kesteloot, Serum bilirubin and 10-year mortality risk in a Belgian population, Cancer Causes Control 12 (10) (2001) 887–894, https://doi.org/10.1023/a:1013794407325.

[19] N. Ishizaka, Y. Ishizaka, E. Takahashi, M. Yamakado, H. Hashimoto, High serum bilirubin level is inversely associated with the presence of carotid plaque, Stroke 32 (2) (2001) 580–583, https://doi.org/10.1161/01.str.32.2.580-b.

[20] T. Inoguchi, Y. Nohara, C. Nojiri, N. Nakashima, Association of serum bilirubin levels with risk of cancer development and total death, Sci. Rep. 11 (1) (2021), 13224, https://doi.org/10.1038/s41598-021-92442-2.

[21] D.E. Baranano, M. Rao, C.D. Ferris, S.H. Snyder, Biliverdin reductase: a major physiologic cytoprotectant, Proc. Natl. Acad. Sci. U. S. A. 99 (25) (2002) 16093–16098, https://doi.org/10.1073/pnas.252626999.

[22] Y. Huang, J. Li, W. Li, N. Ai, H. Jin, Biliverdin/bilirubin redox pair protects lens epithelial cells against oxidative stress in age-related cataract by regulating NF-kappaB/iNOS and Nrf2/HO-1 pathways, Oxid. Med. Cell. Longev. (2022), 7299182, https://doi.org/10.1155/2022/7299182, 2022.

[23] Q. Yao, R. Sun, S. Bao, R. Chen, L. Kou, Bilirubin protects transplanted islets by targeting ferroptosis, Front. Pharmacol. 11 (2020) 907, https://doi.org/10.3389/fphar.2020.00907.

[24] S. Saha, B. Buttari, E. Panieri, E. Profumo, L. Saso, An overview of Nrf2 signaling pathway and its role in inflammation, Molecules 25 (22) (2020), https://doi.org/10.3390/molecules25225474.

[25] L.J. Horsfall, R. Hardy, A. Wong, D. Kuh, D.M. Swallow, Genetic variation underlying common hereditary hyperbilirubinaemia (Gilbert's syndrome) and respiratory health in the 1946 British birth cohort, J. Hepatol. 61 (6) (2014) 1344–1351, https://doi.org/10.1016/j.jhep.2014.07.028.

[26] Z.X. Zhou, J.K. Chen, Y.Y. Hong, R. Zhou, D.M. Zhou, L.Y. Sun, W.L. Qin, T.C. Wang, Relationship between the serum total bilirubin and inflammation in patients with psoriasis vulgaris, J. Clin. Lab. Anal. 30 (5) (2016) 768–775, https://doi.org/10.1002/jcla.21936.

[27] D.T. Tran, Y.Y. Jeong, J.M. Kim, H.B. Bae, S.K. Son, S.H. Kwak, The anti-inflammatory role of bilirubin on "Two-Hit" sepsis animal model, Int. J. Mol. Sci. 21 (22) (2020), https://doi.org/10.3390/ijms21228650.

[28] J. He, G. Jiang, X. Li, Q. Xiao, Y. Chen, H. Xu, G. Liu, A. Lei, P. Zhou, K. Shi, Q. Yang, M. Zhao, Z. Yao, J. Zhou, Bilirubin represents a negative regulator of ILC2 in allergic airway inflammation, Mucosal Immunol. 15 (2) (2022) 314–326, https://doi.org/10.1038/s41385-021-00460-0.

[29] Y. Li, B. Huang, T. Ye, Y. Wang, D. Xia, J. Qian, Physiological concentrations of bilirubin control inflammatory response by inhibiting NF-kappaB and inflammasome activation, Int. Immunopharm. 84 (2020), 106520, https://doi.org/10.1016/j.intimp.2020.106520.

[30] Y. Lin, S. Wang, Z. Yang, L. Gao, Z. Zhou, P. Yu, B. Wu, Bilirubin alleviates alum-induced peritonitis through inactivation of NLRP3 inflammasome, Biomed. Pharmacother. 116 (2019), 108973, https://doi.org/10.1016/j.biopha.2019.108973.

[31] Y. Li, H. Sheng, Z. Yan, B. Guan, S. Qiang, J. Qian, Y. Wang, Bilirubin stabilizes the mitochondrial membranes during NLRP3 inflammasome activation, Biochem. Pharmacol. (2022) 203 115204, https://doi.org/10.1016/j.bcp.2022.115204.

