Self-assembling bile pigments for cancer diagnosis and therapy

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Abstract
Nanomaterials that integrate multiple functions provide promising opportunities for noninvasive and targeted cancer diagnosis and therapy. However, the unclear metabolic pathway to nanomaterials brought difficulties to clinical application. Self-assembling bile pigments are endogenous functional materials with excellent biocompatibility and low toxicity. Functional materials based on endogenous bile pigments provide a decent solution to this dilemma. In this review, the features and functions of self-assembling bile pigments are discussed in detail for cancer diagnosis and treatment applications. Emphases are put on the intrinsic physicochemical characteristics of bile pigments and their applications, including drug delivery, photoacoustic imaging, photothermal therapy, and anti-inflammatory therapy. This review will promote the exploration of these areas and tremendously realize the innovative applications of self-assembling biliverdin /bilirubin nanomaterials toward cancer diagnosis and therapy.

KEYWORDS
self-assembly, endogenous, bile pigments, nanomaterials, cancer diagnosis and therapy

1 | INTRODUCTION

For decades, cancer has been one of the foremost diseases threatening human health.[1] Conventional cancer treatments (surgery,[2] radiotherapy,[3] and chemotherapy[4]) have serious limitations, including severe drug resistance, strong side effects and toxicity, limited therapeutic effects, and high recurrence rate. Nanomedicine has the advantages of high bioavailability and significant therapeutic effect, which provide a new idea for cancer diagnosis and therapy.[5] In particular, in recent years, nanomaterials with good targeting ability and stimuli-responsive properties have been emphasized.[6] Thus far, research on nanomaterials mainly involves inorganic nanomaterials,[7] organic nanomaterials,[8] and polymer materials.[9] Although some of nanomaterials can be disassembled or degraded in some specific environments, their metabolic pathways have not yet clearly demonstrated and this brings great difficulties for the clinical translation.[10,11] It is particularly urgent to develop nanomaterials with clear metabolic mechanism, excellent biocompatibility, and appropriate photophysical properties.

Bile pigments are a group of open-chain tetapyrrole compounds, which are obtained by oxidative degradation and ring-opening reaction of hemoglobin in nature.[12] Bile pigments are generally considered as metabolic products.[13] Recently, physiological roles and biological functions of bile pigments have been gradually explored.[14] Bile pigments show the inhibition of complement-dependent reaction.[15] Biliverdin (BV) and bilirubin (BR) are typical bile pigment molecules and their metabolic mechanisms have been studied in detail.[14,16] The typical amphiphilicity of BV and BR endows them with admirable self-assembly ability. Furthermore, they have chemical structures similar to porphyrins, which endow them with inherent metal-chelating ability and make their functions diverse. BV has a high extinction coefficient at a specific wavelength range (600–900 nm), showing the potential of photothermal therapy (PTT) and photoacoustic imaging (PAI).[17] BR has been found with outstanding anti-inflammatory effect due to its antioxidant capacity, meanwhile, in the atmosphere...
of oxidation, BR can be converted to BV and perform the functions of BV.\(^{16c,18}\)

Self-assembling bile pigments overcome the water-insoluble problem of bile pigments, thus maintaining their biocompatibility and endowing them with stability and targeting ability.\(^{19}\) Through the self-assembly strategy, the size distribution of nanoparticles (NPs) can be accurately regulated, and the drug release can be effectively controlled, so as to improve the bioavailability of bile pigments. High biosafety and clear metabolic pathway are the most basic and important requirements for the transformation of nanomaterials from scientific research to clinical practice.\(^{20}\) Compared with inorganic, organic, and polymer nanomaterials, functional materials based on endogenous molecules show significant superiority.\(^{21}\) Self-assembling bile pigment realizes the integration of multicomponents and multifunctions, and can be used in the early noninvasive diagnosis and treatment of tumors with high safety.

In this review, we highlight recent advances in the area of self-assembling bile pigments in cancer diagnosis and therapy (Figure 1). We first discuss two representative bile pigments, BV and BR, including their endogenous characteristics, structural features, and biological activities. Then, we discuss self-assembling bile pigments toward innovative cancer treatment modalities, including antitumor phototherapies, inflammatory-immunological micro-circumstance treatment, and photoinmunotherapy. Several representative nanomaterials constructed by the self-assembly of bile pigments are discussed in detail. The biosafety and metabolic mechanism of self-assembling BV and BR are emphasized. We believe that the utilization of endogenous bile pigments will show promising prospects in the biomedical fields. The safety and effectiveness of self-assembling bile pigments will bring a new dawn for treating clinical diseases.

