Emerging Nano-Theranostic Strategies against Non-Alcoholic Fatty Liver Disease: a review

Emerging nano-theranostic strategies against non-alcoholic fatty liver disease: A review

Samam Sargazi[1], Mahtab Ghasemi Toudeshkchouei[2], Abbas Rahdar [3], Aisha Rauf[4], Soheil Amani[5], Muhammad Bilal[6], Razieh Behzadmehr[7], Ana M. Díez-Pascual[8], Francesco Baino [9,*]

[1] Research Institute of Cellular and Molecular Sciences in Infectious Diseases, Zahedan University of Medical Sciences, Zahedan, 9816743463, Iran; sgz.biomed@gmail.com

[2] Department of Polymer, School of Chemical Engineering, College of Engineering, University of Tehran, P.O. Box 11155-4563, Tehran 1417613131, Iran, mahtabghasemi2543@gmail.com

[3] Department of Physics, Faculty of Science, University of Zabol, 538-98615, Zabol, Iran, a.rahdar@uoz.ac.ir

[4] Grand Asian University, Sialkot Pakistan Quaid I Azam University, Islamabad, Pakistan, maisha.rauf001@gmail.com

[5] Institute for Advanced Studies in Basic Sciences (IASBS), Zanjan, Iran, soheilamani28@gmail.com

[6] School of Life Science and Food Engineering, Huaiyin Institute of Technology, Huaian 223003, China; bilaluaf@hotmail.com

[7] Department of Radiology, Zabol university of medical sciences, Zabol, Iran, razi.behzadmehr@gmail.com

[8] Universidad de Alcalá, Facultad de Ciencias, Departamento de Química Analítica, Química Física e Ingeniería Química, Ctra. Madrid-Barcelona, Km. 33.6, 28805 Alcaláde Henares, Madrid, Spain; am.diez@uah.es

[9] Institute of Materials Physics and Engineering, Applied Science and Technology Department, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy

* Correspondence: francesco.baino@polito.it

ABSTRACT

As a major global cause of liver disease, non-alcoholic fatty liver disease (NAFLD) is characterized by excessive hepatocellular accumulation of lipids in the liver, elevated levels of hepatic enzymes, and fibrotic evidence. The primary therapies for NAFLD are changing lifestyle or managing comorbid-associated diseases. Lately, nanotechnology has revolutionized the art of nanostructure synthesis for disease imaging, diagnosis, and treatment. Loading drugs into nanocarriers has been established as a promising strategy to extend their circulating time, particularly in treating NAFLD. In addition, considering a master modulator of adipogenesis and lysosomal biogenesis and function, designing novel nanostructures for biomedical applications requires using biodegradable materials. Various nanostructures, including inorganic nanoparticles (NPs), organic-based NPs, metallic nanocarriers, biodegradable polymeric nanocarriers, polymer-hybrid nanocarriers, and lipid-based nanocarriers have been designed for NAFLD treatment, which significantly affected serum glucose/lipid levels and liver function indices. NPs modified with polymers, bimetallic NPs, and superparamagnetic NPs have been used to design sensitive nanosensors to measure NAFLD-related biomarkers. However, certain limitations are associated with their use as diagnostic agents. The purpose of this review article is to shed light on the recent advancements in the field of nanomedicine for the early diagnosis, treatment, and prognosis of this progressive liver disease.

Keywords: Biomaterials; Nanomaterials; Drug delivery, Nanotechnology; Non-alcoholic fatty liver disease (NAFLD).

1. Introduction

As a chronic liver disease and a significant health concern, non-alcoholic fatty liver disease (NAFLD) is generally related to a sedentary lifestyle and hypercaloric diet and is primarily observed in low-level alcohol consumers (alcohol consumption of 30 g alcohol daily) or people who do not consume alcohol [1–3]. The disease refers to a general characteristic in which a large amount of fat accumulates in the liver cells [4]. There are two clinical-pathological entities afflicting individuals with NAFLD, one known as "simple steatosis" and the other referred to as "non-alcoholic steatohepatitis". Non-alcoholic steatohepatitis may progress and become an irreversible wound or injury, hepatocellular carcinoma, or cirrhosis [5]. NAFLD is marked by fatigue and abdominal discomfort in the upper-right quadrant of the body [6]. The disease prevalence in adults is roughly 30% and is directly associated with obesity [7]. The disease occurs in different strata and at different ages but is more common in people aged 35 to 55 [8, 9].

There are two types of NAFLD, i.e. non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver (NAFL) [10]. In NAFL, 5% hepatic steatosis is present without hepatocyte injury, while NASH refers to hepatic steatosis and lobular inflammation accompanied by hepatocyte injury, with or without a fibrotic phenotype [11]. The most dangerous NAFLD complication is liver cirrhosis, the last stage of liver damage (fibrosis) that leads to liver failure [12]. Cirrhosis develops due to liver injury, and when the liver tries to stop the swelling by leaving scars called fibrosis, the swelling persists [13]. Fibrosis has progressed to include more and more liver tissue, which, if left untreated, could result in esophageal vein swelling, bleeding, and rupture, as well as confusion, sleepiness, liver malignancy, and liver failure [14]. Several anomalies similar to metabolic syndrome are present in the pathological process of NAFLD, including increased belly fat, impaired insulin sensitivity, elevated blood pressure, and elevated blood triglycerides [15].