[32] Y. Liu, P. Li, J. Lu, W. Xiong, J. Oger, W. Tetzlaff, M. Cynader, Bilirubin possesses powerful immunomodulatory activity and suppresses experimental autoimmune encephalomyelitis, J. Immunol. 181 (3) (2008) 1887–1897, https://doi.org/10.4049/jimmunol.181.3.1887.

[33] N.M. Khan, T.B. Poduval, Immunomodulatory and immunotoxic effects of bilirubin: molecular mechanisms, J. Leukoc. Biol. 90 (5) (2011) 997–1015, https://doi.org/10.1189/jlb.0211070.

[34] M.E. Vogel, G. Idelman, E.S. Konaniah, S.D. Zucker, Bilirubin prevents atherosclerotic lesion formation in low-density lipoprotein receptor-deficient mice by inhibiting endothelial VCAM-1 and ICAM-1 signaling, J. Am. Heart Assoc. 6 (4) (2017), https://doi.org/10.1161/JAHA.116.004820.

[35] A.J. Thomas, C.S. Ogilvy, C.J. Griessenauer, K.A. Hanafy, Macrophage CD163 expression in cerebrospinal fluid: association with subarachnoid hemorrhage

outcome, J. Neurosurg. 131 (1) (2018) 47–53, https://doi.org/10.3171/2018.2.JNS172828.
[36] Y.Z. Zhao, Z.W. Huang, Y.Y. Zhai, Y. Shi, C.C. Du, J. Zhai, H.L. Xu, J. Xiao, L. Kou, Q. Yao, Polylysine-bilirubin conjugates maintain functional islets and promote M2 macrophage polarization, Acta Biomater. 122 (2021) 172–185, https://doi.org/10.1016/j.actbio.2020.12.047.
[37] F. Rocuts, X. Zhang, J. Yan, Y. Yue, M. Thomas, F.H. Bach, E. Czismadia, H. Wang, Bilirubin promotes de novo generation of T regulatory cells, Cell Transplant. 19 (4) (2010) 443–451, https://doi.org/10.3727/096368909X484680.
[38] T.W. Kim, Y. Kim, W. Jung, D.E. Kim, H. Keum, Y. Son, S. Jon, Bilirubin nanomedicine ameliorates the progression of experimental autoimmune encephalomyelitis by modulating dendritic cells, J. Contr. Release 331 (2021) 74–84, https://doi.org/10.1016/j.jconrel.2021.01.019.
[39] H. Keum, T.W. Kim, Y. Kim, C. Seo, Y. Son, J. Kim, D. Kim, W. Jung, C.H. Whang, S. Jon, Bilirubin nanomedicine alleviates psoriatic skin inflammation by reducing oxidative stress and suppressing pathogenic signaling, J. Contr. Release 325 (2020) 359–369, https://doi.org/10.1016/j.jconrel.2020.07.015.
[40] M. Vuerich, N. Wang, J.J. Graham, L. Gao, W. Zhang, A. Kalbasi, L. Zhang, E. Csizmadia, J. Hristopoulos, Y. Ma, E. Kokkotou, A.S. Cheifetz, S.C. Robson, M.S. Longhi, Blockade of PGK1 and ALDOA enhances bilirubin control of Th17 cells in Crohn's disease, Commun Biol 5 (1) (2022) 994, https://doi.org/10.1038/s42003-022-03913-9.
[41] C.L. Basiglio, S.M. Arriaga, H.F. Pelusa, A.M. Almara, M.G. Roma, A.D. Mottino, Protective role of unconjugated bilirubin on complement-mediated hepatocytolysis, Biochim. Biophys. Acta 1770 (7) (2007) 1003–1010, https://doi.org/10.1016/j.bbagen.2007.03.005.
[42] J. Nano, T. Muka, M. Cepeda, T. Voortman, K. Dhana, A. Brahimaj, A. Dehghan, O.H. Franco, Association of circulating total bilirubin with the metabolic syndrome and type 2 diabetes: a systematic review and meta-analysis of observational evidence, Diabetes Metab. 42 (6) (2016) 389–397, https://doi.org/10.1016/j.diabet.2016.06.002.
[43] R. Takei, T. Inoue, N. Sonoda, M. Kohjima, M. Okamoto, R. Sakamoto, T. Inoguchi, Y. Ogawa, Bilirubin reduces visceral obesity and insulin resistance by suppression of inflammatory cytokines, PLoS One 14 (10) (2019), e0223302, https://doi.org/10.1371/journal.pone.0223302.