2 | FEATURES OF BILE PIGMENTS

Bile pigments are products of endogenous heme decomposition and they show good biocompatibility, low toxicity, and clear metabolic mechanism in vivo.\(^{12a}\)

2.1 | The molecular structure

Bile pigment molecule consists of four pyrrole rings (marked as A, B, C, and D rings in turn), which are bridged by three carbon atoms. According to the number of bridged bonds, bile pigments can be classified as belane (zero double bond, e.g., urobilinogen), bilene (one double bond, e.g., stercobilin), biladiene (two double bonds, e.g., BR), and bilatriene (three double bonds, e.g., BV).\(^{22}\)

For BR, the double bonds are between the A/B ring and the C/D ring respectively. Generally, A/B ring and C/D ring lie in the same plane. The intramolecular hydrogen bond can be established between two planes, so BR is usually insoluble in water. However, when BR is exposed to blue light, the intramolecular hydrogen bond will break, and the solubility of BR in water will be significantly improved. Researchers can utilize this feature to design controllable nanomaterials.\(^{23}\) For BV, all three methylene bonds are double bonds. When the three bridge double bonds are cis (Z-type), BV is spiral structure; when they are trans (E-type), BV is chain structure.\(^{24}\)

Bile pigments have inherent metal-chelating ability.\(^{25}\) The nitrogen atoms of pyrrole rings can provide lone pair electrons to occupy empty d orbit of transition metal ions to form coordination bonds. When BR or BV forms 1:1 complexes with metal ions, the original linear structure will convert into a ring structure (similar to the structure of porphyrin) to be adapted to the chelation with central metal
ions. When bile pigment is combined with Mn$^{2+}$, magnetic resonance imaging (MRI)-guided antitumor phototherapy can be achieved.\textsuperscript{[17]}

2.2 | Metabolic pathway

BV is formed by the oxidation of heme by heme oxygenase (HO-1), the loss of ferrous iron, and CO, and then the opening of porphyrin ring.\textsuperscript{[14c,f,18]} Next, BV will be reduced to BR by biliverdin reductase (BVR). Then, BR will be absorbed in blood by hemoglobin to form unconjugated bilirubin (UCB) and the UCB will diffuse to the cytoplasm/endoplasmic reticulum of hepatocytes. UCB will be converted here by glucuronosyltransferase to bilirubin diglucuronate (BDG) and enters bile. After that, BDG will enter the intestine through the bile capillary, and be hydrolyzed and reduced to bilinogen. Part of the bilinogen (about 10–20%) will enter the sinusoid endothelium through the portal vein of the liver and circulates again. The rest of the bilinogen will be oxidized to urobilinogens and stercobilinogens, which are further excreted through the colon.\textsuperscript{[22]}

2.3 | Biological characteristics

BV and BR are available in mammals bile.\textsuperscript{[26]} BV has two main absorption bands: one is between 350 and 400 nm, called Q band; the other is between 600 and 900 nm, called S band. Absorption more than 650 nm is favorable for in vivo applications, because photons above 650 nm have the least absorption and scattering by biological tissue (i.e., the first biological imaging window, NIR-I, 600–950 nm).\textsuperscript{[27]} The high extinction coefficients of BV in the wavelength range of 600–900 nm make it photothermal advantages. BR is an effective endogenous antioxidant agent, which can clear all kinds of reactive oxygen species (ROS) and play an indispensable role in protecting cells and tissues from oxidative damage.\textsuperscript{[28]} Moreover, BR has intrinsic anti-inflammatory\textsuperscript{[29]} and anti-cancer activities.\textsuperscript{[30]} Due to the existence of intramolecular hydrogen bond, the water solubility of free BR is poor. However, BR can be stimulated by a specific wavelength of light (e.g., 450 nm), and then the water solubility of BR increases significantly with the destruction of intramolecular hydrogen bonds.\textsuperscript{[19b,31]} Furthermore, ROS can oxidize BR to BV,\textsuperscript{[29]} which can also dramatically improve the water solubility of BR. These characteristics provide great potential for construction of self-assembled material with multiple stimulus responsiveness from bile pigments.