It has been established that increasing synthesis of triglyceride pharmacologically will increase the release of very-low-density lipoprotein (VLDL) into the bloodstream. Therefore, by increasing the risk of cardiovascular disease, a treatment for NASH that is unlikely to be feasible. There is a lack of understanding of the lipotoxic species produced in NASH. However, once they are better understood, specific inhibitors or accelerators of their synthesis and removal could prove helpful in the treatment of this condition [16]. Anti-apoptotic, anti-inflammatory and anti-fibrotic agents are currently being used in treatment approaches to manage lipotoxic injury [16].

A liver biopsy is needed to determine whether NAFLD markers such as early fibrosis, inflammation, hepatocyte ballooning exist and where they are located, even though current imaging techniques such as ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI) can diagnose NAFLD [17]. The invasive nature of liver biopsies and their high cost make non-invasive methods to diagnose NASH and liver fibrosis necessary [18]. Recent improvements in the use of nanoparticles (NPs) as smart probes for early detection of metabolic sickness markers have helped tackle this problem and make a diagnosis more quickly. The most popular indicators utilized in nano-sensors include glucose, uric acid, leptin, and other vitamins [19]. Carbon nanomaterials, monometallic nanoparticles (i.e., gold, silver, and platinum NPs), and bimetallic NPs, particularly iron-gold NPs, have been used [19, 20].

NPs have a wide range of applications in nanomedicine with special reference to nano-diagnosis and therapy, including vaccine, gene, and drug administration, due to their unique physiochemical, biochemical, and electrical properties [21–26]. By finely modulating drug distribution, absorption, and metabolism, nanotechnology has helped overcome the constraints of traditional delivery, such as drug safety and efficacy [27–30]. Encapsulating hydrophilic pharmaceuticals like proteins, nucleic acids, or peptides has also improved their stability in biological conditions as well as their transmission through cell membranes and blood flow [31].

Depending on the manufactured material, NPs can be divided into five categories: polymeric NPs, inorganic NPs, lipid-based NPs, bio-inspired NPs, and hybrid NPs [31]. NPs are being utilized to treat various diseases, including cancer, infections, and metabolic disorders such as NAFLD [32, 33]. Previously, Teng and colleagues developed galactose (Gal)/oxidized starch-lysozyme (OSL) (Gal-OSL) nanovehicles for delivering resveratrol (RSV) to the liver tissue. Using covalently conjugated Gal, Gal-OSL NPs were successfully directed RSV into liver tissues, thus increasing hepatic RSV levels *in vivo*. Their findings showed that Gal-OSL/RSV nanovehicles could be a promising formulation to treat NAFLD at early stages [34]. Curcumin was also loaded onto poly(lactic-co-glycolic acid) (PLGA) NPs to improve its stability and bioavailability. The findings demonstrated that NPs increased curcumin bioavailability by 22-folds and reduced the degree of fatty liver [35]. Similarly, PLGA NPs modified with RSV are reported to show excellent stability, solubility, and bioactivity and reduced the severity of NAFLD in the study by Wan et al. [36].

The goal of this article is to shed light on the application of nanomedicine in the management of NAFLD and provide new insights for improving the functional features of NPs to overcome pharmacological constraints. We expect that this review serves as both inspiration and motivation for the ongoing development and design of NPs to treat chronic liver disease.

2. Diagnosis of NAFLD

2.1. Current methods

When a person is suspected of NAFLD by the abnormal ultrasound or biochemical analysis, a complete clinical history is crucial to rule out the possible convictions of steatosis (SA) and its associated conditions. Most of the NAFLD patients are presented with symptoms without any apparent specific cause, such as sleep disturbances and fatigue, and a careful approach regarding the patient's drug history, auto-immune diseases, hepatitis C infection, Nilson's disease, etc. is therefore needed. Other conditions related to NAFLD may include hypogonadism, polycystic ovary disease, hypopituitarism, and hypothyroidism. Clinical assessment of NAFLD or steatosis must include biophysical assessment, blood pressure, waist circumference, body mass index (BMI), biochemical analysis and biomarker analysis [37, 38].

2.1.1. Biochemical and clinical assessments

During routine testing, mildly deranged liver enzymes may be presented, but the clinician must not reck on the abnormal liver biochemical analysis to detect the NAFLD. In fact, almost 80% of patients remain asymptomatic. The liver biochemical tests include alanine aminotransferase (ALT), prothrombin time, albumin, aspartate aminotransferase (AST), and platelet count assay. In NAFLD, levels of both ALT and AST enzymes are elevated 2-5 times while AST/ALT ratio is more than 1 with advanced fibrosis. Alkaline phosphate may also be elevated but is not always a characteristic of NAFLD. Some evidence suggests an increased fructose level in a total of about 60% NAFLD patients, representing the condition of advanced liver fibrosis. Smooth muscle actin antibody and anti-smooth muscle antibody levels are generally positive at the low titer in NAFLD. Lipid profile, urea, electrolytes, HbA1C, and thyroid-stimulating hormone (TSH) may also be assessed to estimate any related metabolic disease. Biochemical tests alone are insufficient to detect NAFLD and/or steatosis accurately. Other specific scores contain various parameters such as fatty liver index (FLI) and steato test [39–43].

