

A Novel Protein-Polysaccharide Oral Nanoemulsion Targeting Activated Hepatic Stellate Cells to Enhance the Therapeutic Effect of Pirfenidone on Fibrosis After Transarterial Chemoembolization for Liver Cancer

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Transarterial chemoembolization (TACE) is considered the main treatment for intermediate and advanced liver cancer. Nevertheless, TACE may aggravate liver fibrosis in these patients, which could affect the therapeutic effect after TACE. Pirfenidone (PFD) exhibits significant antifibrotic effects in the liver, primarily via inhibition of hepatic stellate cells (HSCs) activation. However, owing to the high dose required for effective treatment, oral administration of PFD is associated with several side effects. This study introduces an oral folic acid (FA)-modified protein-polysaccharide PFD nanoemulsion designed to treat post-TACE liver fibrosis via liver targeting. This novel PFD oral nanoemulsion withstands gastrointestinal digestion and ensures the gastrointestinal stability of PFD. Furthermore, this nanoemulsion improves the intestinal permeability and antifibrotic efficacy of PFD at a lower dose via folate receptors expressed on both intestinal epithelial cells and activated HSCs. In conclusion, this FA-modified protein-polysaccharide nanoemulsion presents a promising approach for oral PFD delivery to effectively ameliorate fibrosis after TACE for liver cancer.

after TACE.^[2] The primary characteristic of liver fibrosis is the excessive accumulation of extracellular matrix (ECM) in the liver, resulting in the deterioration of liver structure and function.^[3] As indicated by previous studies, hepatic stellate cells (HSCs) activation plays a crucial role in this process,^[4,5] by driving the progression of liver fibrosis and remodeling intrahepatic structures by stimulating ECM secretion.^[6]

Liver fibrosis is a dynamic and reversible condition. However, early diagnosis of liver fibrosis is challenging because of its subtle clinical manifestations of this condition.^[7,8] Currently, no medications are authorized for the treatment of liver fibrosis in general. Pirfenidone (PFD) is the first broad-spectrum antifibrotic medication to be used clinically in the treatment of idiopathic pulmonary fibrosis (IPF).^[9] PFD possesses distinctive anti-inflammatory, antifibrotic, and

anti-oxidative stress properties that have been shown not only to impede the advancement of chronic fibrosis but also to efficiently mitigate inflammation during the acute phase of IPF.^[10] Consequently, both the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have endorsed the use of PFD in the clinical management of IPF. Previous research also reported that PFD could suppress HSCs activation and diminish

1. Introduction

Liver cancer is the third most common cause of cancer-related deaths worldwide.^[1] In intermediate and advanced liver cancer patients, transarterial chemoembolization (TACE) is a leading treatment. Nevertheless, the use of TACE may aggravate liver fibrosis in these patients, which could affect the therapeutic effect

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the expression of HSP47 and collagen I,^[11] suggesting that this agent has the potential to prevent fibrosis after TACE for liver cancer.

PFD is administered orally in clinical settings.^[12] However, according to the recommended dosing schedule when prescribing Esbriet (PFD), the full daily dose of pirfenidone is nine pills (267 mg each, yellow tablets or white capsules).^[13] These systemic and chronic daily doses can lead to off-target toxicities and significant patient discomfort.^[14–16] Thus, there is an urgent need to achieve better therapeutic outcomes at a lower PFD dosage through targeted distribution and increased cellular uptake. Oral targeting is a noninvasive drug delivery strategy that employs nano-delivery systems to accomplish systemic or remote targeting via the oral route.^[17,18] This method can identify target tissues or cells more accurately and efficiently and offers the advantages of convenient administration and high compliance. Therefore, an orally targeted formulation of PFD is anticipated to achieve effective antifibrotic effects.

Oral targeting necessitates the surface functional design of the delivery system to surmount gastrointestinal barriers and rejection, which improves bioavailability and ensures accumulation at the targeted liver region.^[19] Nanoemulsions are a promising oral drug delivery mechanism that can improve the bioavailability and efficacy of the drug through its favorable absorption properties.^[20] Numerous food proteins, with excellent emulsifying capabilities, can serve as emulsifiers for nanoemulsions.^[21] Zein, a natural plant protein derived from corn gluten meal, has a high content of hydrophobic residues and polar groups, embodying the characteristics of a typical amphiphilic polymer.^[22] The peptide bonds in zein undergo cleavage in a deamidation reaction under acidic or alkaline conditions, producing deamidated zein peptide (ZP).^[23] Deamidated ZP, prepared through alkaline hydrolysis, reportedly has increased hydrophobic residues and polar groups on its surface, thereby enhancing its emulsification properties.^[24] However, the thin and charged nature of most protein interface membranes makes them susceptible to environmental factors in the gastrointestinal tract, including pH, ionic strength, and enzymatic hydrolysis.^[25,26] Dextran (DEX) has been shown to create an expansive spatial network structure during the aqueous phase, which enhances the stability of the delivery system through site resistance.^[27] Consequently, nanoemulsions formulated with ZP and DEX synthesized conjugates are expected to resist gastrointestinal digestion and improve the stability of the delivery system in the gastrointestinal tract.

An oral nanodrug delivery system must not only withstand degradation in the gastrointestinal tract but also penetrate biofilms. Emerging evidence suggests that specific ligands such as folic acid (FA),^[28] vitamin B₁₂,^[27] and transferrin,^[29] enhance the penetration of nanoparticles through the intestinal epithelium via receptor-mediated endocytosis, thereby improving oral

absorption. Among them, FA, a critical water-soluble vitamin characterized by low immunogenicity and high stability, is frequently conjugated to the surface of nanocarriers to boost the delivery of small molecules and protein drugs and to achieve optimal therapeutic outcomes.^[30–32] Given expression of folate receptor (FR)-alpha increases upon HSCs activation,^[33] FA targets not only folate receptors on intestinal epithelial cells to enhance intestinal permeability but also the activated HSCs with overexpressed FR, thereby augmenting the antifibrotic effects of PFD.

In this study, a DEX-FA conjugate was created via an esterification reaction and ZP-DEX-FA conjugates (ZD-FA) were subsequently used to prepare a PFD nanoemulsion (PFD@ZD-FA) designed to treat fibrosis after TACE for liver cancer through oral administration (**Scheme 1**). The results indicated that this FA-modified protein-polysaccharide nanoemulsion efficiently encapsulated PFD and improved its transportation across epithelial cells in the small intestine, leading to improved oral bioavailability of PFD. Moreover, the nanoemulsion extended the residence time of PFD in the gastrointestinal tract and facilitated its accumulation in the targeted liver region, particularly in activated HSCs, resulting in a heightened antifibrotic effect of PFD at a lower dosage. These findings suggest that the ZD-FA nanoemulsion developed in the current study can serve as an oral delivery nanopatform for PFD to achieve liver-targeting and improve the efficacy of PFD in the treatment of fibrosis after TACE for liver cancer.

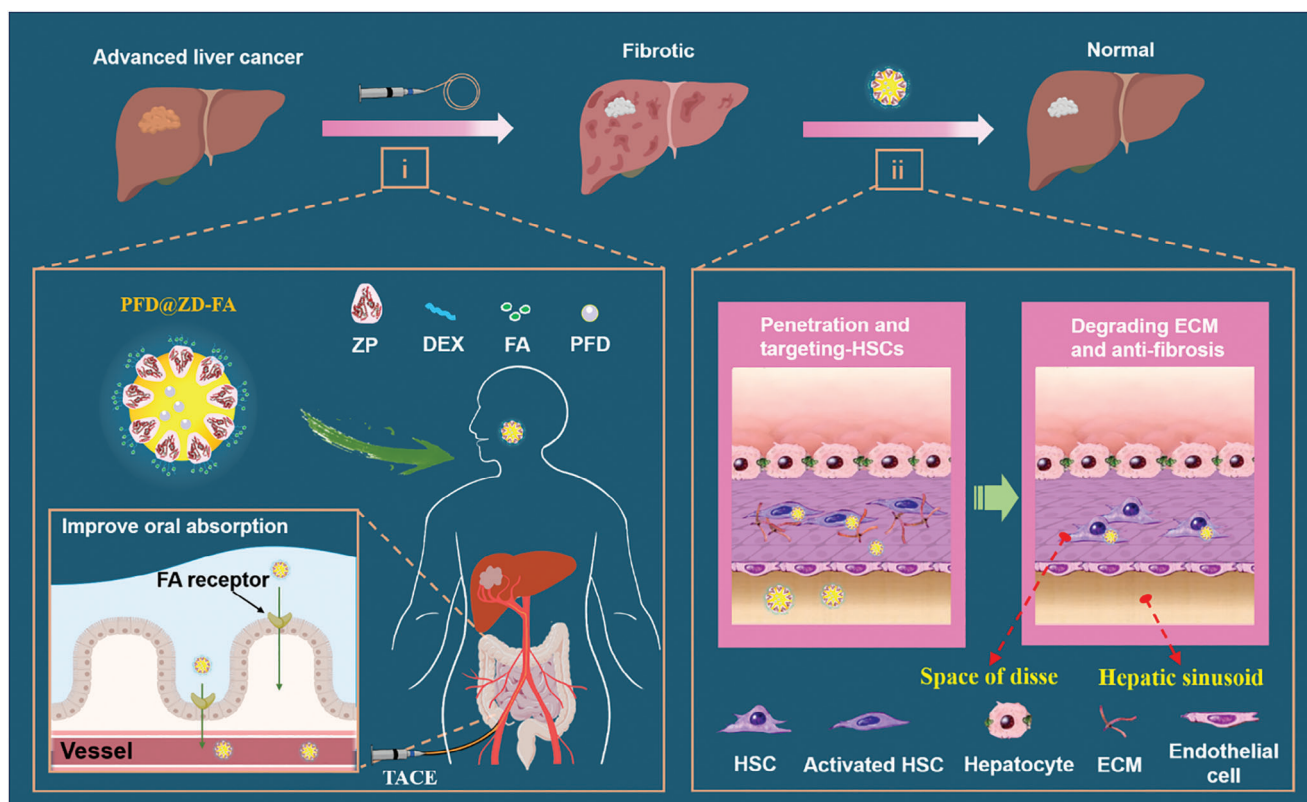
2. Results and Discussion

2.1. Development and Progression of Fibrosis after TACE for Liver Cancer

TACE is the preferred treatment for intermediate and advanced liver cancer. Nevertheless, TACE has been reported by previous clinical studies to cause liver damage and exacerbate liver fibrosis.^[2] At present, the diagnosis of liver fibrosis after TACE is mainly through non-invasive methods such as liver function indicators, computed tomography (CT), and magnetic resonance imaging scans, which cannot accurately reflect the level of fibrosis.^[34] To assess and confirm the degree of liver fibrosis after TACE for liver cancer, we collected pre- and post-TACE blood test results from 189 intermediate and advanced liver cancer patients who had undergone the TACE. The aspartate transaminase-to-platelet ratio index (APRI) and fibrosis 4 score (FIB-4) as the non-invasive markers of fibrosis,^[35] were evaluated in these patients (**Figure 1A,B**). The results indicated that both APRI and FIB-4 were significantly increased after TACE.

Clinical studies showed that the tumor volume of some patients with intermediate liver cancer may be reduced after TACE, and the liver tumor can be removed by surgery. Surgical excision is the leading treatment for early-stage liver cancer. Therefore, in the current study, liver and tumor tissues were collected from these patients to determine the degree of liver fibrosis (**Figure S1**, Supporting Information). Samples were divided into three regions according to tumor location: the para-cancerous liver (within 2 cm of the tumor margin), the junction area between the liver and tumor, and the tumor itself (**Figure 1C**). These samples were analyzed for cellular hardness using atomic force microscopy (AFM) (**Figure 1D–F**), which may be associated with

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Scheme 1. PFD@ZD-FA administered orally is able to overcome the gastrointestinal tract barrier and target activated HSCs in the space of disse, enhancing the antifibrotic effect of PFD.

the therapeutic effect of liver cancer. The findings indicated that liver hardness was 8.8-fold higher in the para-cancerous livers treated with TACE than in para-cancerous livers not treated with TACE (Figure 1D). This increase in tissue hardness was also observed in the junction area and tumor of patients treated with TACE versus those without TACE (Figure 1E,F). According to the section of the gross specimen, the tumor peripheral envelope thickened significantly after TACE treatment, compared to the without TACE group (Figure 1G). Further, staining with Sirius red in the junction area revealed minimal collagen fiber deposition in patients who had not undergone TACE. Conversely, a significant collagen fiber accumulation of was noted in the junction area in patients treated with TACE (Figure 1H,J). The progression of fibrosis is known to be associated with HSCs activation,^[4,5] and α -smooth muscle actin (α -SMA, a biomarker of HSCs activation) expression in the junction area was higher in patients who underwent TACE than in those who did not (Figure 1I,K). In vitro experiments demonstrated that PFD nanoemulsions exhibited less cytotoxicity to hepatocytes compared with HSCs, indicating the specific inhibitory effect of PFD on the HSCs (Figure S2, Supporting Information). Therefore, we speculated that PFD may be effective in mitigating fibrosis after TACE for liver cancer.

2.2. Preparation and Characterization of PFD@ZD-FA

DEX-FA conjugates were synthesized via an esterification reaction involving the γ -carboxylic acid group of FA and the hydroxyl

group of DEX. The $^1\text{H-NMR}$ spectrum of DEX-FA displayed characteristic peaks of both DEX and FA (Figure 2A), with distinct signals of DEX (α protons) at 4.68 ppm. Additionally, characteristic signals of FA at 8.64 ppm (pterin protons, β) and 7.58 and 6.64 ppm (γ and δ aromatic protons) validated the presence of FA residues. Fourier transform infrared spectroscopy (FT-IR) analysis was also employed to verify ZD and ZD-FA. The FT-IR spectra for FA, DEX, ZP, ZD, and ZD-FA are shown in Figure 2B. ZP demonstrated characteristic peaks at 3445, 1656, and 1556 cm^{-1} , corresponding to hydroxyl, amide I, and amide II groups, respectively. Compared to ZP, both ZD and ZD-FA showed reduced absorption intensities at these wave numbers, likely due to the Maillard reaction between the carbonyl groups of DEX and the amino group in ZP, resulting in the depletion of bands associated with these groups. Furthermore, ZD exhibited significantly higher absorption within the wave number range of 1017–1155 cm^{-1} , indicating strong stretching vibrations of the newly formed C–N covalent bond in the conjugates. Notably, the FT-IR spectra of ZD-FA and ZD were similar, potentially due to the overlap of amide I and II peaks of ZP at 1656 and 1556 cm^{-1} with the characteristic peaks of FA at 1694, 1643, and 1484 cm^{-1} .