These intrinsic properties of bile pigments endow them with critical position in anti-inflammatory and anti-tumor therapy applications. The high photon absorption of BV makes it a potential photothermal nanomaterial.\textsuperscript{[17]} Meanwhile, BR can be transformed into BV under certain conditions, so that BR can perform the same function as BV in phototherapy. These characteristics determine that self-assembling bile pigments have great potential in precision medicine. However, bile pigments are easy to be oxidized, so attention should be paid.

2.4 | Mechanism of self-assembly of bile pigments

The self-assembly of bile pigments is driven by weak interactions including hydrogen bonding, $\pi-\pi$ stacking, and hydrophobic interaction. The propionic acid chain of the bile pigments will bond with the oxygen atom at the carbonyl group of another bile pigment molecule to provide hydrogen bonding sites. Due to the presence of $\pi$ electrons on the pyrrole rings and bridged bonds, $\pi-\pi$ stacking between bile pigment molecules can also occur. In the polar solution, when the bile pigment molecules are close to a certain distance, the apolar bile pigment molecules will squeeze out the surrounding orderly arranged solvent molecules and combine with each other through hydrophobic interaction, which will increase the entropy of the system, thus reducing the system-free energy and enhancing stability.\textsuperscript{[17]} Modification of the propionic acid chain of bile pigments can also change its assembly ability to a certain extent. For example, the combination of acrylic acid chains with polysaccharides increases their water solubility, making them easier to form nanospheres.\textsuperscript{[23]}

2.5 | Features of self-assembling bile pigments

Self-assembling bile pigments have higher photostability because the internal pigments in nanostructures isolate oxygen. Compared with monomer of bile pigments, self-assembled bile pigments can load chemotherapeutic drugs for combined treatment to improve the therapeutic efficacy. Self-assembly of bile pigments can provide high delivery efficiency through passive targeting; in addition, bile pigment can also be conjugated with targeting molecules to achieve active targeting. Compared with the monomer, self-assembled bile pigments have better circulation stability in vivo and higher photothermal conversion efficiency due to the supramolecular photothermal effect.\textsuperscript{[21b]} The self-assembly will result in the broadened absorption of bile pigments, which benefits the deeper tissue penetration for cancer treatment.

3 | SELF-ASSEMBLING BILE PIGMENTS FOR DRUG DELIVERY

When small molecule chemo-therapeutic is administered intravenously for cancer treatment, they usually encounter the disadvantages of poor circulation time and unsatisfactory tumor enrichment.\textsuperscript{[32]} In order to overcome these shortcomings, researchers tried to use nanodrug delivery system to prolong the drug circulation time, enhance the drug concentration in the tumor location, reduce their accumulation in organs, and reduce the side effects.\textsuperscript{[6b,33]} The key of drug delivery is to concentrate the drugs in the tumor lesions. Meanwhile, there is no drug leakage in the delivery process, and the carrier does not cause additional toxicity.\textsuperscript{[32b]} The nanomaterials based on hydrophobic BV and BR can well encapsulate Adriamycin, cisplatin, and other highly toxic chemotherapeutic drugs. These nanomaterials can not
only reduce the toxicity of chemotherapeutic drugs but also improve their tumor targeting, and promote their release in tumor microcirculation.\textsuperscript{[34]}

The drugs with polarity similar to bile pigments can be encapsulated into the assembled nanostructures of bile pigments mainly by the $\pi$-$\pi$ stacking and hydrophobic interaction. For instance, Gao and coworkers\textsuperscript{[35]} synthesized a ROS-sensitive PEGylated BR. BR was self-assembled into NPs and encapsulated d-SN38 and d-LND, forming SL@BRNPs in aqueous solution. The obtained NPs can further combine with iRGD and anti-PD-L1 to make the tumor sensitive to chemotherapy and respond to immunotherapy, so as to make it better in synergistic anti-tumor therapy.