More specifically, steato test is based on hepatoglobin, apolipoprotein A1, ALT, gamma-glutamyltransferase (GGT), bilirubin, BMI, cholesterol, macroglobulin, and glucose tests considering the age and gender of the patient. The scoring depends on the presence of ALT, AST, fasting insulin and ALT/AST ratio, type-2 diabetes mellitus (T2DM), etc. Other parameters include triglycerides, liver accumulation products such as waist circumference, gender, and hepatic steatosis indices, including the ALT/AST ratio [40, 43].

2.1.2. Imaging techniques

The most widely available, non-invasive and cheapest method for diagnosing NAFLD is ultrasound analysis. The continued attenuation parameters by the ultrasound can be used to quantify the steatosis, but the reproducibility and reliability of this technique is also a question mark. MRI-based imaging techniques are currently being used to quantify steatosis, but not commonly. MR-based protein density fat fraction diagnosis is the most accurate approach for diagnosing NAFLD. Thus, monitoring of fat and grading of NAFLD are essential for diagnosing NAFLD. Because it depicts the entire liver at a single measurement. Still, the expense and availability are the two restrictions in using the MRI as a diagnostic procedure for NAFLD [44–46].

Transient elastography is another routinely used clinical procedure for diagnosing NAFLD, which measures the liver elasticity in kilopascals (kPa). This imaging technique is a quick procedure with < 5 min duration and immediate result. [47–49]

A combination of serum biomarkers and TE is nowadays used for determining NAFLD stages.

2.1.3. NAFLD fibrosis score

The advanced stage of liver fibrosis with stages 3 or 4 is related to the mortality of NAFLD. Liver blood tests (ALT/AST ratio, platelet index, procollagen 3 peptide) and their predictive negative values are somewhat better than predicted positive values. Other lab tests that can recognize stage 3 and 4 fibrosis include Fibrosis-4, BARD, and fibrosis scores of NAFLD. The non-alcoholic fatty steatosis (NFS) uses variables such as BMI, age, ALT/AST ratio, T2DM, serum albumin, and platelet count and is the advanced and highly accurate method with liver biopsy [50, 51].

2.1.4. Liver biopsy

NAFLD liver staging and diagnosis cannot be performed routinely by liver biopsy because this method is invasive and stressful to patients; therefore, its application has to be carefully planned [52]. Still, this technique remains an essential diagnostic tool to accurately detect the accurate stage of liver disease as the other diagnostic tests do not always lead to the differential diagnosis among diseases. The NAFLD activity score (NAS) can be considered the most crucial diagnostic scoring recommended by the NASH clinical research network. The lobular inflammation score (0-3), hepatocyte ballooning (1-2), and steatosis (0-3). are included in this grading. As part of clinical trials, liver biopsy is frequently used to determine NASH key characteristics [53–55].

2.2. The application of nanomedicine for diagnosis and imaging of NAFLD

The nanomedicine-based drug delivery approach allowed the formulation of therapeutic modalities at the nanoscale using nanotechnology [56]. The nano-vehicles can be of inorganic or organic nature, and their small size enables their easy dispersion in aqueous solution, which is an essential aspect of administrating nanodrugs in peripheral blood [56].

Nanomaterials have tunable shapes and a large surface area-to-volume ratio. They can provide a broad surface area and can be functionalized with various ligands, which improves their pharmacokinetic properties as the circulation time of NPs can be enhanced, for example, by modifying with polyethylene glycol (PEG). Many attached ligands allow the specific attachment of the nanomaterials to different receptors that are present on the cell surface. The labeled nanomaterials can be utilized for theranostic purposes for various clinical conditions [57, 57–59]. A summary of the different approaches is displayed in Figure 1.

2.2.1. Magnetic Nanoparticles

Inorganic magnetic NPs show great promise for both diagnosis and therapy (i.e, theranostics) of many diseases, including cancer by magnetic hyperthermia and NAFLD. Magnetic NPs typically comprise a metal, bimetallic or metal oxide core coated with an organic material, and the whole nano-system provides inimitable optical, magnetic, and electrical properties. The interaction of magnetic NPs with the magnetic field will allow easy penetration of these NPs through the human body, thus enabling their use as a valuable biomedical tool.

Bimetallic NPs are interesting nano-systems and, in the context of liver disease treatment, one study reported the synthesis of graphene oxide-impregnated bimetallic (Au-Fe) NPs for label-free detection of the markers associated with the NAFLD [27].