PFD nanoemulsions were prepared with ZP, ZD, and ZD-FA as emulsifiers, and their physicochemical properties were characterized. The particle size (PS), polydispersity index (PDI), and zeta potential of the nanoemulsions were measured using dynamic laser scattering (DLS). The PS of PFD@Z prepared with ZP as the emulsifier was ≈ 117.77 nm (Figure 2C). Upon incorporating DEX, the PS of PFD@ZD markedly decreased to 28.48 nm.

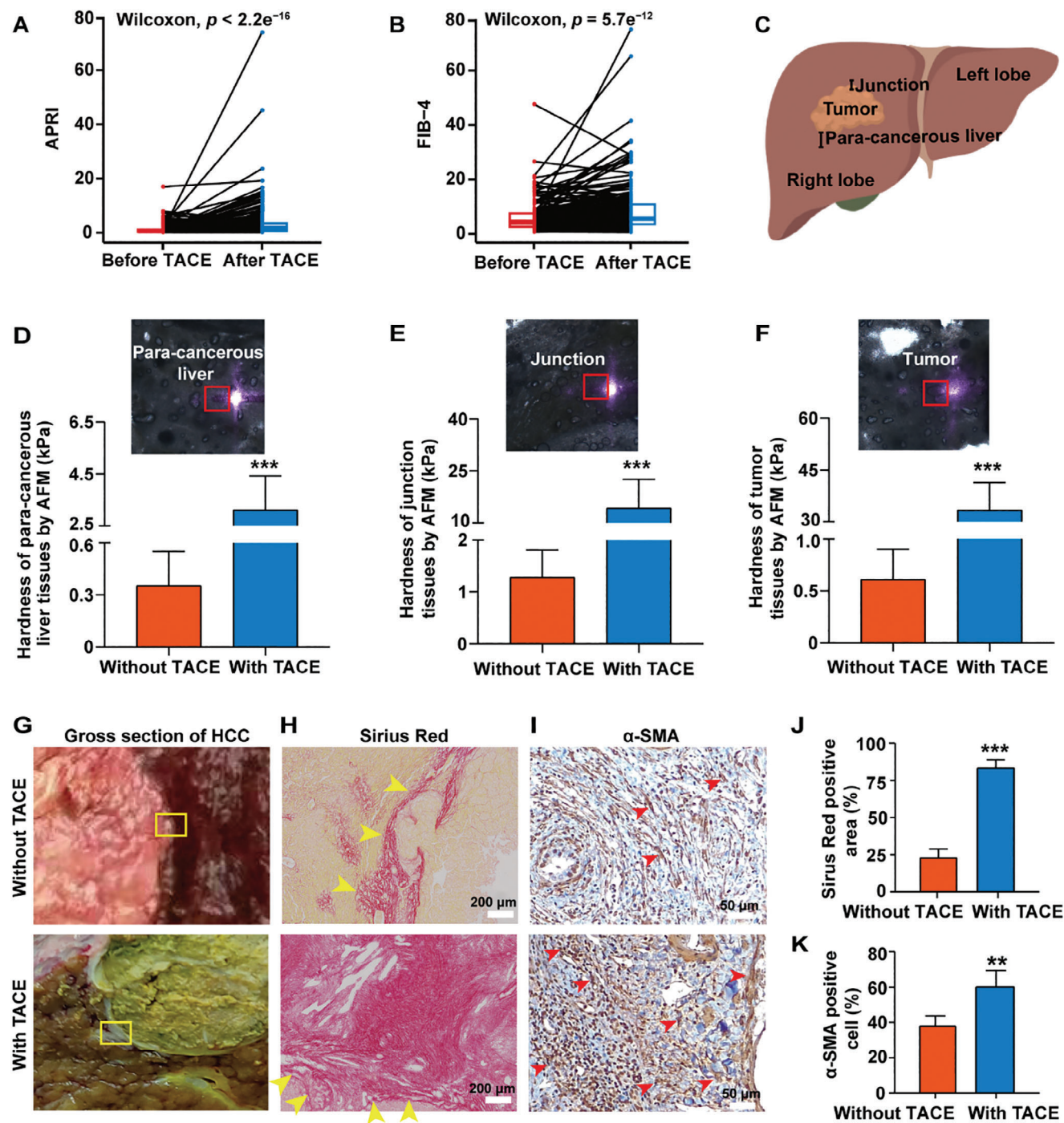


Figure 1. Post-TACE liver fibrosis in patients with HCC. A,B) Line plots demonstrating APRI and FIB-4 values in 189 HCC patients before and after TACE treatment. Dots display individual measurements and lines connect the paired measurements of individual patients. The boxes represent the median and interquartile. C) Liver model diagram indicating para-cancerous liver, the junction area, and tumor. D–F) Tissue hardness was measured in the para-cancerous liver, junction area, and tumor tissues using AFM. Above the bar chart is an image of the position of the cantilever probe on the tissue section under AFM. The red box marks the position of the AFM probe ($n = 4$). G) Both pictures were gross anatomical photographs of the junction between the tumor and the liver. The yellow box represents the interface area ($n = 4$). H) Sirius red staining image of collagen fiber (red) in the junction area in patients who had undergone TACE and patients who had not undergone TACE (yellow arrow) ($n = 4$). Scale bar: 200 μ m. I) Immunohistochemistry analysis of α -SMA in the junction area on resection samples (red arrow) ($n = 4$). Scale bar: 50 μ m. J,K) Relative quantification of Sirius Red positive area and α -SMA positive cells ($n = 4$). Each bar represents the mean $\pm$ SD. ** $p < 0.01$, *** $p < 0.001$ versus without TACE.

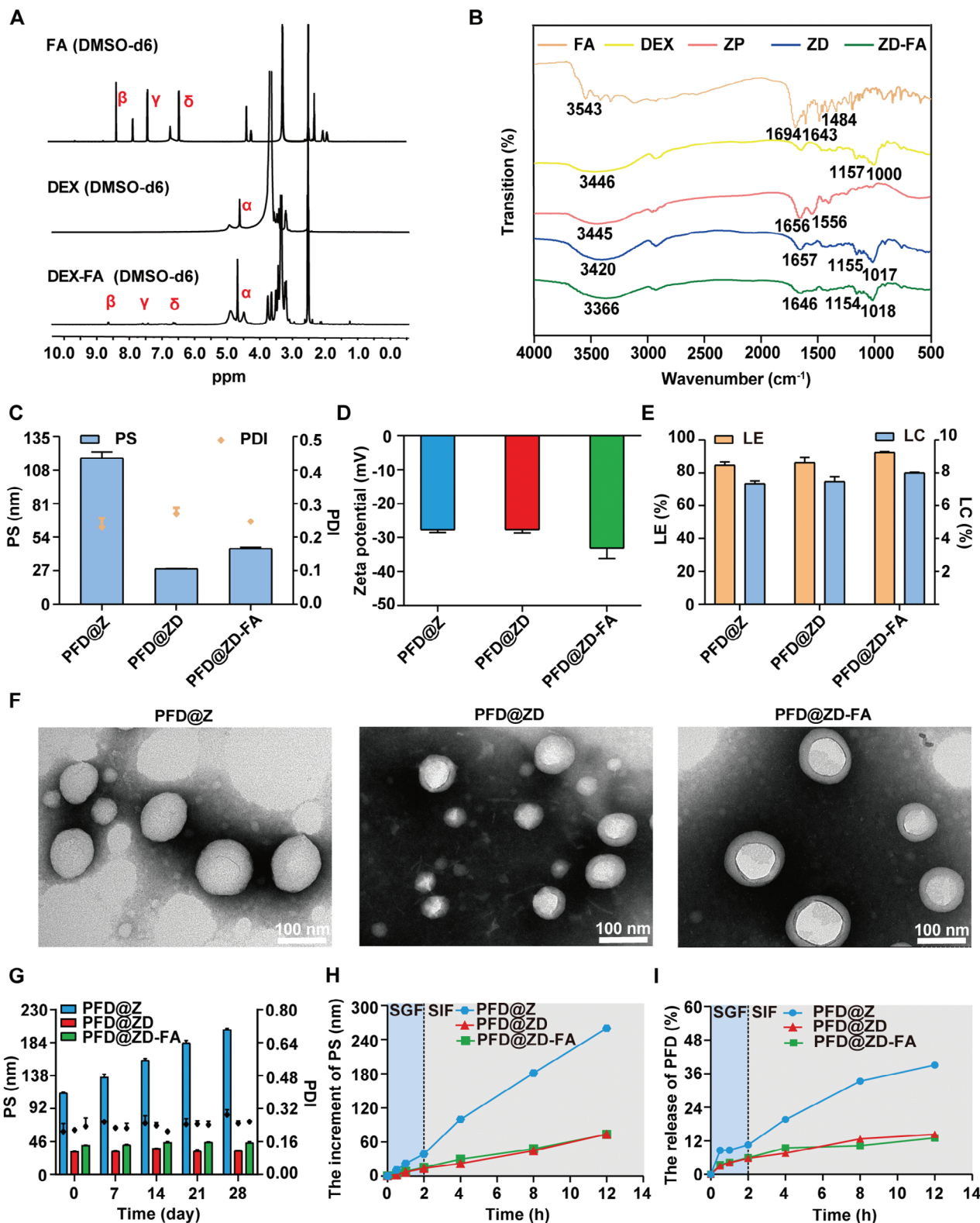


Figure 2. Preparation and characterization of PFD nanoemulsions. A) ^1H -NMR spectra of FA, DEX, and DEX-FA. B) FT-IR spectra of FA, DEX, ZP, ZD, and ZD-FA. C,D) The PS (bars), PDI (dots), and zeta potential of PFD nanoemulsions ($n = 3$). E) The LE and LC of PFD nanoemulsions ($n = 3$). F) TEM images of PFD nanoemulsions. Scale bar: 100 nm. G) PS and PDI changes in PFD nanoemulsions at 4 °C for 28 days ($n = 3$). H) PS changes in PFD nanoemulsions in SGF and SIF incubated for 12 h ($n = 3$). I) In vitro PFD release profiles of PFD nanoemulsions in SGF and SIF for 12 h ($n = 3$). Each bar represents the mean $\pm$ SD.

The PS of PFD@ZD-FA was 44.79 nm, slightly larger than that of PFD@ZD. These results suggest that DEX improves the emulsifying capabilities of ZP, resulting in smaller nanoemulsion droplets, and the introduction of FA has a slight influence on the emulsification efficiency. The PDI of PFD nanoemulsions was less than 0.3 indicating uniform size distribution. All nanoemulsions exhibited a negative charge. DEX had a negligible influence on the potential of nanoemulsion, whereas FA conjugation further decreased the zeta potential (Figure 2D).

PFD nanoemulsions exhibited a high loading efficiency (LE) of PFD, exceeding 80%. The LE for PFD@ZD and PFD@ZD-FA were marginally higher than that of PFD@Z (Figure 2E). Transmission electron microscopy (TEM) revealed the morphology of PFD@Z, PFD@ZD, and PFD@ZD-FA, with all nanoemulsions displaying spherical shapes. PFD@ZD and PFD@ZD-FA shared relatively similar structures with obvious hydration layers (Figure 2F). The stability of nanoemulsions at 4 °C was also assessed by monitoring changes in PS and PDI over 28 days (Figure 2G). The PS of PFD@Z rapidly increased compared with that of PFD@ZD and PFD@ZD-FA. Additionally, both PFD@ZD and PFD@ZD-FA showed less PS change in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) and demonstrated excellent stability (Figure 2H). Furthermore, PFD@Z showed a quick release in SGF and SIF, achieving a cumulative release of 43.39% at 12 h, whereas PFD@ZD and PFD@ZD-FA showed a significantly delayed and reduced cumulative release rate (Figure 2I). This demonstrated the excellent stability of PFD@ZD-FA in low pH conditions and environments in the presence of enzymes.

2.3. Inhibition of HSCs Activation by PFD@ZD-FA

Inhibition of HSCs activation is crucial for reversing liver fibrosis. Activated HSCs metamorphose into myofibroblast-like cells, expressing α -SMA and collagen.^[36] To ascertain whether PFD@ZD-FA inhibits HSCs activation, we investigated the effects of various formulations on activated HSCs proliferation (Figure 3A). Compared to the control group, free PFD reduced HSCs survival rates, whereas PFD@ZD and PFD@ZD-FA significantly decreased these rates with no notable differences among them. Whereas the PFD@ZD-FA group exhibited a higher apoptosis rate (Figure 3B,C). For cell proliferation evaluation, the activity of cells was measured by CCK-8, while the cell apoptosis rate was evaluated by flow cytometry. To fully verify the targeting of FA-modified PFD nanoemulsion on activated HSCs α -SMA level was also detected by immunofluorescence in subsequent studies to reflect the activation state of HSCs (Figure 3D,E). The results showed that the HSCs inhibitory activity of PFD@ZD-FA was higher than that of PFD@ZD, which confirmed that FA-modified PFD nanoemulsions could further enhance the uptake of HSCs of PFD preparations, and thus enhance the pharmacological action of PFD. The protein (Figure 3F) and mRNA expression levels (Figure 3G) of fibrosis-related factors, including α -SMA, collagen I, and collagen III, after treatment with PFD formulations were also assessed and quantified (Figure S3, Supporting Information). The results indicated that PFD@ZD-FA enhances apoptosis and reduces fibrosis-related factor levels in HSCs, effectively inhibiting

their activation and demonstrating superior antifibrotic effects in vitro.

Activation of the tumor growth factor- β (TGF- β)/Smad signaling pathway has also been implicated in HSCs activation during liver fibrosis.^[37] According to the previous reports, the anti-fibrosis effects of PFD have contributed to the inhibition of the TGF- β /Smad signaling pathway (Figure S4, Supporting Information).^[38–40] The protein levels of total and phosphorylated Smad2, Smad3, and combined Smad2/3 were evaluated. PFD treatment led to a reduction of phosphorylated Smad2, Smad3, and Smad2/3 (Figure 3H), and the relative protein level of p-Smad/Smad was calculated (Figure S5, Supporting Information). PFD@ZD-FA nanoemulsion showed similar inhibitory effects on the TGF- β /Smad signaling pathway with LY2109761—an inhibitor of the TGF- β receptor.