[44] G. Wen, L. Yao, Y. Hao, J. Wang, J. Liu, Bilirubin ameliorates murine atherosclerosis through inhibiting cholesterol synthesis and reshaping the immune system, J. Transl. Med. 20 (1) (2022) 1, https://doi.org/10.1186/s12967-021-03207-4.
[45] L. Vitek, C. Tiribelli, Bilirubin: the yellow hormone? J. Hepatol. 75 (6) (2021) 1485–1490, https://doi.org/10.1016/j.jhep.2021.06.010.
[46] L. Vitek, Bilirubin as a signaling molecule, Med. Res. Rev. 40 (4) (2020) 1335–1351, https://doi.org/10.1002/med.21660.
[47] P. Rao, R. Suzuki, S. Mizobuchi, T. Yamaguchi, S. Sasaguri, Bilirubin exhibits a novel anti-cancer effect on human adenocarcinoma, Biochem. Biophys. Res. Commun. 342 (4) (2006) 1279–1283, https://doi.org/10.1016/j.bbrc.2006.02.074.
[48] S.Z. Shahrokhi, F.S.K. Tehrani, S. Salami, Molecular mechanisms of bilirubin induced G1 cell cycle arrest and apoptosis in human breast cancer cell lines: involvement of the intrinsic pathway, Mol. Biol. Rep. 49 (11) (2022) 10421–10429, https://doi.org/10.1007/s11033-022-07757-8.
[49] M. Stoeckius, A. Erat, T. Fujikawa, M. Hiromura, A. Koulova, L. Otterbein, C. Bianchi, E. Tobiasch, Y. Dagon, F.W. Sellke, A. Usheva, Essential roles of Raf/extracellular signal-regulated kinase/mitogen-activated protein kinase pathway, YY1, and Ca2+ influx in growth arrest of human vascular smooth muscle cells by bilirubin, J. Biol. Chem. 287 (19) (2012) 15418–15426, https://doi.org/10.1074/jbc.M111.266510.
[50] R. Ollinger, M. Bilban, A. Erat, A. Froio, J. McDaid, S. Tyagi, E. Csizmadia, A.V. Graca-Souza, A. Liloia, M.P. Soares, L.E. Otterbein, A. Usheva, K. Yamashita, F.H. Bach, Bilirubin: a natural inhibitor of vascular smooth muscle cell proliferation, Circulation 112 (7) (2005) 1030–1039, https://doi.org/10.1161/CIRCULATIONAHA.104.528802.
[51] K.J. Peyton, A.R. Shebib, M.A. Azam, X.M. Liu, D.A. Tulis, Durante W., Bilirubin inhibits neointima formation and vascular smooth muscle cell proliferation and migration, Front. Pharmacol. 3 (2012) 48, https://doi.org/10.3389/fphar.2012.00048.
[52] W. Goessling, S.D. Zucker, Role of apolipoprotein D in the transport of bilirubin in plasma, Am. J. Physiol. Gastrointest. Liver Physiol. 279 (2) (2000) G356–G365, https://doi.org/10.1152/ajpgi.2000.279.2.G356.
[53] J.M. Sarjeant, A. Lawrie, C. Kinnear, S. Yablonsky, W. Leung, H. Massaeli, W. Prichett, J.P. Veinot, E. Rassart, M. Rabinovitch, Apolipoprotein D inhibits platelet-derived growth factor-BB-induced vascular smooth muscle cell proliferated by preventing translocation of phosphorylated extracellular signal regulated kinase 1/2 to the nucleus, Arterioscler. Thromb. Vasc. Biol. 23 (12) (2003) 2172–2177, https://doi.org/10.1161/01.ATV.0000100404.05459.39.
[54] D.E. Kim, Y. Lee, M. Kim, S. Lee, S. Jon, S.H. Lee, Bilirubin nanoparticles ameliorate allergic lung inflammation in a mouse model of asthma, Biomaterials 140 (2017) 37–44, https://doi.org/10.1016/j.biomaterials.2017.06.014.
[55] F.M. Veronese, A. Mero, The impact of PEGylation on biological therapies, BioDrugs 22 (5) (2008) 315–329, https://doi.org/10.2165/00063030-200822050-00004.