However, because of higher bio-safety, magnetic oxide-based NPs are often preferred. In this regard, magnetic iron oxide NPs (Fe_3O_4 and γ-Fe_2O_3) are routinely utilized in nanomedicine due to their ferromagnetic behavior. Specifically, superparamagnetic iron oxide NPs (SPIONs) with a size of 30 nm, as well as ultra-small superparamagnetic iron oxide NPs (USPIONs) with a 10 nm size, have a variety of applications in diagnostic procedures such as imaging of intestines, lymph nodes, cardiovascular systems, and liver [60–65]. The reported circulation half-life of SPIONs and USPIONs is longer (24 h), and because of their small size, they cannot be engulfed by the phagocytes [66]. The magnetic NPs with adjustable anisotropy can be widely used in the field of magnetic hyperthermia [67]. The chemical composition of

ferrite could be modified via doping the ferrite with particular metals, thus tuning their anisotropic magnetic properties [68].

The most effective FDA-approved iron-oxide-based drug is feraheme (furomoxytol), which consists of magnetite NPs coated with carboxydextran that are successfully applied in MRI as an off-label agent [63, 69, 70]. Iron oxide is administered orally, but the low gastric pH can degrade the iron oxide, thus limiting its delivery via the oral route, so there is a need to coat the iron oxide NPs with certain coating agents such as silica oxide to protect from a harsh gastric environment [71, 72]. The magnetic NPs labeled with mesenchymal stromal cells (MSC) provides a new promising therapeutic modality against liver fibrosis (NAFLD and HCC) and for diagnostic purposes [73] as imaging agents related to MRI. The use of feraheme, an off-label agent, for evaluation of the stages of liver fibrosis has significantly increased [74]. Iron oxide NPs act as a negative contrast agent and represents the superparamagnetic effects of T2 [75].

Currently, different types of magnetic NPs with different surface modifications are being produced, but certain limitations are associated with their use as diagnostic agents. Lie et al. conjugated the iron oxide with the indocyanine green (immunofluorescence marker) and targeted the integrin avb3 to diagnose early liver fibrosis [17]. However, the iron may accumulate in the tissues and thus can be very harmful to the bodily systems. One study reported the induction of septic shock and altered liver enzymes after a single dose administration of magnetic NPs, while high doses upregulated some genes related to liver cirrhosis [76, 77]. The iron overload via magnetic NPs induces apoptotic cell death in macrophages via activating the c-Jun N-terminal kinase (JNK) signaling pathway [78].

As a contrast agent, ferumoxytol has been utilized in MRI for the early diagnosis of NAFLD. In this regard, the adverse effects of iron NPs were only limited to allergic, anemia or hypertensive patients [7]. The use of SPIONs in the NAFLD as a contrast agent is based on the ability of the liver macrophages Kupffer cells to uptake the SPIONs with less uptake in case of diseased cells compared to normal cells resulting in reduced MRI signals. Some examples of the SPIONs used as a contrast agent in the NAFLD treatment are given in Table 1.

Table 1: SPIONs and USPIONs for the diagnosis of NAFLD

SPION Type	**Disease**	**Disease model**	**Toxicity**	**Reference**
Dextran coated SPIONs (SPION-dex)	*In-vivo* tracking of liver, intestine etc.	Pig model	Excellent biocompatibility with no cytotoxic effect	[79]

SPION@SiO_2–ICG–RGD	NIR and MRI imaging of liver fibrosis	Mouse model	No detectable toxicity	[17]
Resovist	MRI indicated decreased enhancement in fatty liver disease	NAFLD patients with cirrhosis	-	[80]
Feromoxytol	MRI differentiates the NAFLD from healthy liver cells at a high dose	NAFLD and healthy patients	Off label contrast agent use	[7]
Resovist	The reduction of MRI signal with SPIONs is in inverse proportion to level of NAFLD	NAFLD and healthy patients	-	[81]
Resovist	Effectiveness determination of pioglitazone treatment in NAFLD with MRI	Ob/Ob male mice	-	[82]
Resovist	The signals of MRI reduced from 3h to 15 min with the progression of NAFLD	NAFLD rat model	-	[83]

NAFLD: non-alcoholic fatty liver disease, SPION: superparamagnetic iron oxide NPs; ALT: alanine aminotransferase; AST: aspartate aminotransferase; MRI: magnetic resonance imaging; ICG: indocyanine green; RGD: arginylglycylaspartic acid.

Due to the moderate levels of toxicity associated with iron oxide, there is a need to monitor its circulation and biodistribution in blood. The iron oxide magnetic NPs can be hybridized (i.e., usually coated) with various agents such as non-magnetic oxides, silica dioxide (SiO_2) polymers, as well as noble metals, potentially reducing their toxicity concern [84–87]. The most common organic coating of magnetic NPs is PEG which results in significantly improved plasma half-life of these NPs.

The pharmacokinetic behavior of magnetic NPs is altered with coating because of the alteration in surface charge [88, 89]. In addition to their role in providing dual surfaces for conjugation of the two types of molecules, nanohybrid conjugates play a vital role in the real-time delivery of drugs and intravital fluorescence microscopy for MRI [90]. Citrate-coated USPIONs with an ultrasmall size (12 nm) and outstanding water dispersibility exhibited a critical role as efficient MRI contrast agents in the rat fibrosis model of NAFLD, and the quality of contrast was significantly enhanced after the injection of these USPIONs [91].