Nile red (NR) labeled nanoemulsions (NR/PFD@ZD and NR/PFD@ZD-FA) were prepared (Figure S6, Supporting Information) to investigate the uptake of PFD in LX2 cells after TGF- β activation (Figure 3I; Figure S7, Supporting Information). NR + PFD demonstrated weak fluorescence in LX2 cells, whereas NR-labeled nanoemulsions significantly enhanced fluorescence intensity in LX2 cells, indicating higher cellular uptake due to endocytosis. Notably, the uptake of NR/PFD@ZD-FA by LX2 cells was substantially greater than the uptake of NR/PFD@ZD. Given that FR is overexpressed on the surface of activated LX2, we explored whether the increased uptake of NR/PFD@ZD-FA was mediated by the FA ligand. Preincubation with FA to saturate its receptors on LX2 cells resulted in a marked decrease in cellular uptake of NR/PFD@ZD-FA, suggesting that the FR-mediated pathway plays a significant role in the interaction with LX2 cells. Moreover, nanocarriers of 6–100 nm are more likely to enter the Disse space through the micropores of sinusoidal endothelial cells.^[41,42] The PS of the PFD@ZD-FA is $\approx$ 45 nm, which can increase the penetration of nanomedicines in the Disse space and achieve the targeting of HSCs, which can produce a positive therapeutic effect on liver fibrosis. Besides, the cellular uptake of NR-labeled nanoemulsions by hepatocytes was studied (Figure S8, Supporting Information). There was no significant difference in fluorescence intensity between NR/PFD@ZD, NR/PFD@ZD-FA, and NR/PFD@ZD-FA with FA-preincubated groups. Hence, we suggested that PFD nanoemulsions are efficiently absorbed and delivered to the liver tissue after oral administration and NR/PFD@ZD-FA enhanced the delivery of PFD to liver tissue through FA-modification, especially for activated HSCs.

2.4. Absorption Mechanisms of PFD@ZD-FA in the Gastrointestinal Tract

After confirming the potential of PFD nanoemulsions to inhibit liver fibrosis, the fate of the nanoemulsions was examined after oral administration. To assess the ability of nanoemulsions to promote PFD absorption, we developed an in vitro model composed of a mucus layer and a Caco-2 cell monolayer to mimic the intestinal mucus barrier and epithelial cells with their tight junctions, respectively (Figure 4A). The PFD content that penetrated the mucus layer at 0.5, 1, 2, and 4 h was measured to evaluate the mucus penetration efficiency (Figure 4B). The penetration percentage progressively increased for all PFD nanoemul-

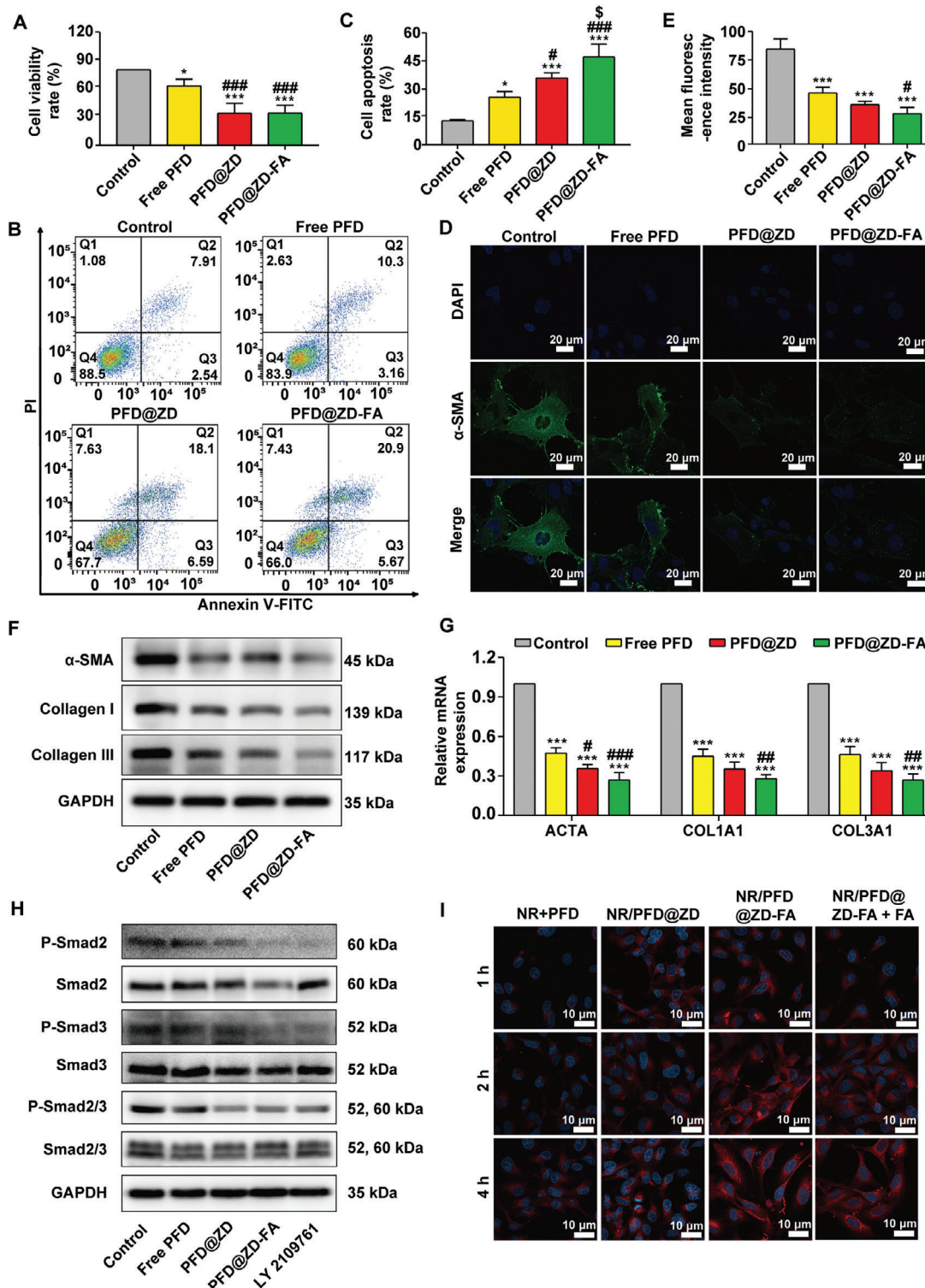


Figure 3. In vitro effect of PFD@ZD-FA on activated HSCs. A) Cell viability of LX2 cells treated with PFD preparations for 48 h was detected using CCK-8 ($n = 3$). B,C) Apoptosis rate in LX2 cells treated with PFD preparations for 48 h was assessed using Annexin V-FITC with flow cytometry ($n = 3$). D,E) Fluorescence intensity statistics and images of α -SMA treated with PFD preparations for 48 h ($n = 3$). Scale bar: 20 μ m. F) The protein levels of α -SMA, collagen I, and collagen III in LX2 cells treated with PFD preparations for 48 h ($n = 3$). G) α -SMA, collagen I, and collagen III mRNA expression in LX2 cells treated with PFD preparations for 48 h ($n = 3$). H) The protein expression of the TGF- β /Smad signaling pathway in LX2 cells after 48 h of treatment with PFD preparations and LY2109761 ($n = 3$). I) NR images in TGF- β activated LX-2 cells treated with NR-labeled nanoemulsions for 1, 2, and 4 h ($n = 3$). Scale bar: 10 μ m. * $p < 0.05$, *** $p < 0.001$ versus control; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ versus free PFD; \$ $p < 0.05$ versus PFD@ZD. Each bar represents the mean $\pm$ SD.

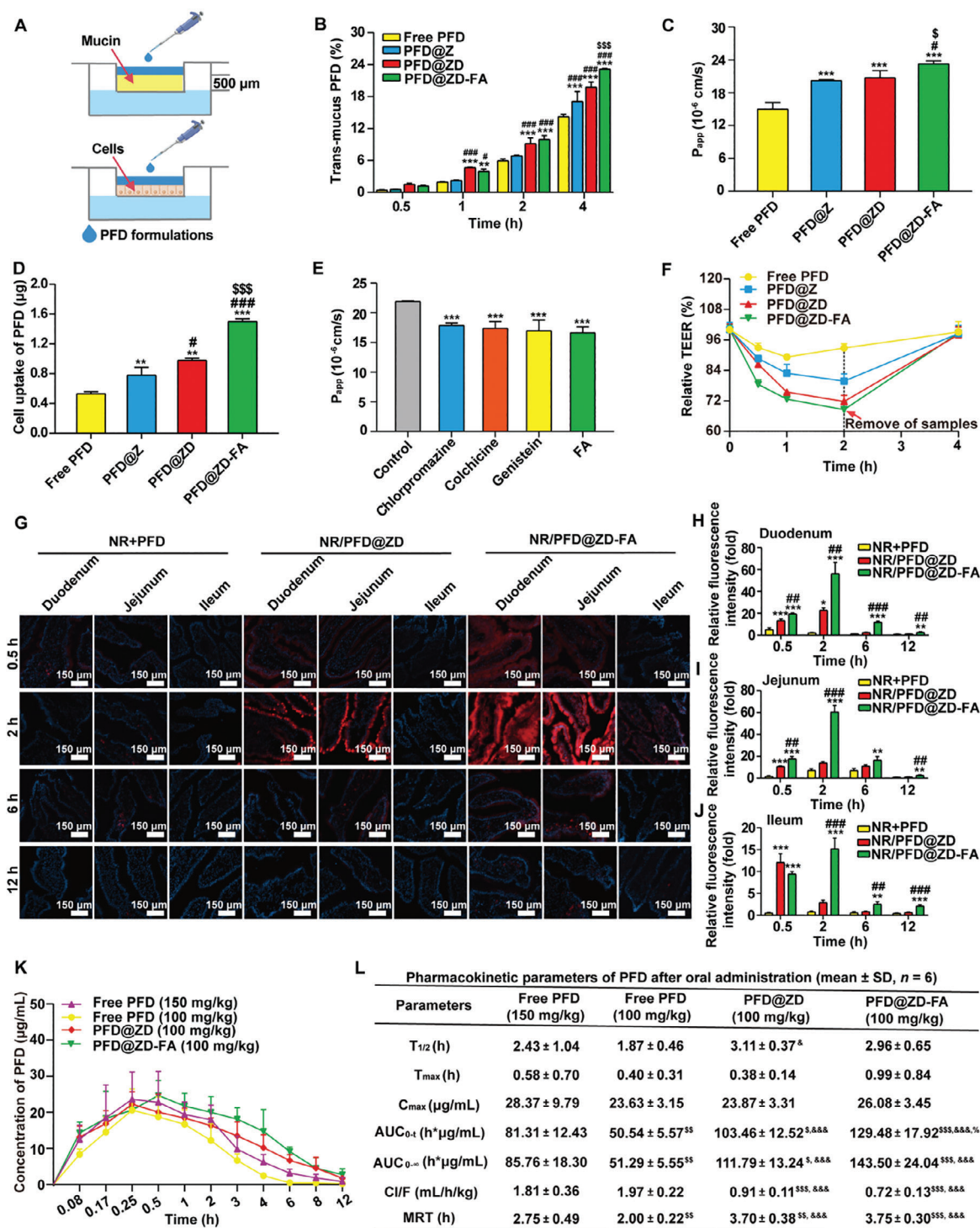


Figure 4. In vitro absorption and pharmacokinetic studies of PFD nanoemulsions. A) Illustration of the Transwell experiment and Caco-2 cell monolayer assay. B,C) Transport of PFD permeability via the mucus layer and P_{app} values of Caco-2 cell monolayer for various PFD preparations ($n = 3$). D) Cellular uptake of PFD preparations in Caco-2 cell monolayer at 2 h ($n = 3$). ^{*} $p < 0.01$, ^{***} $p < 0.001$ versus free PFD; [#] $p < 0.05$ and ^{###} $p < 0.001$ versus PFD@Z; ^s $p < 0.05$ and ^{ss} $p < 0.01$ versus PFD@ZD. E) P_{app} values of PFD preparations in Caco-2 cell monolayer under various inhibitory conditions ($n = 3$). ^{***} $p < 0.001$ versus control. F) Relative TEER changes of the Caco-2 cell monolayer after treatment with PFD preparations ($n = 3$). G) NR-labeled images of duodenum, jejunum, and ileum segments in liver fibrosis model rats at 0.5, 2, 6, and 12 h after oral administration of NR preparations. Scale bar: 150 µm. H–J) Fluorescence intensity statistics of NR from (G) in the duodenum, jejunum, and ileum segments ($n = 3$). ^{*} $p < 0.05$, ^{**} $p < 0.01$, and ^{***} $p < 0.001$ versus NR + PFD; ^{##} $p < 0.01$ and ^{###} $p < 0.001$ versus NR/PFD@ZD. K,L) Plasma concentration-time curves and main pharmacokinetic parameters of PFD following oral administration of various PFD preparations ($n = 6$). ^s $p < 0.05$, ^{ss} $p < 0.01$, and ^{ss} $p < 0.001$ versus free PFD 150 mg kg⁻¹; ^{&&&} $p < 0.001$ versus free PFD 100 mg kg⁻¹; [%] $p < 0.05$ versus PFD@ZD 100 mg kg⁻¹. Each bar represents the mean ± SD.

sions, with significantly higher rates observed for PFD@ZD and PFD@ZD-FA than for others at 1 h. Notably, PFD@ZD-FA exhibited the most substantial penetration effect at 4 h. Mucins in the mucus layer bind to nanoparticles mainly through hydrophobic interactions, but can also be electrostatically attracted to positively charged particles through charge groups, which results in nanoparticles being retained in the mucus layer and unable to traverse the intestinal cell membranes, whereas negatively charged and hydrophilic nanoparticles do not interact with mucins.^[43–45] Therefore, we speculated that PFD@ZD-FA with negative surface charge and hydrophilic modification by DEX, has a significant advantage for penetration the mucus layer. In addition, it has been shown that there are pore sizes between the mucin fibers in the mucus layer, nanoparticles with smaller particle sizes are more conducive to penetrating the mucus.^[46] PFD@ZD-FA has a small PS (≈ 45 nm) and also facilitates penetration of mucus. In summary, the negative charge, hydrophilicity, and small size of PFD@ZD-FA may reduce the repulsive force between the nanoemulsion and mucin thereby enhancing its mucus layer penetration.