[56] S. Yadav, A.K. Sharma, P. Kumar, Nanoscale self-assembly for therapeutic delivery, Front. Bioeng. Biotechnol. 8 (2020) 127, https://doi.org/10.3389/fbioe.2020.00127.
[57] H. Keum, D. Kim, J. Kim, T.W. Kim, C.H. Whang, W. Jung, S. Jon, A bilirubin-derived nanomedicine attenuates the pathological cascade of pulmonary fibrosis, Biomaterials 275 (2021), 120986, https://doi.org/10.1016/j.biomaterials.2021.120986.
[58] J.Y. Kim, D.Y. Lee, S. Kang, W. Miao, H. Kim, Y. Lee, S. Jon, Bilirubin nanoparticle preconditioning protects against hepatic ischemia-reperfusion injury, Biomaterials 133 (2017) 1–10, https://doi.org/10.1016/j.biomaterials.2017.04.011.
[59] Y. Lee, K. Sugihara, M.G. Gillilland 3rd, S. Jon, N. Kamada, J.J. Moon, Hyaluronic acid-bilirubin nanomedicine for targeted modulation of dysregulated intestinal barrier, microbiome and immune responses in colitis, Nat. Mater. 19 (1) (2020) 118–126, https://doi.org/10.1038/s41563-019-0462-9.
[60] L. Kou, H. Huang, Y. Tang, M. Sun, Y. Li, J. Wu, S. Zheng, X. Zhao, D. Chen, Z. Luo, X. Zhang, Q. Yao, R. Chen, Opsonized nanoparticles target and regulate macrophage polarization for osteoarthritis therapy: a trapping strategy, J. Contr. Release 347 (2022) 237–255, https://doi.org/10.1016/j.jconrel.2022.04.037.
[61] Z.W. Huang, Y. Shi, Y.Y. Zhai, C.C. Du, J. Zhai, R.J. Yu, L. Kou, J. Xiao, Y.Z. Zhao, Q. Yao, Hyaluronic acid coated bilirubin nanoparticles attenuate ischemia reperfusion-induced acute kidney injury, J. Contr. Release 334 (2021) 275–289, https://doi.org/10.1016/j.jconrel.2021.04.033.
[62] Z. Huang, Y. Shi, H. Wang, C. Chun, L. Chen, K. Wang, Z. Lu, Y. Zhao, X. Li, Protective effects of chitosan-bilirubin nanoparticles against ethanol-induced gastric ulcers, Int. J. Nanomed. 16 (2021) 8235–8250, https://doi.org/10.2147/IJN.S344805.
[63] Y. Lee, S. Lee, S. Jon, Biotinylated bilirubin nanoparticles as a tumor microenvironment-responsive drug delivery system for targeted cancer therapy, Adv. Sci. 5 (6) (2018), 1800017, https://doi.org/10.1002/advs.201800017.
[64] M. Yu, D. Su, Y. Yang, L. Qin, C. Hu, R. Liu, Y. Zhou, C. Yang, X. Yang, G. Wang, H. Gao, D-T7 peptide-modified PEGylated bilirubin nanoparticles loaded with cediranib and paclitaxel for antiangiogenesis and chemotherapy of glioma, ACS Appl. Mater. Interfaces 11 (1) (2019) 176–186, https://doi.org/10.1021/acsami.8b16219.
[65] P. Rathinaraj, G. Muthusamy, N.R. Prasad, S. Gunaseelan, B. Kim, S. Zhu, Folate-gold-bilirubin nanoconjugate induces apoptotic death in multidrug-resistant oral carcinoma cells, Eur. J. Drug Metab. Pharmacokinet. 45 (2) (2020) 285–296, https://doi.org/10.1007/s13318-019-00600-9.
[66] V.J. Pizzuti, D. Viswanath, S.E. Torregrosa-Allen, M.P. Currie, B.D. Elzey, Y.Y. Won, Bilirubin-coated radioluminescent particles for radiation-induced photodynamic therapy, ACS Appl. Bio Mater. 3 (8) (2020) 4858–4872, https://doi.org/10.1021/acsabm.0c00354.
[67] D. Yoo, W. Jung, Y. Son, S. Jon, Glutathione-responsive gold nanoparticles as computed tomography contrast agents for hepatic diseases, ACS Appl. Bio Mater. 4 (5) (2021) 4486–4494, https://doi.org/10.1021/acsabm.1c00224.