Several coating materials such as chitosan, citrate, dextran, and polyvinyl acetate-methylacrylic acid were employed to produce more compatible SPIONs for diagnosing liver fibrosis in NAFLD [91–93]. Another nano-assembly of SPIONs consisted of Au-NPs and SPIONs that enabled the dual imaging modalities of this nanoparticulate system, i.e., MRI and computed tomography, to effectively distinguish between the healthy, NAFLD, AFLD, and cirrhosis liver cells in the rat model [94]. It was also observed that SPIONs linked to cyclic-RGD peptides demonstrated more excellent selectivity against integrin αvβ3 leading to enhanced MRI signal [77]. Another *in-vivo* study on Resovist in ob/ob mouse model examined the effectiveness of the pioglitazone in NAFLD management via the MRI technique. The signal fervency was reduced as the NAFLD disease progressed, and pioglitazone treatment improved the signal enhancement of SPIONs in MRI, which proved the possible recovery of phagocytic function of the Kupffer cells [82]. The assessment of collagen or elastin via non-invasive assessment in the detection of liver fibrosis can be visualized by employing MRI-based techniques [95]. There is no established marker for early diagnosis of the fibrotic liver.

Liquid biopsy by the detection of Pro-C3 can be very promising in detecting early fibrosis. Other biomarkers of liver fibrosis diagnosis include non-coding RNA, microbiomes, and micro-RNAs (miRNAs) [77, 96]. The non-invasive imaging modalities can be employed to screen such biomarkers. To detect fibrotic liver lesions using MRI, a USPION-based targeted contrast agent has also been used [91]. The in-situ imaging of the over-expressed miRNA is the advanced NAFLD diagnostic strategy. One study involved the synthesis of the Au-NPs coupled with the quantum dots and black hole quencher 2 (BHQ2) via hybridization of DNA in order to image the miRNA. The prepared low background fluorescent probe was employed for the targeted imaging of miRNA for diagnosing NAFLD [97].

Magnetic particle imaging (MPI) is an alternative technique to directly image the distribution of magnetic NPs and can be a remarkable strategy to manage liver diseases. The magnetic NPs can be used as important biosensors for detecting liver diseases like NAFLD [98–100]. The USPIONs are bound to the arginine glycine aspartic acid (RGA) and can thus detect the stage of liver fibrosis by employing MRI [10]. The

fluorescence imaging-based USPION-SiO_2 conjugated indocyanine green dye-based RGA targeting is remarkably improved for diagnosis liver fibrosis theranostic approach [101].

Patients with NAFLD overexpress some miRNAs in their hepatocytes, which link NAFLD and hepatocellular carcinoma [102]. It can be an essential biomarker for estimating the pathogenesis and progress of NAFLD by monitoring its levels in NAFLD patients. Different fluorescent nanoprobes have also been devised for the imaging of these miRNA in hepatocytes such as a bioresponsive and degradable nanohybrid, liposomal nanohybrid decorated with red emitting carbon dots. Here, this system was localized for tumor imaging and light-mediated tumor growth inhibition [88].

2.2.2. Quantum dots

Quantum dots (QDs) offer unique and attractive fluorescent properties that are different from conventional organic dyes, including their strong fluorescence, broad excitation spectrum, and long-term photostability [103]. They are being utilized as energy acceptors and donors in several ways of fluorescent resonance energy transfer (FRET) probes. Various QDs, including semiconductor nanocrystals and nanometals (e.g. gold QDs), have been fabricated and tested in biomedicine for imaging, living system sensing, and drug delivery [104–109] [110]. In the context of NAFLD treatment, Chai et al. [97] developed a one-donor–two-acceptor nanoprobe by assembling gold NPs coupled with BHQ2 (AuBHQ) and quantum dots (QDs) through DNA hybridization. The QD fluorescence was quenched up to 82.8% simultaneously by the gold NPs and the BHQ2 via nanometal surface energy transfer and fluorescence resonance energy transfer, thus reducing the background signals for target imaging. Such low-background fluorescent nanoprobe was successfully applied for imaging the target miR-21 in NAFL cells by catalyzing the disassembly of QDs with the AuBHQ and the fluorescence recovery of QDs. The proposed approach showed promise for early treatment of NAFLD and deserves further investigation.