Jon and his coworkers prepared BR nanoparticles (BRNPs) with a diameter of about 105 nm by the self-assembly of PEGylated BR and stable amide bond.\textsuperscript{[19b]} The results show that BRNPs can decompose in 10 and 1 min, respectively, after being stimulated by ROS and a certain wavelength of laser (450 nm and 650 nm), indicating that BRNPs have the potential to act as a responsive drug carrier (Figure 2A). In general, the loading capacity of DOX on nanocarriers is less than 10%.\textsuperscript{[36]} In this work, as both BR and DOX have aromatic planar rings, DOX can be easily encapsulated into BRNPs with an efficiency of nearly 100% and a maximum loading capacity up to 23% (wt%). In the subsequent of animal experiments, the tumor growth of DOX@BRNPs group was inhibited by 55.0%, about twice than that of free DOX (Figure 2B).

To further improve the targeting performance, the researchers modified the NPs with biotin (DOX@bt-BRNPs) in the subsequent improvements (Figure 2C).\textsuperscript{[37]} DOX@bt-BRNPs accumulates preferentially in Hela tumors through the passive enhanced permeability and retention (EPR) effect and the specific biotin–biotin transporter interaction after intravenous injection, thus producing a stronger anticancer effect than free DOX and un-targeting modified ones. In addition, the NPs can also be in response to ROS in the tumor micro-environment. The uptake of DOX@bt-BRNPs in tumor was much higher than that of free DOX and DOX@BRNPs, while that in liver, lung, and other organs was much lower (Figure 2D). In vivo experiments showed that DOX@bt-BRNPs significantly enhanced the anti-tumor effect and inhibited tumor growth by 93%, which were 1.7 times than that of DOX@BRNPs. When BR was exposed to 450 nm or 650 nm light, the intramolecular hydrogen bond of BR was destroyed, and the water solubility of BR increased, so that drugs carried in BRNPs could be released. In this work, the authors made full use of the photosensitivity of BR. It is worth noting that BRNPs are completely composed of PEGylated BR, which can be synthesized on a large scale as a pure chemical entity after regular column chromatography.
When BR was exposed to light or ROS, the intramolecular hydrogen bonds would be destroyed and the solubility would increase. Utilizing this property, BR and photosensitizer can be co-assembled into NPs. When the co-assembled NPs were exposed to light, BRNPs would be transformed into nanofibers, which would be better retained in the tumor. For example, Gao and his group members synthesized Ce6/BR-FFVLK-PEG chimeric molecules with both hydrophilic and hydrophilic ends through the series connection of hydrophobic Chlorin e6 (Ce6), BR, short peptide (Phe-Phe-Val-Leu-Lys, FFVLK, to form hydrogen bond), and hydrophilic polyethylene glycol (PEG) (Figure 3A).[38] The molecule can assemble into micelles in aqueous solution, and then transform into nanofibers under a 650 nm laser irradiation (Figure 3B). This means that during laser irradiation, nanofibers are formed in situ in the tumors, and remain for a long time for sustained drug release. A ROS-responsive paclitaxel dimer with thioketal linker (PTX2-TK) was chosen as a model drug so as to carry out combinational chemo-photodynamic treatment for breast cancer. Evaluations in vitro and in vivo reveal that the PTX2-TK@Ce6/BR-FFVLK-PEG shows significant tumor suppression. As BR can react with ROS and consume ROS, the ROS shielding effect caused by BR could indeed prevent the nonspecific phototoxicity. Although BR consumes the ROS generated by photosensitizer, the residual ROS remains important. Upon irradiation, they almost induce 100% of cell death, thus achieving the remarkable cytotoxicity.