[68] D.Y. Lee, S. Kang, Y. Lee, J.Y. Kim, D. Yoo, W. Jung, S. Lee, Y.Y. Jeong, K. Lee, S. Jon, PEGylated bilirubin-coated iron oxide nanoparticles as a biosensor for magnetic relaxation switching-based ROS detection in whole blood, Theranostics 10 (5) (2020) 1997–2007, https://doi.org/10.7150/thno.39662.
[69] J.H. Correia, J.A. Rodrigues, S. Pimenta, T. Dong, Z. Yang, Photodynamic therapy review: principles, photosensitizers, applications, and future directions, Pharmaceutics 13 (9) (2021), https://doi.org/10.3390/pharmaceutics13091332.
[70] X. Jiang, B. Du, J. Zheng, Glutathione-mediated biotransformation in the liver modulates nanoparticle transport, Nat. Nanotechnol. 14 (9) (2019) 874–882, https://doi.org/10.1038/s41565-019-0499-6.
[71] P. Fathi, P. Moitra, M.M. McDonald, M.B. Esch, D. Pan, Near-infrared emitting dual-stimuli-responsive carbon dots from endogenous bile pigments, Nanoscale 13 (31) (2021) 13487–13496, https://doi.org/10.1039/d1nr01295a.
[72] X. Jiang, Q. Yao, X. Xia, Y. Tang, M. Sun, Y. Li, H. Zheng, A. Cai, H. Zhang, V. Ganapathy, R. Chen, L. Kou, Self-assembled nanoparticles with bilirubin/JPH203 alleviate imiquimod-induced psoriasis by reducing oxidative stress and suppressing Th17 expansion, Chem. Eng. J. 431 (2022), https://doi.org/10.1016/j.cej.2021.133956.
[73] D.Y. Lee, J.Y. Kim, Y. Lee, S. Lee, W. Miao, H.S. Kim, J.J. Min, S. Jon, Black pigment gallstone inspired platinum-chelated bilirubin nanoparticles for combined photoacoustic imaging and photothermal therapy of cancers, Angew Chem. Int. Ed. Engl. 56 (44) (2017) 13684–13688, https://doi.org/10.1002/anie.201707137.
[74] B. Fullagar, W. Rao, C. Gilor, F. Xu, X. He, C.A. Adin, Nano-encapsulation of bilirubin in pluronic F127-chitosan improves uptake in beta cells and increases islet viability and function after hypoxic stress, Cell Transplant. 26 (10) (2017) 1703–1715, https://doi.org/10.1177/0963689717735112.
[75] Q. Yang, C. Zhou, Q. Zhao, Z. Chu, D.P. Yang, N. Jia, Sonochemical assisted synthesis of dual functional BSA nanoparticle for the removal of excessive bilirubin and strong anti-tumor effects, Mater Sci Eng C Mater Biol Appl 100 (2019) 688–696, https://doi.org/10.1016/j.msec.2019.03.042.
[76] S. Xue, X. Zhou, W. Sang, C. Wang, H. Lu, Y. Xu, Y. Zhong, L. Zhu, C. He, J. Ma, Cartilage-targeting peptide-modified dual-drug delivery nanoplatform with NIR laser response for osteoarthritis therapy, Bioact. Mater. 6 (8) (2021) 2372–2389, https://doi.org/10.1016/j.bioactmat.2021.01.017.
[77] Q. Yao, Z.W. Huang, Y.Y. Zhai, M. Yue, L.Z. Luo, P.P. Xue, Y.H. Han, H.L. Xu, L. Kou, Y.Z. Zhao, Localized controlled release of bilirubin from beta-cyclodextrin-conjugated epsilon-polylysine to attenuate oxidative stress and inflammation in transplanted islets, ACS Appl. Mater. Interfaces 12 (5) (2020) 5462–5475, https://doi.org/10.1021/acsami.9b18986.
[78] Q. Yao, Y. Shi, X. Xia, Y. Tang, X. Jiang, Y.W. Zheng, H. Zhang, R. Chen, L. Kou, Bioadhesive hydrogel comprising bilirubin/beta-cyclodextrin inclusion complexes promote diabetic wound healing, Pharm. Biol. 59 (1) (2021) 1139–1149, https://doi.org/10.1080/13880209.2021.1964543.
[79] Q. Yao, X. Jiang, Y.Y. Zhai, L.Z. Luo, H.L. Xu, J. Xiao, L. Kou, Y.Z. Zhao, Protective effects and mechanisms of bilirubin nanomedicine against acute pancreatitis,