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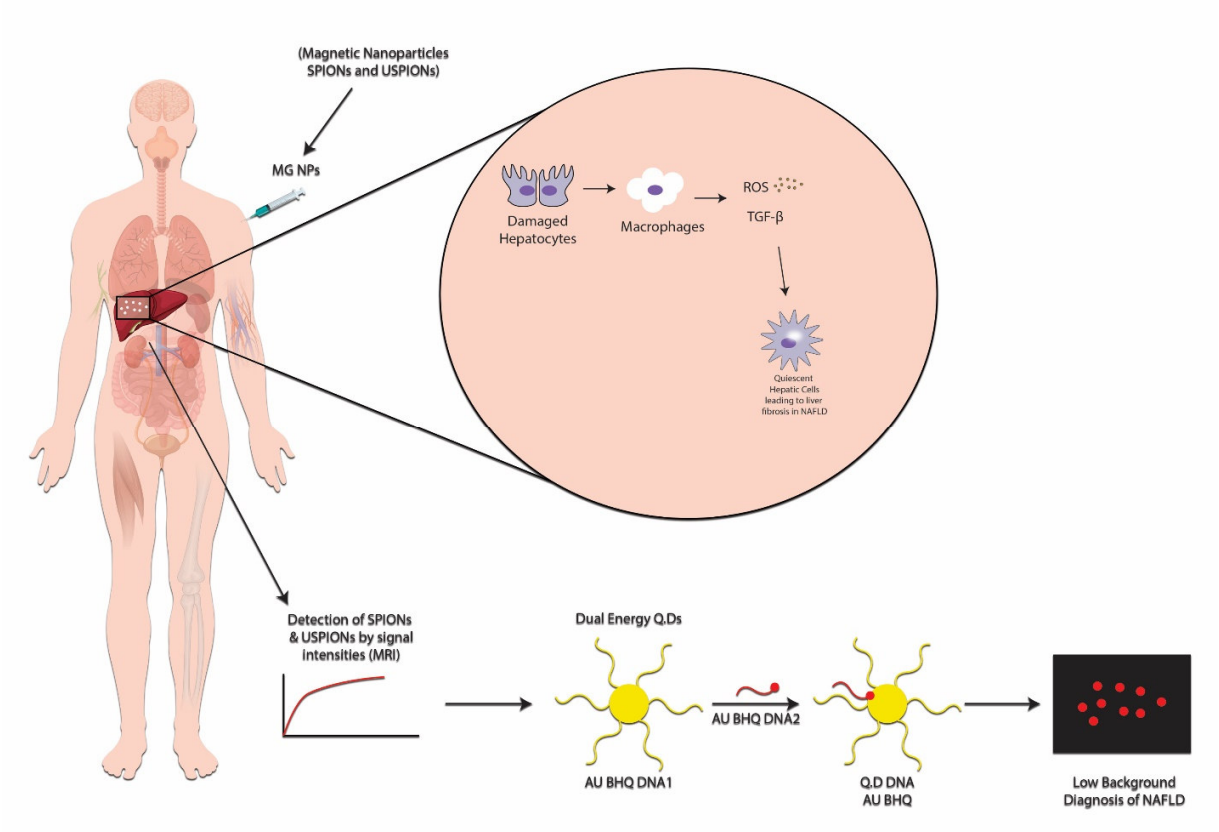

Figure 1: Diagram depicting the role of different nano-formulations in the diagnosis of NAFLD. MG NPs: magnetic NPs, QD BHQ DNA (DNA hybrid Au-NPs and QDs).

3. Nanostructures for NAFLD treatment

The liver is an essential organ that performs many functions required to maintain healthy cellular processes and maintain normal homeostasis [111]. In addition, liver hepatocytes in a healthy adult have a very slow turnover rate, and they can rapidly regenerate the liver cells upon loss of mass. In addition, an impaired liver is inherently resistant to insulin, the hormone that increases free fatty acids and increases lipogenesis [35, 36, 112]. As described in Figure 2, hepatocytes (parenchymal cells) are determined as the primary structural constituent of the liver.

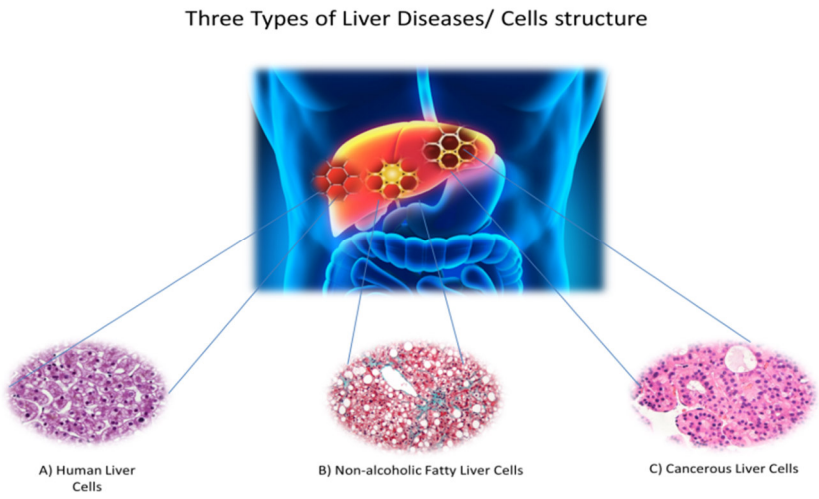

Figure 2. A) Schematic representation of different liver cells. Significant (centrilobular) macrovesicular steatosis (white/clear round/oval gaps), B) moderate fibrosis (green) with hepatocytes staining red are seen in the liver. Macrovesicular steatosis is characterized by a massive lipid accumulation that distorts the cell's nucleus. C) Liver cells with hepatocellular carcinoma (HCC).