Furthermore, we employed the apparent permeability coefficient (P_{app}) as a key metric to quantify the transport of PFD nanoemulsions across the Caco-2 cell monolayer (Figure 4C). Compared to free PFD, nanoemulsions significantly enhanced transintestinal epithelial cell permeability, particularly in the PFD@ZD-FA group. Besides, we also examined the role of transcellular pathways in PFD penetration by assessing the cellular uptake of PFD formulations by Caco-2 cells. Notably, nanoemulsions increased the cellular uptake of PFD, especially for PFD@ZD-FA (Figure 4D). To further explore the cellular uptake mechanisms of PFD@ZD-FA by introducing various uptake inhibitors and found that active ingredient, active ingredient, genistein, and free FA substantially reduced the P_{app} value of PFD. These findings suggest that PFD@ZD-FA is primarily absorbed through the macropinocytosis, clathrin, caveolin, or FR-mediated pathway (Figure 4E). Lastly, we measured the transepithelial electrical resistance (TEER) of the cell monolayer membrane to assess the integrity of the monolayer membrane and to examine the effects of nanoemulsions on cell tight junctions, which are crucial for paracellular transport (Figure 4F). The application of PFD nanoemulsions to the apical side of the cell monolayer markedly decreased the TEER, which gradually recovered upon removal of formulations. Overall, these results indicate that the penetration of PFD@ZD-FA may be facilitated by transcellular and paracellular pathways.

NR-labeled nanoemulsions were also used to study the small intestine distribution in rats. Briefly, rats were euthanized at 0.5, 2, 6, and 12 h after oral administration, and their small intestines were subsequently harvested and examined. Duodenum, jejunum, and ileum sections were analyzed for fluorescence intensity using a confocal laser scanning microscope (CLSM) (Figure 4G–J). The findings indicated that the overall fluorescence of the nanoemulsions surpassed that of NR + PFD. NR/PFD@ZD-FA demonstrating intensity superior to that of NR/PFD@ZD within the small intestinal segments. Notably, the fluorescence of NR + PFD in the duodenum was markedly diminished at 2 h after administration, where the nanoemulsions maintained varying degrees of NR fluorescence, particularly in the NR/PFD@ZD-FA group. The persistence of fluorescence

may be attributed to the enhanced resistance of nanoemulsions to enzymatic degradation and slower release of the drug in gastric and intestinal fluids, facilitating early retention within the small intestine. Additionally, the presence of FR, which is highly expressed on the apical brush border membrane of the duodenum and proximal jejunum, may account for the increased NR distribution in the villi of these segments in the NR/PFD@ZD-FA group. In summary, NR/PFD@ZD-FA has the potential to enhance the transepithelial transport of NR in the duodenum and jejunum, thereby improving the bioavailability of loaded drugs. The result also confirms the ability of NR/PFD@ZD-FA to penetrate the mucus to reach the small intestinal epithelial cells for absorption.

2.5. Pharmacokinetic Studies of PFD

To study the pharmacokinetic behavior of PFD in rats with liver fibrosis, free PFD was orally administrated in rats at a dose of 100 or 150 mg kg^{−1}, and PFD@ZD and PFD@ZD-FA were administered at a dose of 100 mg kg^{−1}. Subsequently, the plasma drug concentration-time curve (Figure 4K) and the main pharmacokinetic parameters were obtained (Figure 4L). High-dose free PFD (150 mg kg^{−1}) increased the mean residence time (MRT) and the area under the curve ($AUC_{0-\infty}$) by 1.38- and 1.67-fold, respectively, compared to the 100 mg kg^{−1} dose, with minimal difference in clearance (CL/F). PFD@ZD and PFD@ZD-FA significantly extended MRT by 1.85- and 1.88-fold, respectively, and enhanced $AUC_{0-\infty}$ by 2.18- and 2.80-fold, respectively. Furthermore, these groups demonstrated a decrease in apparent plasma clearance, with a CL/F of 46.19% for PFD@ZD and 36.55% for PFD@ZD-FA compared to the free PFD group at 100 mg kg^{−1}. These results suggest that the nanoemulsions are effective in extending the circulation time of PFD and reducing the renal and hepatic clearance rates. MRT values for PFD@ZD and PFD@ZD-FA were similar, but the PFD@ZD-FA group demonstrated greater increases in maximum concentration (C_{max}) and $AUC_{0-\infty}$. This suggests that PFD@ZD-FA enhanced the oral absorption of PFD, possibly due to FR-mediated endocytosis facilitating intestinal transport.

2.6. Biodistribution Analysis

Above all, we investigated the biodistribution of PFD@ZD-FA in normal rats. After oral administration, the heart, liver, spleen, lung, kidney and small intestine were collected at 0.25, 0.5, and 2 h, respectively, and the drug concentrations were detected (Figure S9, Supporting Information). The accumulation of PFD nanoemulsion in the small intestine was much higher than that of free PFD. Importantly, the small intestine distribution of PFD@ZD-FA is higher than that of PFD@ZD. This result may be attributed to the fact that nanoemulsion prolonged the retention time of PFD in the small intestine and FA promoted the absorption of PFD nanoemulsion through FR-mediated endocytosis. However, there was no significant difference in the distribution of free PFD and PFD nanoemulsions in the liver of normal rats.

Furthermore, we used a CT scan with lipiodol as the contrast agent to monitor the spatial and temporal distribution of PFD

nanoemulsion in rats with liver fibrosis. We prepared lipiodol-encapsulated nanoemulsions including Lipiodol/PFD@ZD and Lipiodol/PFD@ZD-FA (Figure S10, Supporting Information). CT scout images revealed a high-density shadow of lipiodol in the gastric cavity at 0.08 h after the oral administration of lipiodol solution, with some of the lipiodol progressing from the gastric cavity to the small intestine (Figure 5A). The residence time of lipiodol in the small intestine was ≈ 4 h. After 6 h, lipiodol had moved to the large intestine and was excreted from the body after 24 h. The retention time of Lipiodol/PFD@ZD and Lipiodol/PFD@ZD-FA in the small intestine was extended to over 6 h. At 24 h, the nanoemulsions were still present in the colon. Transverse slices of CT scan (Figure 5B) and quantitative analysis of CT values of at least three transverse slices in the region of interest (ROI) (Figure 5C) were implemented for 24 h. Specifically, the liver CT values for Lipiodol/PFD@ZD and Lipiodol/PFD@ZD-FA were substantially higher than those for the Lipiodol + PFD group at various time intervals. At 24 h, the CT value for Lipiodol/PFD@ZD-FA in the liver was 95.62, which was ≈ 1.39 times higher than that of the Lipiodol + PFD group and 1.22 times higher than that of the Lipiodol/PFD@ZD. These findings indicated that Lipiodol/PFD@ZD-FA can maintain prolonged retention in the small intestine to enhance the absorption of PFD and facilitate liver accumulation.

To further verify the distribution of nanoemulsions in the fibrosis liver rats model after intestinal absorption, the rats were euthanized, and their livers were isolated 6 h after oral administration. The uptake of NR-labeled nanoemulsions by the activated HSCs in the liver was observed and quantified, in which the activated HSCs region was labeled by α -SMA and distinguished by the cell morphology (Figure 5D,E). The NR + PFD exhibited very weak fluorescence within the activated HSCs region, whereas the fluorescence intensity of NR/PFD@ZD-FA in the activated HSCs region was 5.73 times greater than that of NR + PFD and 1.8 times greater than that of NR/PFD@ZD.

2.7. Application of PFD@ZD-FA to Liver Fibrosis

In the previous studies on liver fibrosis treatment by PFD, the oral dose of PFD in rats is usually 250–500 mg kg⁻¹.^[47–49] Our preliminary experimental results showed that free PFD was effective against liver fibrosis at the dosage of 250 mg kg⁻¹ in the rat fibrosis liver (Figure S11, Supporting Information). To reduce the dosage and minimize the toxicity, PFD at a dosage of 100 or 150 mg kg⁻¹ was administered daily to the liver fibrosis rat model for 4 weeks. A positive correlation between liver hardness and the extent of liver fibrosis was established by a previous report.^[50] We measured the liver tissue hardness using ultrasonic shear wave elastography (SWE) (Figure 6A). The results indicated an increase in liver hardness in the model (liver fibrosis rat) group compared to the normal group (healthy rat). Treatment with PFD preparations demonstrated a dose-dependent reduction in liver hardness, validating the antifibrotic effect of PFD. The PFD@ZD-FA group notably showed a 67.55% reduction in liver hardness relative to the model group. To elucidate the mechanisms underlying PFD in alleviating liver fibrosis, the liver tissue from euthanized rats was examined, and the hardness of liver tissues was assessed using AFM. Our findings confirmed that

PFD treatment decreased the overall liver hardness by reducing the cell hardness (Figure 6B). Furthermore, the indicators of liver cell damage including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were detected after treatments. PFD@ZD-FA led to a reduction in serum AST and ALT levels (Figure 6C,D). It was noted that PFD@ZD-FA treatment reduced the liver-to-body weight ratio in comparison to the model group and other PFD treatments, further corroborating its protective effect on liver function (Figure S12, Supporting Information).

Histological examination revealed that a smooth liver surface in the normal group displayed a smooth liver surface, whereas the model group exhibited thickening and a gray-white discoloration of the liver capsule (Figure 6E). Treatment with PFD preparations resulted in a decrease in the thickness of the liver capsule and a reduction in the grayish-white surface discoloration, suggesting an ameliorative effect on liver fibrosis. These observations were substantiated by Sirius red, Masson's trichrome, and immunohistochemical staining of liver tissue sections. Hematoxylin and eosin (H&E) staining indicated well-arranged liver cells radiating from the central vein in the normal group. In contrast, the model group displayed increased cell volume, lighter cytoplasmic staining, mild liver edema, and fibrous tissue formation near the portal area. Increased collagen fiber deposition in the model group was evident with Sirius red and Masson's trichrome staining. Quantitative analyses of these staining results showed that the extent of fibrosis was mitigated by PFD treatments, particularly with PFD@ZD-FA (Figure 6F,G). Collagen III and α -SMA secreted by activated HSCs contribute to ECM remodeling and fibrosis progression.^[51] The level of collagen III (Figure 6H) and α -SMA (Figure 6I) were downregulated after PFD treatment. PFD@ZD-FA showed pronounced effects due to its enhanced oral bioavailability and targeted action on activated HSCs, leading to the inhibition of fibrosis. The safety and biocompatibility of the PFD nanoemulsion treatments were supported by the absence of significant lesions in the heart, lung, spleen, and kidney of the treated rats (Figure S13, Supporting Information). In the model group, we observed an increase in monocytes and neutrophils and a decrease in lymphocytes. The PFD treatment facilitated the normalization of blood cell counts, potentially reflecting the hepatoprotective effects of PFD preparations (Figure S14, Supporting Information).

2.8. Exploring the Therapeutic Effects of PFD on Post-TACE Liver Fibrosis

We determined the therapeutic potential of PFD against fibrosis after TACE for liver cancer using an orthotopic VX2 liver tumor rabbit model (Figure 7A). The growth of VX2 liver tumors was monitored via ultrasound, and tumors measuring 1.5–2.0 cm in diameter were selected to undergo TACE (Figure S15, Supporting Information). Rabbits received either high-dose (135 mg kg⁻¹) or low-dose (90 mg kg⁻¹) PFD, along with PFD@ZD (90 mg kg⁻¹) and PFD@ZD-FA (90 mg kg⁻¹) nanoemulsions intragastrically, once daily for 3 weeks, starting 1 week before and continuing for 2 weeks after TACE. Ultrasound monitoring demonstrated a significant reduction in liver tumor volume after TACE compared to the model group (VX2 liver tumor rabbits) (Figures S15 and S16, Supporting Information). Oil Red O staining (Figure S17,

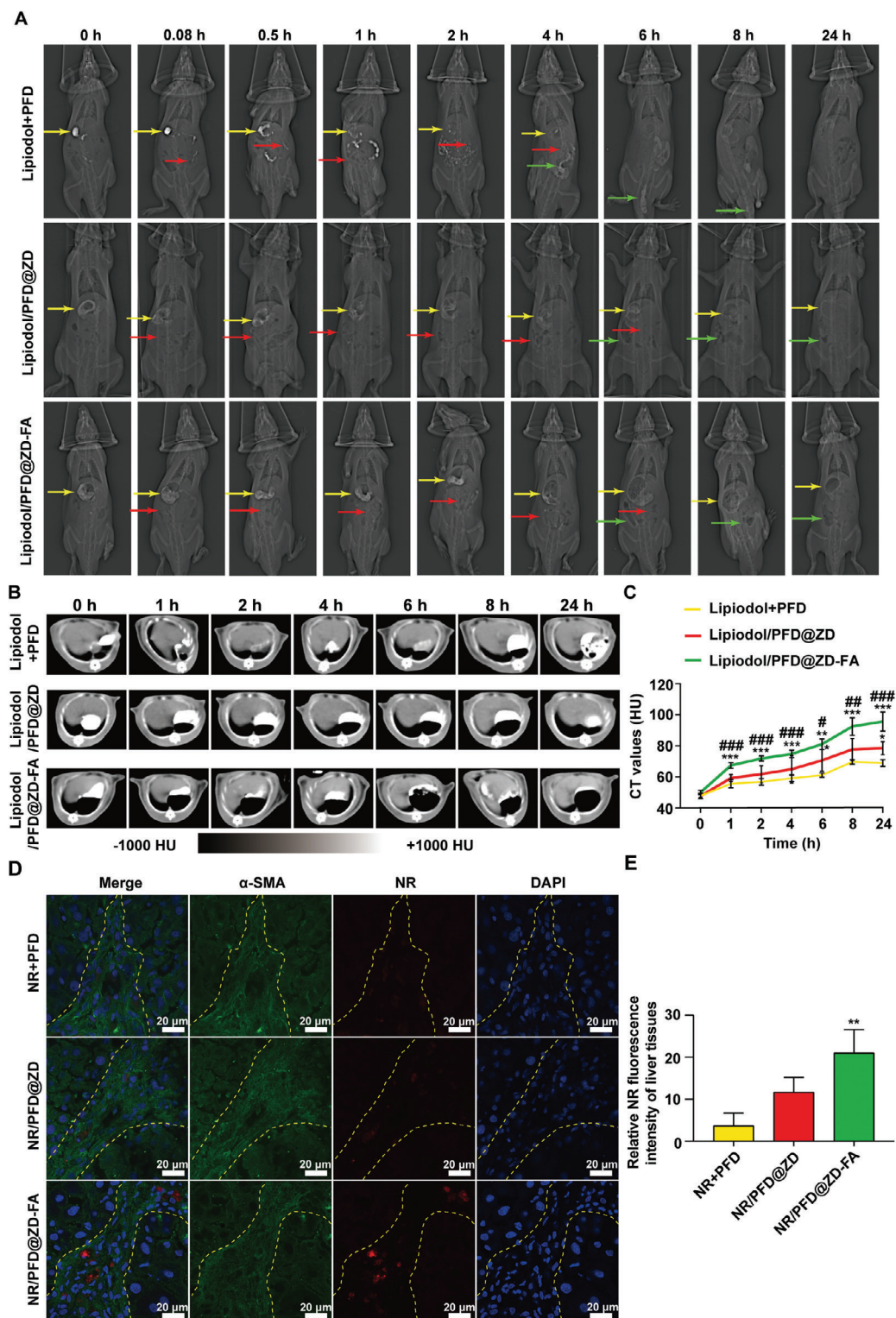


Figure 5. Intestinal distribution and liver targeting of lipiodol-labeled nanoemulsions in CCl_4 -induced liver fibrosis model rats by CT scan. A) Representative images of CT scout indicating the stomach (yellow arrows), small intestine (red arrows), and colon (green arrows) at 0, 0.08, 0.5, 1, 2, 4, 6, 8, and 24 h after oral administration of Lipiodol + PFD, Lipiodol/PFD@ZD, and Lipiodol/PFD@ZD-FA ($n = 5$). B) Representative images of on transverse slice of the CT scan ($n = 5$). C) Quantitative analysis of CT values of at least three transverse slices in the ROI at various times ($n = 5$). $^{**}p < 0.01$, and $^{***}p < 0.001$ versus Lipiodol + PFD; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ versus Lipiodol/PFD@ZD. D) Colocalization images of NR and α -SMA in liver tissues in rats with CCl_4 -induced liver fibrosis after 6 h of treatment with oral NR-labeled nanoemulsions ($n = 3$). Scale bar: 20 μm . E) Fluorescence intensity statistics for NR in (D). $^{**}p < 0.01$ versus NR + PFD. Each bar represents the mean $\pm$ SD.