J. Contr. Release 322 (2020) 312–325, https://doi.org/10.1016/j.jconrel.2020.03.034.
[80] D. Dekker, M.J. Dorresteijn, M.E.B. Welzen, S. Timman, P. Pickkers, D.M. Burger, P. Smits, F. Wagener, F.G.M. Russel, Parenteral bilirubin in healthy volunteers: a reintroduction in translational research, Br. J. Clin. Pharmacol. 84 (2) (2018) 268–279, https://doi.org/10.1111/bcp.13458.
[81] S.Z. Alshawwa, A.A. Kassem, R.M. Farid, S.K. Mostafa, G.S. Labib, Nanocarrier drug delivery systems: characterization, limitations, future perspectives and implementation of artificial intelligence, Pharmaceutics 14 (4) (2022), https://doi.org/10.3390/pharmaceutics14040883.
[82] W. Yang, L. Wang, E.M. Mettenbrink, P.L. DeAngelis, S. Wilhelm, Nanoparticle toxicology, Annu. Rev. Pharmacol. Toxicol. 61 (2021) 269–289, https://doi.org/10.1146/annurev-pharmtox-032320-110338.
[83] S. Pareek, A.S. Flegle, D. Boagni, J.Y. Kim, D. Yoo, A. Trujillo-Ocampo, S.E. Lee, M. Zhang, S. Jon, J.S. Im, Post transplantation bilirubin nanoparticles ameliorate murine graft versus host disease via a reduction of systemic and local inflammation, Front. Immunol. 13 (2022), 893659, https://doi.org/10.3389/fimmu.2022.893659.
[84] R. Ben-Amotz, J. Bonagura, M. Velayutham, R. Hamlin, P. Burns, C. Adin, Intraperitoneal bilirubin administration decreases infarct area in a rat coronary ischemia/reperfusion model, Front. Physiol. 5 (2014) 53, https://doi.org/10.3389/fphys.2014.00053.
[85] B. Bakrania, E.F. Du Toit, K.H. Wagner, J.P. Headrick, A.C. Bulmer, Pre- or post-ischemic bilirubin ditaurate treatment reduces oxidative tissue damage and improves cardiac function, Int. J. Cardiol. 202 (2016) 27–33, https://doi.org/10.1016/j.ijcard.2015.08.192.
[86] Y. Lee, H. Kim, S. Kang, J. Lee, J. Park, S. Jon, Bilirubin nanoparticles as a nanomedicine for anti-inflammation therapy, Angew Chem. Int. Ed. Engl. 55 (26) (2016) 7460–7463, https://doi.org/10.1002/anie.201602525.
[87] M.J. Kim, Y. Lee, S. Jon, D.Y. Lee, PEGylated bilirubin nanoparticle as an anti-oxidative and anti-inflammatory demulcent in pancreatic islet xenotransplantation, Biomaterials 133 (2017) 242–252, https://doi.org/10.1016/j.biomaterials.2017.04.029.
[88] J. Dolai, K. Mandal, N.R. Jana, Nanoparticle size effects in biomedical applications, ACS Appl. Nano Mater. 4 (7) (2021) 6471–6496, https://doi.org/10.1021/acsanm.1c00987.
[89] S. Samimi, N. Maghsoudnia, R.B. Eftekhari, F. Dorkoosh, Chapter 3 - lipid-based nanoparticles for drug delivery systems, in: S.S. Mohapatra, S. Ranjan, N. Dasgupta, R.K. Mishra, S. Thomas (Eds.), Characterization and Biology of Nanomaterials for Drug Delivery, *Elsevier*, 2019, pp. 47–76.
[90] E. Joseph, G. Singhvi, Chapter 4 - multifunctional nanocrystals for cancer therapy: a potential nanocarrier, in: A.M. Grumezescu (Ed.), Nanomaterials for Drug Delivery and Therapy, *William Andrew Publishing*, 2019, pp. 91–116.
[91] W. Ai, S. Bae, Q. Ke, S. Su, R. Li, Y. Chen, D. Yoo, E. Lee, S. Jon, P.M. Kang, Bilirubin nanoparticles protect against cardiac ischemia/reperfusion injury in mice, J. Am. Heart Assoc. 10 (20) (2021), e021212, https://doi.org/10.1161/JAHA.121.021212.