4 | SELF-ASSEMBLING BILE PIGMENTS FOR LIGHT-INDUCED THERAPEUTICS

Due to excellent photothermal conversion efficiency, bile pigments are often used in light-induced therapeutics including PTT and PAI. PTT takes use of materials with high photothermal conversion efficiency, which can accumulate in the tumor tissue and realizes the conversion of light energy to heat energy under the irradiation of an external light source (usually NIR light), so as to kill cancer cells by local hyperthermia.[6a,39] PAI is a nonionizing and noninvasive imaging modality based on the photoacoustic effect. PAI utilizes photothermal materials to absorb short pulse laser and transforms the optical signal to an ultrasonic signal to realize noninvasive imaging, capable of visualizing optical
Abundant NIR-absorbing nanomaterials, ranging from organic to inorganic nanomaterials, have successfully demonstrated their PAI and/or PTT performance in vivo,[39a,b,d,41] However, these nonbiodegradable or slowly degradable nanomaterials including Au nanorods,[42] carbon tubes,[43] or polymer NPs[44] will cause potential side effects arising from long-term residence, which may greatly hinder their translation into clinical applications. In fact, it has been reported that 30–99% of NPs will accumulate in the liver, and long-term accumulation of NPs has been proved to have negative effects on liver and kidney.[45] Therefore, there is an urgent need to develop PAI and PTT nanomaterials with high NIR conversion efficiency, non-toxic, degradable, and clear metabolic pathways. Endogenous bile pigments will be excellent options.

Jon and his coworkers reported a cisplatin (a model Pt ion source) chelated BR-based NP (cisPt@BRNP) using the intrinsic metal-chelating ability of BR, as new photon-nanodrugs for combined PAI and antitumor PTT.[19a] In this work, cisplatin and BRNPs were simply mixed to prepare cisPt@BRNPs with approximately 150 nm in size. Inspired by the structure of BR in black pigment gallstones, the lone-pair electrons of BR in NPs may participate in the coordination with the Pt II center (Figure 4A). In animal experiments, cisPt@BRNPs achieved effective tumor visualization through PAI on a HT-29 human colorectal cancer xenograft tumor model. The authors confirm that the blood circulation time of cisPt@BRNPs is about 2.5 times than that of cisplatin, and it can be accumulated in the tumor through EPR effect. At 1 h postinjection, a perceptible signal distinguishable from the background emerged, and then reached a maximum value at 6 h (Figure 4B). The tumors were irradiated with an 808 nm laser (1.0 W cm$^{-2}$ for 10 min) for PTT of cancer. Obvious temperature elevation was observed in tumors treated with the cisPt@BRNP, which increased to 55$^\circ$C within 10 min, whereas the temperature was only mildly elevated in mice treated with PBS (Figure 4C). The tumors in mice treated with cisPt@BRNPs and 808 nm laser irradiation almost disappeared on day 9 (Figure 4D). This biocompatible nanomedicine based on bile pigment exhibited tumor visualization potential and remarkable therapeutic efficacy through PTT.

The high biocompatibility of BV can be fully utilized in the diagnosis. Pan and coworkers developed biodegradable BV nanoparticles (BVNPs) with inherent PAI capabilities.[34] They report a synthesis method of fully biodegradable BVNPs, which exhibit photoacoustic properties in vivo without dyes or metal-chelation. BVNPs were developed through a nano-precipitation strategy by mixing biliverdin hydrochloride with stoichiometric amounts of 1-ethyl-3-(3-(dimethylamino) propyl) carbodiimide and 2, 2'-ethylenedioxy bis (ethylamine). They found that the reaction products were highly dependent on the reaction media. Water-BVNPs, MES-BVNPs, and NaCl-BVNPs can be obtained in water, 2-(N-morpholino) ethanesulfonic acid buffer, and in aqueous NaCl solution, respectively (Figure 5A). PAI experiments show that NPs accumulate in lymph nodes, indicating the potential application value of NPs as photoacoustic agents for sentinel lymph node detection (Figure 5B). After imaging, BV was reduced to water-insoluble BR by BVR and completely degraded in vivo (56%, 77%, and 73% degradation in 24 h for water-BVNPs, MES-BVNPs, and NaCl BVNPs, respectively) (Figure 5C–E). This nanosystem based on the self-assembly of bile pigments can completely disappear from the biological system, which will promote the clinical transformation of drugs based on endogenous pigments.