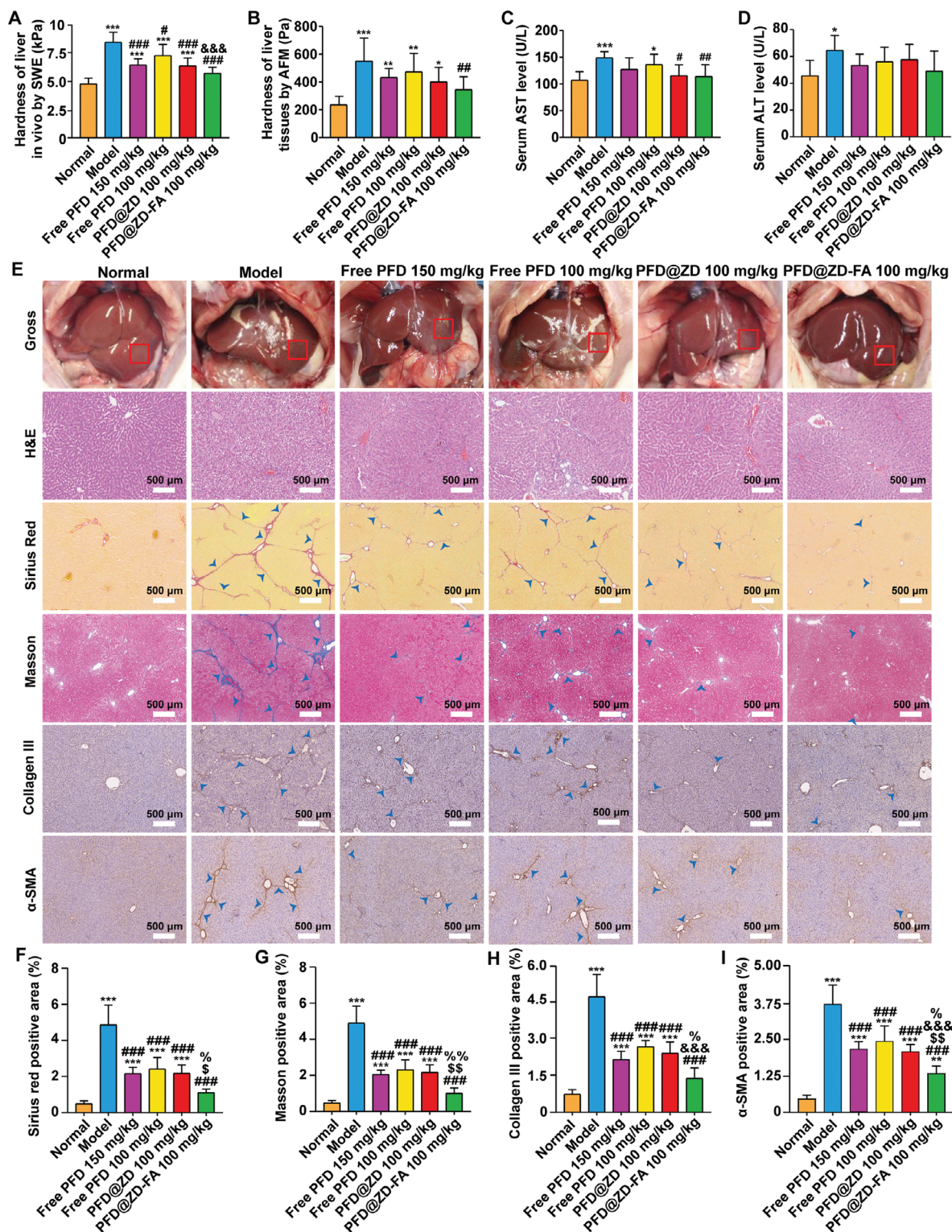


Figure 6. Efficacy of PFD nanoemulsions against liver fibrosis in rats with CCl₄-induced liver fibrosis. A) Hardness of liver in rats detected with SWE (n = 6). B) Hardness of liver fibrous tissue in the liver measured with AFM (n = 6). C, D) AST and ALT levels in rat serum (n = 6). E) Representative images of rat liver anatomy, and liver sections assessed with H&E, Sirius red, Masson's trichrome, collagen III, and α-SMA immunohistochemical analysis after PFD treatment (n = 6). Scale bar: 500 μm. F–I) Quantitative analysis of rat liver assessed with Sirius red, Masson's trichrome, collagen III and α-SMA immunohistochemical analysis (n = 6). *p < 0.05, **p < 0.01, and ***p < 0.001 versus normal; #p < 0.05, ##p < 0.01, and ###p < 0.001 versus model; \$p < 0.05, \$\$p < 0.01 versus free PFD 150 mg kg⁻¹; &&p < 0.001 versus free PFD 100 mg kg⁻¹; %p < 0.05 and %%p < 0.01 versus PFD@ZD 100 mg kg⁻¹. Each bar represents the mean ± SD.

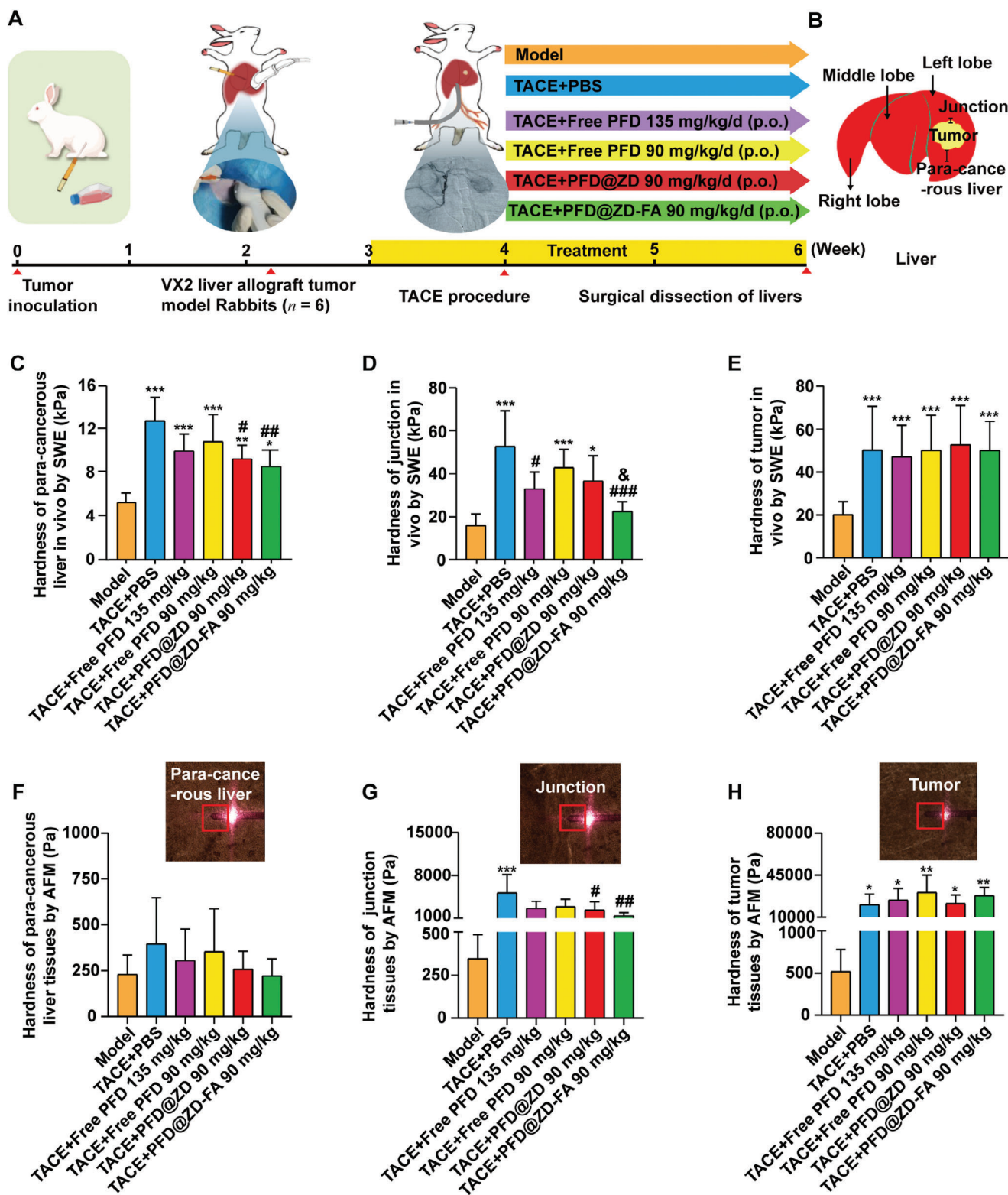


Figure 7. Evaluation of tissue hardness after TACE and treatment with PFD nanoemulsions in postoperative liver fibrosis rabbit VX2 liver tumor model. A) Illustration of VX2 rabbit model and treatment. B) Schematic illustration of the liver lobe of a rabbit. C–E) Hardness of para-cancerous liver, junction, and tumor tissue assessed using SWE ($n = 6$). F–H) Hardness of tissues measured using AFM in para-cancerous liver, junction, and tumor tissues. Above the bar chart is an image of the position of the cantilever probe on the tissue section under AFM ($n = 6$). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ versus model; # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$ versus TACE + PBS; & $p < 0.05$ versus TACE + free PFD 135 mg kg⁻¹. Each bar represents the mean $\pm$ SD.

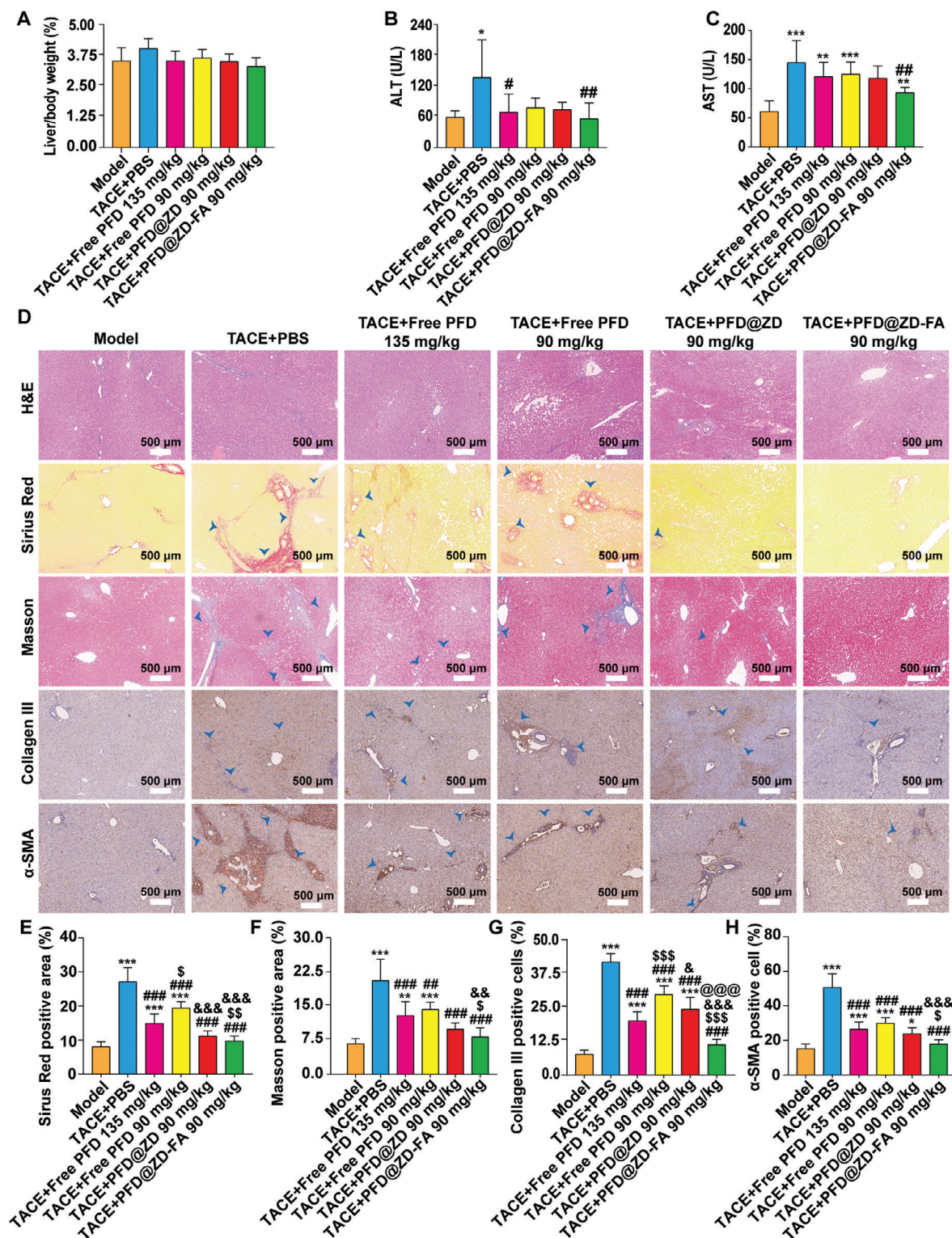


Figure 8. Evaluation of the efficacy of PFD nanoemulsions for treating post-TACE liver fibrosis in a rabbit VX2 liver tumor model. A) Percentage of liver/body weight of rabbit (n = 6). B,C) Rabbit serum AST and ALT levels (n = 6). D) Representative images of the para-cancerous liver were assessed with H&E, Sirius red, Masson's trichrome, collagen III and α-SMA immunohistochemical analysis (n = 6). Scale bar: 500 μm. E,F) Quantitative analysis of Masson and Sirius red staining (n = 6). G,H) Immunohistochemical quantitative analysis of collagen III and α-SMA (n = 6). *p < 0.05, **p < 0.01, and ***p < 0.001 versus model; #p < 0.05, ##p < 0.01, and ###p < 0.001 versus TACE + PBS; \$p < 0.05, \$\$p < 0.01, and \$\$\$p < 0.001 versus TACE + free PFD 135 mg kg⁻¹; &p < 0.05, &&p < 0.01, and &&&p < 0.001 versus TACE + free PFD 90 mg kg⁻¹; @p < 0.05, @@p < 0.01, and @@@p < 0.001 versus TACE + free PFD@ZD 90 mg kg⁻¹. Each bar represents the mean ± SD.