Several methods that researchers have introduced, such as using hydrogen-rich water (HRW), electrolyzed-alkaline water (EAW) [113], and active vitamin D (VD) [114], led to undesirable effects while treating NAFLD. Nanostructures coated with bioactive molecules could provide a more productive and acceptable approach for treating NAFLD. Since NAFLD is a silent cause of death, nanotechnology-based strategies have been shown effective to reduce hepatic lipid deposition blood triglyceride lipid levels, thus inhibiting NAFLD. Herein, hepatoprotective effects of nanomaterials on triglyceride accumulation and lipotoxicity must be evaluated towards cellular and rodent models that probably exhibit their influences on the cell viability and GI_{25} value in HepG2 steatotic liver cancer cells. Additionally, 77 proteins associated with NAFLD are of utmost importance due to their interaction and association with a disease, and they can be translated into NAFLD clinical practice [115, 116].

In this line, nanoformulations have attracted great consideration because of their recent advantages on NAFLD treatment with manageable features to develop innovative nanomedicine systems for improving liver functions via reducing AST and ALT levels in blood [27, 117–119]. Specifically, it was claimed necessary to increase the safety and physicochemical properties of conventional drugs/herbal medicines, their physical, chemical, and physiological stability, as well as their ability to selectively target NAFLD. As a result, various liver nanosystems were made by considering the NP size and shape, zeta potential, rate of dissolution, and fabrication method (Figure 3) [20, 120]. At present, many inorganic- and organic-based NPs, including polymeric and metallic nanocomposites, nanoemulsions, liposomes, micelles, and other

unique nanostructured materials, are being evaluated for treating NAFLD or liver fibrosis via targeted delivery of small drug-like molecules, short-interfering RNAs, antibodies, etc. [121–123].

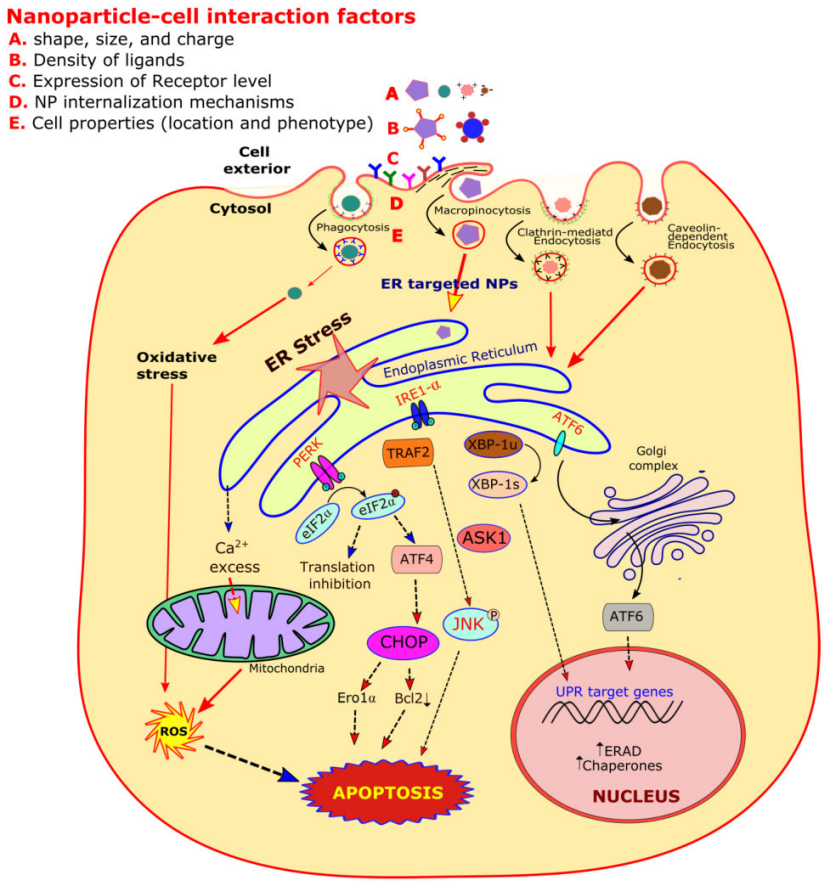

Figure 3. Au-NPs used to target liver cells selectively. The anti-obesity and anti-diabetic properties of generated Au-NPs from Smilax glabra were confirmed by restoring the internal membrane, nucleus, and cytoplasm [120].