[92] C.W. Choi, H. Keum, S. Yang, B.M. Kang, D. Kim, H. Piao, J.W. Kim, B.R. Kim, S.W. Youn, S. Jon, Bilirubin nanomedicine alleviates inflammation and angiogenesis in a rosacea mouse model, Advanced Therapeutics (2022), https://doi.org/10.1002/adtp.202200223.
[93] L. Shan, W. Fan, W. Wang, W. Tang, Z. Yang, Z. Wang, Y. Liu, Z. Shen, Y. Dai, S. Cheng, O. Jacobson, K. Zhai, J. Hu, Y. Ma, D.O. Kiesewetter, G. Gao, X. Chen, Organosilica-based hollow mesoporous bilirubin nanoparticles for antioxidation-activated self-protection and tumor-specific deoxygenation-driven synergistic therapy, ACS Nano 13 (8) (2019) 8903–8916, https://doi.org/10.1021/acsnano.9b02477.
[94] S.X. Chen, F. Xue, Y. Kuang, S. Chen, D. Sheng, H. Chen, A self-activating nanovesicle with oxygen-depleting capability for efficient hypoxia-responsive chemo-thermo cancer therapy, Biomaterials (2021) 269 120533, https://doi.org/10.1016/j.biomaterials.2020.120533.
[95] C. Wang, J. Yang, C. Dong, S. Shi, Glucose oxidase-related cancer therapies, Advanced Therapeutics 3 (10) (2020), 2000110, https://doi.org/10.1002/adtp.202000110.
[96] Y. Li, L. Zhao, X.F. Li, Targeting hypoxia: hypoxia-activated prodrugs in cancer therapy, Front. Oncol. (2021) 11 700407, https://doi.org/10.3389/fonc.2021.700407.
[97] P. Spagnolo, E. Balestro, S. Aliberti, E. Cocconcelli, D. Biondini, G.D. Casa, N. Sverzellati, T.M. Maher, Pulmonary fibrosis secondary to COVID-19: a call to arms? Lancet Respir. Med. 8 (8) (2020) 750–752, https://doi.org/10.1016/S2213-2600(20)30222-8.
[98] T.D. Hinds Jr., J.F. Creeden, D.M. Gordon, D.F. Stec, M.C. Donald, Stec D.E., bilirubin nanoparticles reduce diet-induced hepatic steatosis, improve fat utilization, and increase plasma beta-hydroxybutyrate, Front. Pharmacol. 11 (2020), 594574, https://doi.org/10.3389/fphar.2020.594574.
[99] Y. Wang, M. Pu, J. Yan, J. Zhang, H. Wei, L. Yu, X. Yan, Z. He, 1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid acetoxymethyl ester loaded reactive oxygen species responsive hyaluronic acid-bilirubin nanoparticles for acute kidney injury therapy via alleviating calcium overload mediated endoplasmic reticulum stress, ACS Nano (2022), https://doi.org/10.1021/acsnano.2c08982.
[100] S. Lee, Y. Lee, H. Kim, D.Y. Lee, S. Jon, Bilirubin nanoparticle-assisted delivery of a small molecule-drug conjugate for targeted cancer therapy, Biomacromolecules 19 (6) (2018) 2270–2277, https://doi.org/10.1021/acs.biomac.8b00189.
[101] Y. Lee, S. Lee, D.Y. Lee, B. Yu, W. Miao, S. Jon, Multistimuli-responsive bilirubin nanoparticles for anticancer therapy, Angew Chem. Int. Ed. Engl. 55 (36) (2016) 10676–10680, https://doi.org/10.1002/anie.201604858.
[102] R. Liu, Y. An, W. Jia, Y. Wang, Y. Wu, Y. Zhen, J. Cao, H. Gao, Macrophage-mimic shape changeable nanomedicine retained in tumor for multimodal therapy of breast cancer, J. Contr. Release 321 (2020) 589–601, https://doi.org/10.1016/j.jconrel.2020.02.043.
[103] Y. Zhou, F. Tong, W. Gu, S. He, X. Yang, J. Li, Y.D. Gao, H. Gao, Co-delivery of photosensitizer and diclofenac through sequentially responsive bilirubin nanocarriers for combating hypoxic tumors, Acta Pharm. Sin. B 12 (3) (2022) 1416–1431, https://doi.org/10.1016/j.apsb.2021.12.001.