Supporting Information) was used to assess lipiodol deposition in tumors after TACE, with visible deposition seen in treated VX2 tumors but none seen in the model group, suggesting that TACE effectively accomplished chemoembolization and tumor growth inhibition.

Subsequently, we compared liver fibrosis between normal and tumor-bearing rabbits by assessing liver hardness and performing histological staining. The findings indicated that tumor growth did not induce liver fibrosis in rabbits (Figure S18, Supporting Information). We divided rabbit livers into three segments: the para-cancerous liver, junction area, and tumor (Figure 7B). Using *in vivo* ultrasonic SWE, we scanned and quantified tissue hardness in these three segments (Figure 7C–E) and found that TACE tended to increase the tissue hardness across all segments. PFD treatment significantly decreased the hardness in the para-cancerous liver and junction area but had a minimal effect on the tumor. The rabbits were then euthanized and liver sections were extracted for AFM analysis (Figure 7F–H), which confirmed that PFD treatment slightly attenuated the tissue hardness in the para-cancerous liver tissues and substantially reduced the hardness of cells in the junction area, without altering tumor tissue cell hardness. The PFD@ZD-FA formulation (90 mg kg^{−1}) demonstrated the most potent antifibrotic effect in rabbits after TACE.

The liver-to-body weight ratio in rabbits was assessed to evaluate the antifibrotic effects after TACE for liver cancer. The results indicated no significant differences between the groups (Figure 8A). Serum ALT and AST levels were significantly increased after TACE, suggesting that TACE may induce stress-related liver cell injury. The PFD@ZD-FA group exhibited a notable reduction in these serum levels compared to the other groups (Figure 8B,C), suggesting a superior mitigating effect on fibrosis after TACE for liver cancer. Subsequently, these observations were further assessed using H&E, Sirius red, Masson's trichrome, and immunohistochemical staining of para-cancerous liver tissues (Figure 8D). H&E staining indicated well-arranged liver cells radiating from the central vein in the model group. In contrast, the TACE group demonstrated mild liver edema and fibrous tissue formation near the portal area. Increased collagen fiber deposition in the TACE group was evident with Sirius red and Masson's trichrome staining. Quantitative analyses of these staining results (Figure 8E,F) showed that the extent of fibrosis was mitigated by PFD treatment, particularly PFD@ZD-FA. PFD@ZD-FA downregulated the level of collagen III (Figure 8G) and α -SMA (Figure 8H) due to its enhanced oral bioavailability and targeting ability to activate HSCs after TACE. Furthermore, we stained the junction area (Figure S19A, Supporting Information). H&E staining revealed extensive necrosis of tumor cells after TACE, with some viable tumor cells remaining peripherally. A proliferation of interstitial cells and fibrous tissue was observed at the junction area. Both Sirius red and Masson's trichrome staining demonstrated a reduction in fibrosis of the liver tissue after treatment with PFD formulations, with PFD@ZD-FA showing the most reduced fibrotic area (Figure S19B,C, Supporting Information). These preparations variably decreased the expression of collagen III and α -SMA in fibrotic regions (Figure S19D,E, Supporting Information), especially PFD@ZD-FA. Collectively, PFD treatment effectively ameliorated post-TACE liver fibrosis, with PFD@ZD-FA being the

most effective, likely due to its high oral bioavailability and superior targeting of activated HSCs. No significant lesions were detected in the heart, lung, spleen, or kidney of rabbits after PFD treatment (Figure S20, Supporting Information). In the model group, monocytes and neutrophils increased, with a corresponding decrease in lymphocytes. After treatment with PFD preparations, blood cell counts returned to normal levels, suggesting the potential protective effect of PFD on liver function (Figure S21, Supporting Information).

In this study, the group receiving a high- or low-dose of free PFD or PFD@ZD experienced gastrointestinal reactions and photoallergies, whereas no significant gastrointestinal reactions or photoallergies were observed in the group receiving low-doses PFD@ZD-FA nanoemulsion among rabbits or rats (Tables S1 and S2, Supporting Information). A previous study reported that oral administration of PFD is associated with several side effects.^[14–16,52] A low oral PFD dose is therefore recommended for the treatment of liver fibrosis after TACE.

3. Conclusion

In this study, we developed an FA-modified protein-polysaccharide nanoemulsion (PFD@ZD-FA) for the oral targeted delivery of PFD to treat fibrosis after TACE for liver cancer. The findings indicate that PFD@ZD-FA significantly improved the stability of PFD both *in vivo* and *in vitro*, facilitated its penetration through the mucus layer and Caco-2 monolayer, and prolonged its retention in the gastrointestinal tract, thereby improving oral absorption. PFD@ZD-FA effectively increased the accumulation of PFD in the liver and targeted delivery to activated HSCs via FA-mediated endocytosis, leading to the successful inhibition of liver fibrosis in rats. Moreover, an orthotopic transplant tumor rabbit model was used to establish the model of fibrosis after TACE for liver cancer. In this model, PFD@ZD-FA treatment demonstrated superior antifibrotic effects compared to other PFD formulations, outperforming even high-dose free PFD. Notably, rats in the PFD@ZD-FA group did not experience any adverse reactions. Overall, this research thoroughly evaluated the physicochemical properties and antifibrotic efficacy of orally administered PFD nanoemulsions and PFD@ZD-FA showed promising potential in the treatment of fibrosis after TACE for liver cancer.

4. Experimental Section

Materials: FA, DEX (molecular weight: 10 kDa), Zein, NR, CCl₄, and olive oil were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) was obtained from Haoyuan Chemexpress Co., Ltd. (Shanghai, China). 4-Dimethylaminopyridine (DMAP) was purchased from Adamas-beta (Shanghai) Chemical Reagent Co., Ltd. (Shanghai, China). PFD was obtained from Continent Pharmaceutical Co., Ltd. (Beijing, China). Lipiodol was obtained from Hengrui Medicine Co., Ltd. (Jiangsu, China). Pirarubicin was purchased from Shenzhen Wanle Co., Ltd. (Guangdong, China). Other analytical-grade organic reagents used in this study were purchased from Macklin Biochemical Co., Ltd.

Cells and Animals: The experimental protocols involving animals were approved by the Animal Welfare and Ethics Committee of Guizhou Medical University (Approval No. 17021161, 1900932).

Rabbit VX2 tumor tissues and human hepatic stellate LX2 cells were obtained from Guangzhou Jennio Biotech Co., Ltd. (Guangdong, China). Human hepatocyte cell LO2 was obtained from SUNNCELL Biotech Co., Ltd. (Wuhan, China). A Caco-2 human colon cancer cell line was obtained from Procell Life Science & Technology Inc. (Wuhan, China). LX2 and LO2 cells were cultured in high-glucose Dulbecco's modified Eagle medium (DMEM, Gibco, China) containing 10% fetal bovine serum (FBS, Gibco) and 1% active ingredient/active ingredient (Gibco). Caco-2 cells were cultured in MEM medium (Procell) with 20% FBS (CellMax, Lanzhou, China) and 1% active ingredient/active ingredient (Gibco). All cells were incubated in 5% CO₂ at 37 °C. New Zealand white rabbits (2.0–2.5 kg) and Sprague–Dawley rats (male, 200–250 g) were purchased from the Experimental Animal Center of Guizhou Medical University (Certificate No. SCXK [Guizhou] 2023-0002).

Patients: Informed written consent from all participants or next of kin was obtained after fully understanding the content, purpose, and potential risks of the study. All protocols involving humans were approved by the Human Trial Ethics Committee of Guizhou Medical University (Approval No. 2023 [118]).

Blood samples were continuously collected from 189 HCC patients who underwent their first TACE treatment at the Affiliated Hospital of Guizhou Medical University between January 2018 and December 2023. Among the 189 patients, there were 157 males and 32 females, with a median age of 61 years (interquartile range: 52–70 years).

In addition, liver and tumor tissue samples were collected from eight patients who underwent liver resection at the Affiliated Hospital of Guizhou Medical University between June 2023 and May 2024. Of these eight patients, four patients with intermediate-stage HCC who underwent liver resection with preoperative TACE were classified into the “with TACE” group, and the other four patients with early-stage HCC who underwent liver resection only were classified into the “without TACE” group. The eight patients included six men and two women, with a median age of 58 years (interquartile range: 51–69 years). These eight patients include six males and two females with a median age of 58 years (Interquartile range: 51–69 years).

Evaluation of Fibrosis after TACE for Human HCC: Pre-TACE and post-TACE APRI and FIB-4 indices were assessed in 189 patients, and serum was collected to measure the ALT, AST, and platelet (PLT) levels before and at 1 week after TACE. APRI and FIB-4 indices were calculated using the following equations (with the upper limit of normal [ULN] for AST set at 40 U/L):

$$\text{APRI} = \frac{\text{AST}}{\text{ULN}} \div \text{PLT} \times 100 \quad (1)$$

$$\text{APRI} = \frac{\text{AST}}{\text{ULN}} \div \text{PLT} \times 100 \quad (2)$$

From the eight patients who underwent surgical resection, tissue samples were collected from the para-cancerous liver (within 2 cm of the tumor margin), the junction between the tumor and liver, and the tumor itself. The obtained samples were stained with Sirius red (Anhui Leagene Reagent Biologicals, China) and immunohistochemistry, described in the Supporting Information in detail.

AFM Measurement of Tissue Elasticity: Frozen samples of human, rabbit, and rat liver or tumor tissues were cut into 50 μm sections. The sections were placed in phosphate buffer solution (PBS) at 25 °C and were then scanned on the stage of an AFM (Nano Wizard 4 XP, Bruker Corp., Zurich, Switzerland). Nitride silicon rectangular microcantilevers with an elastic modulus of 0.06 N m⁻¹ and spherical silicon nitride probes with a tip radius of curvature 6 μm (MLCT-10, Bruker Corp.) were used, with the microcantilever probes placed in the tissues. Force-distance curves were obtained, and Young's modulus was calculated using the Hertz contact model and JPK DP Data Processing software to evaluate tissue elasticity.

Synthesis of DEX-FA: FA (0.4586 g, 1.04 mmol) was dissolved in 20 mL dimethyl sulfoxide. EDCI (0.199 g, 1.04 mmol), and DMAP (0.127 g, 1.04 mmol) were added and activated with stirring (500 rpm) in the dark for 30 min at 37 °C. DEX (4 g, 0.4 mmol) was then added and the mixture underwent reaction using magnetic stirring (500 rpm) in the dark at 37 °C for 24 h. The resulting solution was then purified by dialysis (MWCO,

3.5 kDa) using 200-fold volume PBS (0.01 M, pH 7.4) and was changed every 2 h, until the color of the dialysis medium changed from yellow to colorless. After that, the dialysis medium was replaced with deionized water to continue dialysis until 24 h. Finally, DEX-FA was obtained by freeze-drying the solution. DEX-FA was then characterized using ¹H-NMR (AVANCE NEO 600 MHz, Bruker Corp.).

Synthesis of ZD-FA: ZD-FA was prepared via the Maillard reaction. In brief, DEX-FA and ZP were dissolved in deionized water at a mass ratio of 6:1, thoroughly mixed and the pH was adjusted to 8.8 with 1 mol of NaOH. Then, the mixed solution was partitioned and freeze-dried. Subsequently, the freeze-dried powder was then placed in a closed container with saturated KBr and underwent reaction at 60 °C and 79% humidity for 24 h to obtain ZD-FA. Similarly, The ZD was prepared from DEX and ZP using the same method. To confirm the formation of ZD and ZD-FA, FT-IR spectra of FA, DEX, ZP, ZD, and ZD-FA in KBr tablets were recorded using an infrared spectral spectrometer (IS50, Thermo Fisher Scientific Inc., PA, USA).

Preparation of PFD@ZD-FA: ZD-FA was prepared as a 10 mg mL⁻¹ (equivalent ZP concentration) solution with deionized water, and the pH was adjusted to 4.5 with 1 mol of HCl (aqueous phase). PFD was dissolved in medium-chain triglyceride to prepare 100 mg mL⁻¹ (oil phase). The oil and aqueous phases were mixed at an oil-water volume ratio of 1:4. This solution was vortexed for 5 min and then sonicated at 65 W for 3 min (3-s pulse and 3-s pause) in an ice-water bath to produce a primary emulsion. **The primary emulsion was homogenized using a microinjection high-pressure homogenizer (NanoGenizer 20K, Genizer LLC, LA, USA) at 6000 psi for three cycles to obtain PFD@ZD-FA.** PFD@Z and PFD@ZD were prepared by the same method using ZP and ZD as emulsifiers.