BV shows strong light absorption in the range of 600–900 nm, which can effectively convert light energy to local hyperthermia, realizes the thermal ablation of cancer cells, and eventually eliminates the tumor tissues. Due to the inherent metal-chelating ability of bile pigments, we can combine the self-assembling bile pigments with functional metal ions such as Mn$^{2+}$ or Gd$^{3+}$ to perform multiple diagnosis and treatment functions. Recently, Yan’s group developed a photothermal agent based on self-assembling BV.[17] In this work, the authors take BV, Z-His-obzl (ZHO), and Mn$^{2+}$ as building blocks for the supramolecular self-assembly of
**Figure 5** (A) The synthesis method and nanoparticle size of BVNPs (water-BVNPs, MES-BVNPs, and NaCl-BVNPs). (B) PAI of sentinel lymph nodes using BVNPs in vivo. The mice were administered BVNPs via hock injection in the hind limb. Excitation laser was set as 680 nm. LN, lymph node; LV, lymphatic vessel. In vivo degradation of (C) water-BVNPs, (D) MES-BVNPs, and (E) NaCl-BVNPs for PAI acquisition wavelengths of 680, 720, and 750 nm along with time.[34] Copyright 2019, American Chemical Society

BVNPs (ZBNPs) with NIR absorption (Figure 6A). Compared with BV monomer, BV agent exhibited better photostability and higher photothermal conversion efficiency. For example, after laser irradiation (730 nm, 0.3 W cm\(^{-2}\)) for 10 min, the monomer BV was photooxidized, almost lost the near-infrared absorption, while the NIR absorption of ZBNPs only slightly decreased. It shows that BVNPs can greatly inhibit the photodegradation process. In vivo data reveal that the BVNPs can selectively accumulate in tumors and act as multimodal contrast for tumor visualization through both PAI and MRI (Figure 6B and C). Besides, experiments in vivo showed that BVNPs have excellent photothermal conversion capability. After intravenously 6 h postinjected with 5% glucose solution or BVNPs, the tumors of MCF-7 tumor bearing mice were irradiated by 730 nm laser for 10 min (0.3 W cm\(^{-2}\)). The temperature of tumors in BVNPs groups raised to \(\sim 58^\circ\text{C}\), in contrast, the temperature of tumors in blank group raised to only \(\sim 43^\circ\text{C}\) (Figure 6B). After PTT, tumor growth was significantly inhibited in BVNPs groups (Figure 6C). The BVNPs exhibited high biocompatibility and biodegradability with superior biosafety, and they were almost completely cleared at 72 h after the injection through liver and gallbladder circulation.

Intriguingly, the near-infrared fluorescent emission can be achieved when BV is bound to specific proteins and thus they can be used for bioimaging and diagnosis.[46] The conformation of BV is stabilized in the protein and the energy of excited state cannot be dissipated by intramolecular vibrational relaxation. Narikawa’s group engineered cyanobacteriochrome photoreceptors and successfully inserted BV in them to emit near-infrared fluorescence in mammalian liver.[46a]

**5 | SELF-ASSEMBLING BILE PIGMENTS FOR ANTI-INFLAMMATION OR CANCER IMMUNOTHERAPY**

The inflammatory reaction can damage host tissues, cause organ dysfunction, and even lead to tumor or tumor metastasis and recurrence. In some cases, inflammation can modulate signaling pathways and lead to chronic disease.[6c,47] Treatment for inflammation is often dissatisfactory, because
the inflammatory microenvironment is synergistic by many molecules, so it may not be enough to only target one or several molecules. Therefore, anti-inflammatory treatment must target a range of inflammatory sources, such as bacterial flora, to achieve the desired therapeutic effect.

Recent report by Moon’s group shows the prospect of oral BRNPs regulating the intestinal barrier, microbiome, and immune response. In this work, water-soluble BRNPs (HABN) were prepared by coupling BR with hyaluronic acid (HA). The NPs can be targeted to inflammatory colonic epithelial cells and pro-inflammatory macrophages through the interaction between HA and CD44. In addition, BR core endowed HABN and hyaluronidase resistance and strong ROS scavenging ability, preventing colon epithelial cell apoptosis. Animal experiments show that HABN can effectively inhibit the development of colitis in mice and repair the epithelial cell barrier. In addition, HABN also regulates the intestinal microbiota, increasing the overall richness and diversity and significantly increasing the number of *A. muciniphila*, *Clostridium XIVa*, and *Lactobacillus*, which are beneficial intestinal bacteria. The work shows that oral nanomaterials can target the inflammatory colon and directly regulate the gut’s response to colitis. Since intestinal flora disorders and intestinal barrier functions are highly correlated with systemic diseases, this strategy may provide a convenient template for the treatment of other inflammatory diseases.