[104] S.P. Surendran, R.G. Thomas, M.J. Moon, R. Park, J.H. Lee, Y.Y. Jeong, A bilirubin-conjugated chitosan nanotheranostics system as a platform for reactive oxygen species stimuli-responsive hepatic fibrosis therapy, Acta Biomater. 116 (2020) 356–367, https://doi.org/10.1016/j.actbio.2020.09.014.
[105] X. Zhao, C. Huang, M. Su, Y. Ran, Y. Wang, Z. Yin, Reactive oxygen species-responsive celastrol-loaded : bilirubin nanoparticles for the treatment of rheumatoid arthritis, AAPS J. 24 (1) (2021) 14, https://doi.org/10.1208/s12248-021-00636-3.
[106] L. Lin, L.V. Wang, The emerging role of photoacoustic imaging in clinical oncology, Nat. Rev. Clin. Oncol. 19 (6) (2022) 365–384, https://doi.org/10.1038/s41571-022-00615-3.
[107] C.E. Ahlfors, R.P. Wennberg, J.D. Ostrow, C. Tiribelli, Unbound (free) bilirubin: improving the paradigm for evaluating neonatal jaundice, Clin. Chem. 55 (7) (2009) 1288–1299, https://doi.org/10.1373/clinchem.2008.121269.
[108] H. Huang, M. Guo, N. Liu, C. Zhao, H. Chen, X. Wang, S. Liao, P. Zhou, Y. Liao, X. Chen, X. Lan, J. Chen, D. Xu, X. Li, X. Shi, L. Yu, Y. Nie, X. Wang, C.E. Zhang, J. Liu, Bilirubin neurotoxicity is associated with proteasome inhibition, Cell Death Dis. 8 (6) (2017), e2877, https://doi.org/10.1038/cddis.2017.274.
[109] X. Li, Y. Li, S. Lv, H. Xu, R. Ma, Z. Sun, Y. Li, C. Guo, Long-term respiratory exposure to amorphous silica nanoparticles promoted systemic inflammation and progression of fibrosis in a susceptible mouse model, Chemosphere 300 (2022), 134633, https://doi.org/10.1016/j.chemosphere.2022.134633.
[110] G.S. Kang, P.A. Gillespie, A. Gunnison, A.L. Moreira, K.M. Tchou-Wong, L.C. Chen, Long-term inhalation exposure to nickel nanoparticles exacerbated atherosclerosis in a susceptible mouse model, Environ. Health Perspect. 119 (2) (2011) 176–181, https://doi.org/10.1289/ehp.1002508.
[111] Z. Lyu, S. Ghoshdastidar, K.R. Rekha, D. Suresh, J. Mao, N. Bivens, R. Kannan, T. Joshi, C.S. Rosenfeld, A. Upendran, Developmental exposure to silver nanoparticles leads to long term gut dysbiosis and neurobehavioral alterations, Sci. Rep. 11 (1) (2021) 6558, https://doi.org/10.1038/s41598-021-85919-7.
[112] P. Rivera Gil, G. Oberdorster, A. Elder, V. Puntes, W.J. Parak, Correlating physico-chemical with toxicological properties of nanoparticles: the present and the future, ACS Nano 4 (10) (2010) 5527–5531, https://doi.org/10.1021/nn1025687.
[113] X. Shi, Z. Wang, W. Ren, L. Chen, C. Xu, M. Li, S. Fan, Y. Xu, M. Chen, F. Zheng, W. Zhang, X. Zhou, Y. Zhang, S. Qiu, L. Wu, P. Zhou, X. Lv, T. Cui, Y. Qiao, H. Zhao, W. Guo, W. Chen, S. Li, W. Zhong, J. Lin, S. Yang, LDL receptor-related protein 1 (LRP1), a novel target for opening the blood-labyrinth barrier (BLB), Signal Transduct. Targeted Ther. 7 (1) (2022) 175, https://doi.org/10.1038/s41392-022-00995-z.
[114] L.P. Wu, D. Wang, Z. Li, Grand challenges in nanomedicine, Mater Sci Eng C Mater Biol Appl 106 (2020), 110302, https://doi.org/10.1016/j.msec.2019.110302.
[115] M.A. Younis, H.M. Tawfeek, A.A.H. Abdellatif, J.A. Abdel-Aleem, H. Harashima, Clinical translation of nanomedicines: challenges, opportunities, and keys, Adv. Drug Deliv. Rev. 181 (2022), 114083, https://doi.org/10.1016/j.addr.2021.114083.