Characterization of PFD@ZD-FA: PFD nanoemulsions were diluted 20-fold with deionized water and their PS, PDI, and zeta potential of the nanoemulsions were evaluated using a DLS instrument (90Plus-PALS, Brookhaven Instruments Co., Ltd., NY, USA). The morphology of nanoemulsions was observed using TEM (H7800, Hitachi, Ltd., Tokyo, Japan). The nanoemulsions were stained negatively on copper grids with 1% phosphotungstic acid and observed at an accelerating voltage of 120 kV. The drug LE and loading capacity (LC) of PFD were determined using high-performance liquid chromatography (HPLC) with a Ultraviolet detector (LC-16, Shimadzu Instruments Co., Ltd., Kyoto, Japan). For the detection of PFD, a mobile phase of acetonitrile/water (65:35, v/v) was used and detected at λ = 316 nm. The LE (%) and LC (%) of PFD were calculated using the following equations:

$$\text{LE (wt.\%)} = \frac{\text{weight of loaded drug}}{\text{weight of feeding drug}} \times 100 \quad (3)$$

$$\text{LC (wt.\%)} = \frac{\text{weight of loaded drug}}{\text{theoretical total weight of nanoemulsion}} \times 100 \quad (4)$$

The changes in PS and PDI of PFD@Z, PFD@ZD, and PFD@ZD-FA were monitored by a DLS method at 4 °C for 28 days to evaluate the placement stability of the nanoemulsions. Besides, the gastrointestinal stability of PFD nanoemulsions in vitro were investigated. Briefly, PFD@Z, PFD@ZD, and PFD@ZD-FA were each mixed with SGF at the initial 2 h. Then, the solutions were transferred to the SIF, and these mixtures were placed in a shaking incubator (100 rpm min⁻¹, 37 ± 0.5 °C). The change in PS of the nanoemulsions was determined using a DLS method at predetermined time points. For the evaluation of PFD release, PFD@Z, PFD@ZD, and PFD@ZD-FA were placed in a dialysis bag (MWCO, 3.5 kDa) and dispersed in 20 mL of SGF at the initial 2 h. This solution was then transferred to the SIF and placed in a shaking incubator (100 rpm min⁻¹, 37 ± 0.5 °C). The medium (0.5 mL) was collected at predetermined time points, and the same amount of fresh medium was supplemented. Finally, the amount of PFD released was analyzed using HPLC.

In Vitro Effect of PFD@ZD-FA on Activated HSCs: LX2 cells in the logarithmic growth phase were studied in vitro. Cells were treated with 10 ng mL⁻¹ TGF-β (6062712, Dyuo) for 24 h. Cells were then treated with free PFD, PFD@ZD, or PFD@ZD-FA (4 mmol/L PFD) for 48 h.

Cell proliferation activity was detected by adding CCK-8 reagent (MA0218-2, Meilunbio) 10 μ L to each well and incubating for 2 h, after which the optical density value at 450 nm was measured using a microplate reader (SpectraMax Absorbance Reader Cmax Plus, Molecular Devices, China).

For the apoptosis assay, cells were collected after treatment according to the instructions of the assay kit (MULTI SCIENCES, AT101-100-kit, China) after treatment. 5 μ L Annexin V-FITC and 10 μ L PI were added to each tube containing cells. The cells were then analyzed using a flow cytometer (BD Accuri C6 Plus, USA) with the following parameters: excitation = 488 nm, emission = 530 nm for Annexin V-FITC and excitation = 535 nm, emission = 615 nm for PI.

For immunofluorescence analysis, LX2 cells after PFD preparation treatment were fixed with 4% paraformaldehyde and blocked with 10% goat serum at room temperature for 1 h. The cells were then incubated overnight at 4 °C with anti- α -SMA antibody (ab7817, 1:300, Abcam), followed by incubation at room temperature for 1 h with goat anti-mouse IgG H&L Alexa Fluor 488 secondary antibody (ab150113, 1:300, Abcam). DAPI (C0065, Solarbio) was used to stain the nuclei for 5 min, and a mounting medium with antifade fluorescence (S2100, Solarbio) was applied to seal the coverslips. Images were captured using a CLSM (LSM 900, Carl Zeiss AG, Oberkochen, German).

The mRNA levels of ACTA, COL1A1, and COL3A1 were detected with real-time quantitative PCR. RNA was extracted using the RNA rapid extraction kit (Shanghai Yishan Biotechnology Co., Ltd., RN001, China). The extracted RNA was reverse transcribed into cDNA using an RNA reverse transcription kit (TaKaRa, RR820A, Japan). Following the manufacturer's instructions for the real-time fluorescence quantitative PCR kit (TaKaRa, RR047A, Japan), the reaction system was prepared and detected using a real-time PCR instrument (Eco Illumina, Gene Company Limited, China).

The protein levels of collagen I, collagen III, and α -SMA were detected by western blot. LX2 cells in the logarithmic growth phase were seeded into a 10-cm dish. Subsequently, cells were treated with PFD preparation or Ly2109761 (TGF- β signaling pathway inhibitor) 100 μ M. After cell treatment, proteins were extracted using a cell lysis buffer containing 1% protein phosphatase inhibitor (P1260, Solarbio) and PMSF (P0100, Solarbio). The protein concentration was determined using the BCA protein concentration determination kit (PC0020, Solarbio). Equal amounts of protein were loaded onto SDS-PAGE gels and transferred onto PVDF membranes. The membranes were then blocked with 5% nonfat milk at room temperature for 2 h. They were subsequently incubated overnight at 4 °C with the following primary antibodies α -SMA (ab7817, 1:1000, Abcam), collagen I (AB138492, 1:5000, Abcam), collagen III (abs120021, 1:1000, Absin), smad 2 (#5339, 1:1000, CST), P-smad 2 (#18338, 1:1000, CST), smad 3 (#9523T, 1:1000, CST), P-smad 3 (#9520, 1:1000, CST), smad2/3 (CST, #8685, 1:1000), P-smad2/3 (#8828, 1:1000, CST), and GAPDH (abs137959, 1:5000, Absin). After primary antibody incubation, the membranes were incubated at room temperature for 1 h with HRP-conjugated goat antirabbit IgG secondary antibody (Ab6712, 1:10000, Abcam) and goat antimouse IgG secondary antibody (bs-0296-HRP, 1:10000, Bioss). Chemiluminescent detection was performed using ECL substrate (KF8003, Affinity, China), and analysis was conducted using Image J software.

The targeting effect of drug nanoemulsions on LX2 cells was investigated using CLSM. The LX2 cells were inoculated into confocal petri dishes at a density of 5×10^4 per well until adhesion. NR + PFD, NR/PFD@ZD, or NR/PFD@ZD-FA (10 μ g mL⁻¹ NR and 4 mM PFD) were then added and incubated for 1, 2, or 4 h. In addition, for blocking experiments, LX2 cells were preincubated with free FA (10 μ g mL⁻¹) for 30 min, and NR/PFD@ZD-FA was then added. Four percent paraformaldehyde was added for fixation and DAPI staining. Subsequently, the samples were assessed using CLSM.

Permeation Across Mucus Layer In Vitro: The permeability of nanoemulsions across the mucus layer was measured using the Transwell system. Briefly, porcine gastric mucin was dissolved in PBS to prepare a 10 mg mL⁻¹ mucin solution, and 0.2 mL of mucin was added to the upper chambers of the Transwell (12 inserts/24 well plates, pore size 3 μ m, Milli-

pore, Billerica, MA, USA). The lower chambers were filled with 0.8 mL PBS and 0.2 mL free PFD, PFD@Z, PFD@ZD, or PFD@ZD-FA (1 mg mL⁻¹ PFD) was then added above the mucin in the upper chambers. After incubation at 37 °C, 0.5 mL of solution was collected from the lower chambers at 0.5, 1, 2, and 4 h. The concentration of PFD in the lower chambers was determined using HPLC, and the ratio of transmucosal PFD in different groups was calculated.

Transcellular Permeation Assessment on the Caco-2 Cell Monolayer: Caco-2 cells exhibit similar structural and functional properties characteristic of small intestine enterocytes, thus the EMA and FDA recommend being used for the in vitro model for predicting the bioavailability of drugs.^[53,54] Herein, Caco-2 cells were used as a model of gastrointestinal epithelium in vitro. Briefly, Caco-2 cells were seeded on 24-well Transwell plates (pore size 0.4 μ m) at a density of 5×10^4 cells per well and then cultured in MEM medium with 20% FBS for 21 days. The TEER in Caco-2 cell monolayer was measured using a voltohmmeter (Millicell ERS-2, Millipore Co., Ltd.). The TEER values that reached 500 Ω ·cm² were used in the following experiment. To prepare samples, free PFD, PFD@Z, PFD@ZD, and PFD@ZD-FA (1 mg mL⁻¹ PFD) were each diluted with PBS. The medium was then removed and equilibrated with prewarmed PBS for 30 min at 37 °C. The PBS was replaced with 0.2-mL samples in the upper chambers and 0.8 mL of fresh PBS in the lower chambers. After 2 h of incubation, 0.5 mL of the solution was withdrawn from the lower chambers and quantitatively analyzed using HPLC to determine the content of PFD. The P_{app} of PFD was calculated as follows:

$$P_{app} = \frac{Q}{AC_0t} \quad (5)$$

where Q is the cumulative amount (ng) of the PFD transported into the lower chambers, A is the Caco-2 cell monolayer area (cm²), C_0 is the initial PFD concentration (ng mL⁻¹) in the upper chambers, and t is the duration time (s) of the permeability experiment. For the study of intracellular uptake, the cells were harvested, lysed, and extracted with acetonitrile. The cellular uptake of PFD was then quantified using HPLC. In addition, different inhibitors were also added in the permeability experiments to investigate the transcellular mechanism of PFD@ZD-FA. Briefly, the culture medium was removed and incubated with prewarmed PBS for 30 min. The Caco-2 monolayers were then washed and incubated at 37 °C with PBS containing active ingredient (4 μ g mL⁻¹), active ingredient (10 μ g mL⁻¹), genistein (100 μ g mL⁻¹), and FA (10 μ g mL⁻¹) for 30 min. Subsequently, the treatment was replaced with PFD@ZD-FA (0.2 mL, 10 mg mL⁻¹ PFD) for 2 h. The lower chambers solution was collected, quantified and the P_{app} values of PFD were calculated. Lastly, the TEER values were measured to assess the impact of PFD preparations on Caco-2 monolayer integrity. First, the initial TEER values of the Caco-2 cell monolayer were measured. The upper-chamber medium was then replaced with 0.2 mL of PFD preparations (1 mg mL⁻¹ PFD) and TEER values were determined at 0.5, 1, and 2 h. After 2 h, the PFD preparations were withdrawn from the upper chambers and replaced with PBS. The TEER values of the Caco-2 cell monolayer were measured again at 4 h after different treatments were then measured.

Distribution of PFD@ZD-FA in the Gastrointestinal Tract: For imaging, NR-labeled nanoemulsions were prepared (described in the Supporting Information). CCl₄-induced liver fibrosis model rats (described in the Supporting Information) were randomly divided into three groups ($n = 3$) and treated with NR + PFD, NR/PFD@ZD, or NR/PFD@ZD-FA (2 mg kg⁻¹ NR and 100 mg kg⁻¹ PFD) by oral gavage. The rats were then euthanized at 0.5, 2, 6, or 12 h after administration. The duodenum, jejunum, and ileum were removed, opened, and gently rinsed with PBS. After fixation with 4% paraformaldehyde, frozen sections were created and stained with DAPI. Fluorescence images of the small intestine sections were observed and photographed using CLSM.

Pharmacokinetic Study: CCl₄-induced liver fibrosis model rats were randomly divided into four groups ($n = 6$) and treated with free PFD (150 or 100 mg kg⁻¹), PFD@ZD (100 mg kg⁻¹ PFD), or PFD@ZD-FA (100 mg kg⁻¹ PFD) via oral gavage. At a predetermined time, the rats in each group were anesthetized, and blood samples (0.5 mL) were collected from

the jugular vein through an indwelling catheter. Blood samples were centrifuged at $3000 \times g$ for 5 min to separate plasma, and 100 μL of the plasma was mixed with 400 μL of acetonitrile. This mixture was centrifuged at $10\,000 \times g$ for 10 min at 4°C , and then the supernatant was removed and analyzed using HPLC. The pharmacokinetic data were analyzed using the WinNonlin pharmacokinetic software version 8.3 (Pharsight, Mountain View, CA, USA).

Evaluation of Intestinal Distribution and Liver Targeting of PFD@ZD-FA in Rats: For imaging, lipiodol-labeled nanoemulsions were prepared (described in the Supporting Information). CCl_4 -induced liver fibrosis model rats were randomly divided into three groups ($n = 5$) and treated with Lipiodol + PFD, Lipiodol/PFD@ZD, or Lipiodol/PFD@ZD-FA (2 mL kg^{-1} lipiodol and 100 mg kg^{-1} PFD) via oral gavage. Abdominal CT scans were then performed at 0, 0.08, 0.17, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h after gavage under anesthesia maintained with a small animal anesthesia machine (R500, RWD Life Science Co., Ltd., Shenzhen, China) using 1% isoflurane. Rats were scanned using a spiral CT machine (Optima CT 680 Expert, GE, USA). Rats in prone position were scanned from the dome of the diaphragm to the lower edge of the liver while breathing peacefully. Scanning parameters: tube voltage 120 kV, tube current 420 mA, slice thickness 3.5 mm, slice interval 3.5 mm, pitch 0.8, field of view 22 cm, scanning speed 0.75 s r^{-1} , matrix 512×512 . The standard algorithm was used to reconstruct the coronal image of the continuous layer. The reconstructed layer thickness was 0.625 mm and the reconstructed distance was 0.625 mm. CT scout images were used to observe the distribution of nanoemulsions in the gastrointestinal tract. Transverse CT images were obtained to measure the CT values of the liver. The region of interest was placed in the right lobe of the liver, 2 mm^2 in size, avoiding artifacts. Multiple measurements were taken, and the mean value was calculated.

Assessment of Targeting Capability to Activated HSCs: CCl_4 -induced liver fibrosis model rats were randomly divided into three groups ($n = 3$) and treated with NR + PFD, NR/PFD@ZD, or NR/PFD@ZD-FA (2 mg kg^{-1} NR and 100 mg kg^{-1} PFD) via oral gavage. After sacrificing the rats, the livers were collected at 6 h after gavage and fixed in 4% paraformaldehyde and blocked with 10% goat serum blocking solution for 1 h. The samples were then incubated with α -SMA antibody (ab7817, Abcam) overnight at 4°C and goat anti-mouse IgG H&L (Alexa Fluor 488) secondary antibody (ab150113, Abcam) for 1 h at room temperature. After staining with DAPI, the sections were covered with an antifluorescence quenching mounting agent. All slices were examined using CLSM.

Evaluation of Treatment Efficacy for Liver Fibrosis: Rats were given standard rodent food and water within the experimental period and randomly divided into six groups ($n = 6$) based on treatment: normal (no induced liver fibrosis; treated with PBS), model (induced liver fibrosis; treated with PBS), free PFD 150 mg kg^{-1} , free PFD 100 mg kg^{-1} , PFD@ZD 100 mg kg^{-1} , and PFD@ZD-FA 100 mg kg^{-1} . Rats were injected with CCl_4 (CCl_4 and olive oil in a 1:3 ratio at a dose of $0.1\text{ mL}/100\text{ g}$ body weight) twice weekly for 8 weeks for establishing the fibrosis model, and subsequently treated with PFD preparation via oral gavage once daily for 4 weeks. After treatment, the hardness of liver tissue was measured by ultrasonic SWE. Blood samples were collected for testing the levels of serum ALT and AST. Liver and tumor tissue samples were also subjected to H&E staining, Sirius red staining, and Masson's trichrome staining, as well as immunohistochemical analysis of α -SMA and collagen III to determine the degree of tissue fibrosis. Please refer to Supporting Information for the above experimental details.