With the ability of scavenging ROS, bile pigment can be used in kinds of inflammation. For example, Lee’s group demonstrated that bile pigment can be used to treat allergic pneumonia (Figure 7). It is important that the hydrophilic modification and assembly design of bile pigments can greatly improve the therapeutic effect through pharmacokinetic experiments. They also demonstrated the effect of BR in allogeneic transplantation. These results reflect the necessity of modification and self-assembly design of bile pigments.

**6 CHALLENGES AND OPPORTUNITIES**

In summary, we reviewed the functional materials based on the self-assembly of endogenous bile pigments, especially the representative BV and BR. The latest progress of self-assembling bile pigments for multimodal imaging and nanomedical therapy against cancer is discussed and summarized in Table 1. Bile pigments have high biosafety and clear metabolic pathways, which can be used in the treatment of multiple tumors at various sites, especially...
FIGURE 7 Synthesis and effects of BRNPs in murine experimental asthma. (A) PEG-BR was synthesized from bilirubin and PEG. (B) The overall scheme for the induction of airway inflammation and application of BRNPs in mice. (C) Pharmacokinetic profiles of BRNPs and UCB. (D) Sections of lungs were stained with PAS. Arrows indicate metaplastic goblet cells. Copyright 2020, Elsevier B.V

TABLE 1 Summary of the self-assembling bile pigments for cancer diagnosis and therapy

| Bile pigments | Modifiers /conjugates | Drug loaded | Nanoformulation | Features | Reference |
|---------------|-----------------------|-------------|----------------|----------|-----------|
| BR            | PEG2000/PEG3400-Biotin| DOX, cisplatin | Nanoparticles | Multi-responsive drug delivery | [19a,b, 37] |
| BR            | FFVLK-PEG             | PTX         | Nanoparticles to Nanofibers | Targeted drug delivery | [38] |
| BV            | His-containing peptide, Mn$^{2+}$ | \ | Nanoparticles | Supramolecular self-assembly, NIR absorption, PAI/MRI | [17] |
| BV            | MES, NaCl             | \ | Nanoparticles | Biodegradation, PAI | [34] |
| BR            | HA                    | \ | Nanoparticles | Anti-inflammatory, targeted modulation of intestinal microflora | [23] |

those located in sensitive areas and difficult to be surgically removed (such as cancer of eyes, brain, pancreas, and prostate). Self-assembling bile pigments will also play key roles in metastatic neck squamous cell carcinoma and highly metastatic lung cancers.

In addition to high biocompatibility and clear metabolic mechanism, self-assembling bile pigments show unparalleled advantages. For drug delivery, due to the strong antioxidant capacity of BV/BR, it can protect the active drugs (such as indoles) from oxidation. In phototherapy, self-assembling bile pigments (BV/BR) exhibit excellent targeting ability and high photothermal transformation efficiency. Further modification of BV or BR can increase its absorption, further enhance the penetration depth of laser in vivo, and achieve better imaging and therapeutic effect. Furthermore, BV/BR shows inherent anti-inflammatory or anti-oxidation ability. BVNPs/BRNPs can achieve the integration of anti-inflammatory with tumor immunotherapy. Bile pigment has anti-inflammatory activity, can inhibit the release of inflammatory related signal molecules, reverse the inflammatory microenvironment of tumors, achieve the enhancement of immune and antitumor effect, and effectively prevent tumor metastasis and recurrence. Besides, BV can be used as near infrared chromophore in some proteins. It may be expired to use BV as a building block to realize drug loading and in situ fluorescence detection at the same time. Although the nanomaterials of bile pigments can be facilely synthesized by a self-assembly protocol, for their clinical translation, one needs to consider the scale-up procedures that involve the engineering technique. Due to the complexity of nucleation of self-assembling bile pigments, we should pay more attention to the diffusion of mass and energy in order to obtain the nanomaterials with controlled quality in batch for the formulation. Actually, the engineering of self-assembled nanomaterials remains a formidable challenge nowadays, thus more efforts should be devoted by a combination of scientists and engineers. Nevertheless, we believe that this review can greatly promote the exploration of these aspects, and ultimately realize the innovative application of self-assembling bile pigment materials in the field of tumor diagnosis and therapeutics.

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