Evaluation of Treatment Efficacy for Post-TACE Liver Fibrosis: In addition, the efficacy of PFD preparations against liver fibrosis after TACE was also assessed in this study. First, a rabbit VX2 liver allograft tumor model was established under percutaneous ultrasound guidance. TACE procedure was then performed after the tumor had been implanted for 2 weeks. Then the post-TACE liver fibrosis model rabbits were randomly divided into six groups ($n = 6$) based on treatment: model (VX2 liver tumor rabbit), TACE + PBS, TACE + free PFD 135 mg kg^{-1} , TACE + free PFD 90 mg kg^{-1} , TACE + PFD@ZD 90 mg kg^{-1} , and TACE + PFD@ZD-FA 90 mg kg^{-1} . These treatments were administered via oral gavage 1 week before and 2 weeks after TACE treatment. After treatment, the hardness of the liver and tumor measurements were performed using ultrasonic SWE.

Rabbit blood samples were collected for blood count and blood biochemistry (including ALT and AST). Liver and tumor tissue samples were used for various pathological staining, including H&E staining, Sirius red staining, Masson's trichrome staining, and immunohistochemical analysis of α -SMA and collagen III. For more details, see the Supporting Information.

Statistical Analysis: Results were expressed as mean $\pm$ SD from sample numbers (n). Comparisons between two groups of unpaired samples were performed using independent sample t -tests. Wilcoxon signed-rank tests were used to compare pre- and post-TACE values of two noninvasive liver fibrosis markers (APRI and FIB-4) within the same individual. The differences detection of the mean values between multiple groups were determined via one-way ANOVA, with the Tukey test. Two-sided testing, p value < 0.05 was used to indicate significance. Samples were randomly assigned to processing orders, and the investigators were blinded to sample group allocation during the analysis of experimental outcomes. GraphPad Prism (Version, 10.4.1) was used for statistical analysis.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Z.C., S.X., and Y.L. contributed equally to this work. Z.-M. C., S. X., and Y. L. conceived the idea, carried out the experiments, performed the formal analysis, and wrote the original draft. H.-Z.H., X.-L.J., C.-E.-Y.H., and Y.-H.Z. carried out the animal experiments. R.-B.S. and J.-Q.P. analyzed the data. L.T., P.L., Y.C., and S.Z. designed the experiments, provided the funding resources, and revised the manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

folic acid, liver fibrosis, pirfenidone, protein-polysaccharide, transarterial chemoembolization

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- [1] F. Bray, M. Laversanne, H. Sung, J. Ferlay, R. L. Siegel, I. Soerjomataram, A. Jemal, *CA Cancer J. Clin.* **2024**, *74*, 229.
- [2] H. Li, C. Liang, D. Kuang, G. Huang, M. Zhang, P. Chen, Q. Zheng, W. Xu, J. Ren, X. Han, X. Duan, *Cancer Biol. Ther.* **2023**, *24*, 2166335.
- [3] T. Kisseleva, D. Brenner, *Nat. Rev. Gastroenterol. Hepatol.* **2021**, *18*, 151.
- [4] T. Higashi, S. L. Friedman, Y. Hoshida, *Adv. Drug Delivery Rev.* **2017**, *121*, 27.
- [5] I. Mederacke, C. C. Hsu, J. S. Troeger, P. Huebener, X. Mu, D. H. Dapito, J. P. Pradere, R. F. Schwabe, *Nat. Commun.* **2013**, *4*, 2823.
- [6] S. Wang, S. L. Friedman, *J. Hepatol.* **2020**, *73*, 210.
- [7] M. A. Karsdal, H. Krarup, J. M. Sand, P. B. Christensen, J. Gerstoft, D. J. Leeming, N. Weis, O. B. Schaffalitzky de Muckadell, A. Krag, *Aliment. Pharmacol. Ther.* **2014**, *40*, 233.
- [8] R. Masuzaki, T. Kanda, R. Sasaki, N. Matsumoto, M. Ogawa, S. Matsuoka, S. J. Karp, M. Moriyama, *Int. J. Mol. Sci.* **2020**, *21*, 4906.
- [9] P. W. Noble, C. Albera, W. Z. Bradford, U. Costabel, M. K. Glassberg, D. Kardatzke, K. T. E. Jr, L. Lancaster, S. A. Sahn, J. Szwarcberg, D. Valeyre, R. M. du Bois, *Lancet.* **2011**, *377*, 1760.
- [10] J. Behr, A. Prasse, M. Kreuter, J. Johow, K. F. Rabe, F. Bonella, R. Bonnet, C. Grohe, M. Held, H. Wilkens, P. Hammerl, D. Koschel, S. Blaas, H. Wirtz, J. H. Ficker, W. Neumeister, N. Schönfeld, M. Claussen, N. Kneidinger, M. Frankenberger, S. Hummler, N. Kahn, S. Tello, J. Freise, T. Welte, P. Neuser, A. Günther, RELIEF investigators, *Lancet Respir. Med.* **2021**, *9*, 476.
- [11] X. H. Xiang, T. P. Jiang, S. Zhang, J. Song, X. Li, J. Y. Yang, S. Zhou, *Mol. Med. Rep.* **2015**, *12*, 309.
- [12] J. L. Poo, A. Torre, J. R. Aguilar-Ramírez, M. Cruz, L. Mejía-Cuán, E. Cerda, A. Velázquez, A. Patiño, C. Ramírez-Castillo, L. Cisneros, F. Bosques-Padilla, L. Hernández, F. Gasca, F. Flores-Murrieta, S. Treviño, G. Tapia, J. Armendariz-Borunda, L. E. Muñoz-Espinosa, *Hepatol. Int.* **2020**, *14*, 817.
- [13] M. Ghumman, D. Dhamecha, A. Gonsalves, L. Fortier, P. Sorkhdini, Y. Zhou, J. U. Menon, *Eur. J. Pharm. Biopharm.* **2021**, *164*, 1.
- [14] H. E. Jo, S. Randhawa, T. J. Corte, Y. Moodley, *Drugs Aging.* **2016**, *33*, 321.
- [15] L. H. Lancaster, J. A. de Andrade, J. D. Zibrak, M. L. Padilla, C. Albera, S. D. Nathan, M. S. Wijsenbeek, J. L. Stauffer, K. U. Kirchgaessler, U. Costabel, *Eur. Respir. Rev.* **2017**, *26*, 170057.
- [16] M. Molina-Molina, J. G. Shull, V. Vicens-Zygmunt, P. Rivera-Ortega, K. Antoniou, F. Bonella, E. Renzoni, A. M. Russell, T. M. Maher, A. Vancheri, A. Bachs, V. Avilés, J. Palma, G. Bermudo, G. Suarez-Cuartin, C. Tebé, R. Rigo-Bonnin, A. Montes-Worboys, M. Wijsenbeek, C. Vancheri, *Eur. Respir. J.* **2023**, *62*, 2300262.
- [17] Z. Zhang, J. Qi, Y. Lu, W. Wu, H. Yuan, *Med. Res. Rev.* **2021**, *41*, 2590.
- [18] R. Han, H. He, Y. Lu, H. Lu, S. Shen, W. Wu, *J. Control Release.* **2024**, *370*, 256.
- [19] H. Spleis, M. Sandmeier, V. Claus, A. Bernkop-Schnürch, *Adv. Colloid Interface Sci.* **2023**, *313*, 102848.
- [20] Y. Ozogul, G. T. Karsli, M. Durmuş, H. Yazgan, H. M. Oztop, D. J. McClements, F. Ozogul, *Adv. Colloid Interface Sci.* **2022**, *304*, 102685.
- [21] R. Mozafarpour, A. Koocheki, M. A. Sani, D. J. McClements, H. M. Mehr, *Adv. Colloid Interface Sci.* **2022**, *309*, 102768.
- [22] L. André de Almeida Campos, A. Francisco Silva Neto, M. Cecília Souza Noronha, M. Ferreira de Lima, I. M. Ferro Cavalcanti, N. S. Santos-Magalhães, *Int. J. Pharm.* **2023**, *635*, 122754.
- [23] L. Li, P. Yao, *Food Chem.* **2020**, *319*, 126577.
- [24] L. J. Zhou, G. R. Xu, Z. Zhang, H. X. Li, P. Yao, *Colloids Surf. A.* **2017**, *540*, 150.
- [25] A. Teo, K. K. Goh, J. Wen, I. Oey, S. Ko, H. S. Kwak, S. J. Lee, *Food Chem.* **2016**, *197*, 297.
- [26] S. Caballero, G. Davidov-Pardo, *Food Chem.* **2021**, *338*, 128083.
- [27] Y. Chen, J. Wang, J. Xu, J. Zhang, S. Xu, Q. Zhang, J. Huang, J. Peng, H. Xu, Q. Du, Z. Gong, *ACS Nano.* **2023**, *17*, 9090.
- [28] J. Li, B. Chen, T. Yu, M. Guo, S. Zhao, Y. Zhang, C. Jin, X. Peng, J. Zeng, J. Yang, X. Song, *Pharmacol. Res.* **2020**, *157*, 104796.
- [29] D. Yang, Y. Feng, Y. Yuan, L. Zhang, Y. Zhou, A. C. Midgley, Y. Wang, N. Liu, G. Li, X. Yao, D. Liu, *ACS Nano.* **2024**, *18*, 7455.
- [30] L. Xu, Q. Bai, X. Zhang, H. Yang, *J. Control Release.* **2017**, *252*, 73.
- [31] C. Chen, S. Du, W. Zhong, K. Liu, L. Qu, F. Chu, J. Yang, X. Han, *Nano Conver.* **2022**, *9*, 52.
- [32] H. Cheng, S. Guo, Z. Cui, X. Zhang, Y. Huo, J. Guan, S. Mao, *Int. J. Pharm.* **2021**, *596*, 120297.
- [33] L. Xiang, X. Wang, Y. Shao, Q. Jiao, J. Cheng, X. Zheng, S. Zhou, Y. Chen, *ACS Appl. Mater. Interfaces.* **2023**, *15*, 2030.
- [34] R. Loomba, L. A. Adams, *Gut.* **2020**, *69*, 1343.
- [35] T. J. Hydes, O. J. Kennedy, K. Glyn-Owen, R. Buchanan, J. Parkes, D. J. Cuthbertson, P. Roderick, C. D. Byrne, *Clin. Gastroenterol. Hepatol.* **2024**, *22*, 1657.
- [36] J. J. Yang, J. Wang, Y. Yang, Y. Yang, J. Li, D. Lu, C. Lu, *Eur. J. Pharmacol.* **2022**, *922*, 174900.
- [37] X. Liao, X. Ruan, P. Yao, D. Yang, X. Wu, X. Zhou, J. Jing, D. Wei, Y. Liang, T. Zhang, S. Qin, H. Jiang, *J. Transl. Med.* **2023**, *21*, 778.
- [38] D. C. Rockey, P. D. Bell, J. A. Hill, *N. Engl. J. Med.* **2015**, *372*, 1138.
- [39] X. Zhu, M. Cao, K. Li, Y. T. Chan, H. F. Chan, Y. W. Mak, H. Yao, J. Sun, M. T. Ong, K. K. Ho, C. W. Lee, O. K. Lee, P. S. Yung, Y. Jiang, *Bioact. Mater.* **2024**, *39*, 255.
- [40] Q. Lv, J. Wang, C. Xu, X. Huang, Z. Ruan, Y. Dai, *Mol. Med.* **2020**, *26*, 49.
- [41] K. M. Tsoi, S. A. MacParland, X. Z. Ma, V. N. Spetzler, J. Echeverri, B. Ouyang, S. M. Fadel, E. A. Sykes, N. Goldaracena, J. M. Kathis, J. B. Conneely, B. A. Alman, M. Selzner, M. A. Ostrowski, O. A. Adeyi, A. Zilman, I. D. McGilvray, W. C. Chan, *Nat. Mater.* **2016**, *15*, 1212.
- [42] W. Poon, Y. N. Zhang, B. Ouyang, B. R. Kingston, J. L. Y. Wu, S. Wilhelm, W. C. W. Chan, *ACS Nano.* **2019**, *13*, 5785.
- [43] W. Shan, X. Zhu, M. Liu, L. Li, J. Zhong, W. Sun, Z. Zhang, Y. Huang, *ACS Nano.* **2015**, *9*, 2345.
- [44] P. Lundquist, P. Artursson, *Adv. Drug Deliv. Rev.* **2016**, *106*, 256.
- [45] P. C. Griffiths, B. Cattoz, M. S. Ibrahim, J. C. Anuonye, *Eur. J. Pharm. Biopharm.* **2015**, *97*, 218.
- [46] M. Xu, Y. Qi, G. Liu, Y. Song, X. Jiang, B. Du, *ACS Nano.* **2023**, *17*, 20825.
- [47] J. A. Silva-Gomez, M. Galicia-Moreno, A. Sandoval-Rodriguez, H. O. Miranda-Roblero, S. Lucano-Landeros, A. Santos, H. C. Monroy-Ramirez, J. Armendariz-Borunda, *Int. J. Mol. Sci.* **2021**, *22*, 11360.
- [48] C. Li, R. Han, L. Kang, J. Wang, Y. Gao, Y. Li, J. He, J. Tian, *Sci. Rep.* **2017**, *7*, 40523.
- [49] X. Y. Zhao, X. Zeng, X. M. Li, T. L. Wang, B. E. Wang, *Clin. Exp. Pharmacol. Physiol.* **2009**, *36*, 963.
- [50] V. Y. Leung, J. Shen, V. W. Wong, J. Abrigo, G. L. Wong, A. M. Chim, S. H. Chu, A. W. Chan, P. C. Choi, A. T. Ahuja, H. L. Chan, W. C. Chu, *Radiology.* **2013**, *269*, 910.
- [51] M. T. Hove, A. Smyris, R. Booiijk, L. Wachsmuth, U. Hansen, L. Alic, C. Faber, C. Höltke, R. Bansal, *Bioact. Mater.* **2024**, *39*, 406.
- [52] B. Kerget, G. Çil, Ö. Araz, F. Alper, M. Akgün, *Med. Clin. (Barc.)* **2023**, *160*, 525.
- [53] M. Kus, I. Ibragimow, H. Piotrowska-Kempisty, *Pharmaceutics.* **2023**, *15*, 2523.
- [54] P. Artursson, K. Palm, K. Luthman, *Adv. Drug Deliv. Rev.* **2001**, *46*, 27.