3.1. Inorganic-based non-metallic nanostructures

In order to favor chronic changes in the complex physiological roles of the microbial communities related to commensal bacteria, collectively called "intestinal dysbiosis", with the determination of their consequences on immune functions, inorganic NPs have notably attracted researchers' attention. In fact, these types of nanomaterials may affect the gut microbiota because they can directly interact with the immune system and cause inflammatory responses [31]. Nanomaterials that mediated hepatoxicity have also been utilized in the treatment of liver diseases [124]; they include engineered nanoformulations consisting of metal oxides or metal cores such as silver (Ag), zinc oxide (ZnO) [125], zirconia (ZrO_2) [126], multi-walled carbon nanotube (MWCNT) [127], mineral acid extracts like arjunolic acid from Terminalia

arjuna [128], and positively charged titanium dioxide (TiO_2) NPs [129]. In detail, Kermanizadeh and his coworkers [130] compared the beneficial effects of some inorganic-based NPs for NAFLD treatment using a single exposure procedure. In recent studies, the coexposure to MWCNTs and heavy metal ions from lead acetate (PbAc) indicated significant aggravation of the non-alcoholic steatohepatitis phenotype along with hepatic lipid peroxidation, accompanied by upregulation of inflammatory cytokines in a mice model of NAFLD [131]. Therefore, genotoxicity and cytotoxicity of these nanomaterials, as well as their capability to change cytokine secretion and lipid peroxidation, were investigated to examine whether they can be replaced with conventional *in vitro* single cell hepatic model.

Furthermore, nanomaterial translocation proved beneficial to distal organs following various exposure routes, with the liver collecting a large portion of the total dose received. Chen's research team investigated the possible effects of orally ingested TiO_2 NPs on nutritional and lipid metabolism and actually found an impact on lipid metabolism because of the strong induction of oxidative stress [129]. The effects of other metal oxide NPs, like ZnO, were also studied. Because ZnO can be activated by thioacetamide (TAA), ZnO NPs relieved the decrease of hepatic or renal reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and lowered tissue malondialdehyde (MDA, an indicator for lipid peroxidation). Nevertheless, ZnO NPs treatment significantly reduced liver enzymes [gamma-glutamyltransferase (GGT), AST, ALT], inflammatory cytokines (TNF-α, IL-6), and markers of kidney function, including creatinine, urea, and uric acid. Thus, ZnO NPs are considered potential tools in NAFLD treatment due to their ability to decrease TAA toxicity through inhibiting oxidative stress [132]. Accordingly, inorganic NPs with specific optical, electrical, and magnetic features have uncountable advantages on NAFLD treatments. All in all, lacking biocompatibility of some of these NPs stopped investigation in the preclinical stage of studies [133].

A pathogenic mechanism with efficacy in equilibrating hepatic ROS is needed against excessive production of different reactive oxygen species (ROS) [134]. For this reason, nanocrystalline cerium oxide (CeO_2 NPs) can be proposed as a novel antioxidant agent as it showed hepatoprotective properties in experimental liver disease along with better agent loading capacity. Also, CeO_2 demonstrated anti-obesity effects and anti-inflammatory properties. Besides, CeO_2 nanostructures yielded the decline in the serum levels of pro-inflammatory cytokines (IL-1β, IL-12Bp40) in rats based on Kobyliak's research, and then restored IL-4, IL-10, and TGF-β levels compared with the control rats [135, 136].

Similarly, Carvajal et al. has investigated the therapeutic effects of CeO_2 NPs to control NAFLD and observed that the size and content of hepatocyte lipid droplets markedly decreased following treatment. As a result of the high hepatic levels of triglycerides and cholesterol esters, the expression of several cytokines involved in adipokine and chemokine signaling pathways will enhance the hepatic lipid accumulation of

saturated and unsaturated fats [137]. In addition, mefenamic acid (MFA) in terms of the anti-inflammatory agent can synergistically improve complex uptake, distribution, and biostability at the time of combination with CeO_2. Herein, Eilenberger and coworkers studied a CeO_2-based supramolecular complex that encapsulated well-standardized HepG2 cells, resulting in a significant anti-inflammatory response at nanocytotoxic levels [21]. CeO_2 NPs induced changes in fatty acid metabolism, as shown in Figure 4, and then HepG2 cell uptake them along with oxidative stress reduction and increasing the cell viability. As a result, CeO_2 NPs add up lipogenesis and the content of various fatty acids that contain more than 18 carbon atoms, affecting elongase and desaturase activities. Parra-Robert and her colleague reported that using CeO_2 NPs in NAFLD treatment is not be practical, whereas HepG2 cells are not protected against antioxidants and interfere with initiation of the kinase signaling pathway trigged by free radicals. So, it is essential for these NPs to efficiently encourage specific metabolic changes for minimizing fatty acids content in steatotic conditions [138, 139].

In order to evaluate inorganic-based nanocarrier toxicity accurately, high-throughput/-content (HT/C) techniques are employed. With this aim, a set of particles was selected comprising fourteen nanostructures of CeO_2, Ag, TiO_2, ZnO, and SiO_2 NPs with dissimilar coatings and surface features at different concentrations. After treatment, the reverse transcription-polymerase chain reaction (RT-PCR) should be carried out to determine ALT gene expression in liver cells. Then, triglyceride content in the blood should be quantified with an assay kit. As evaluated in the literature, metallodrugs are activated via redox reactions or ligand substitution, highlighting their role in preclinical developments for more efficient NAFLD treatment [140].

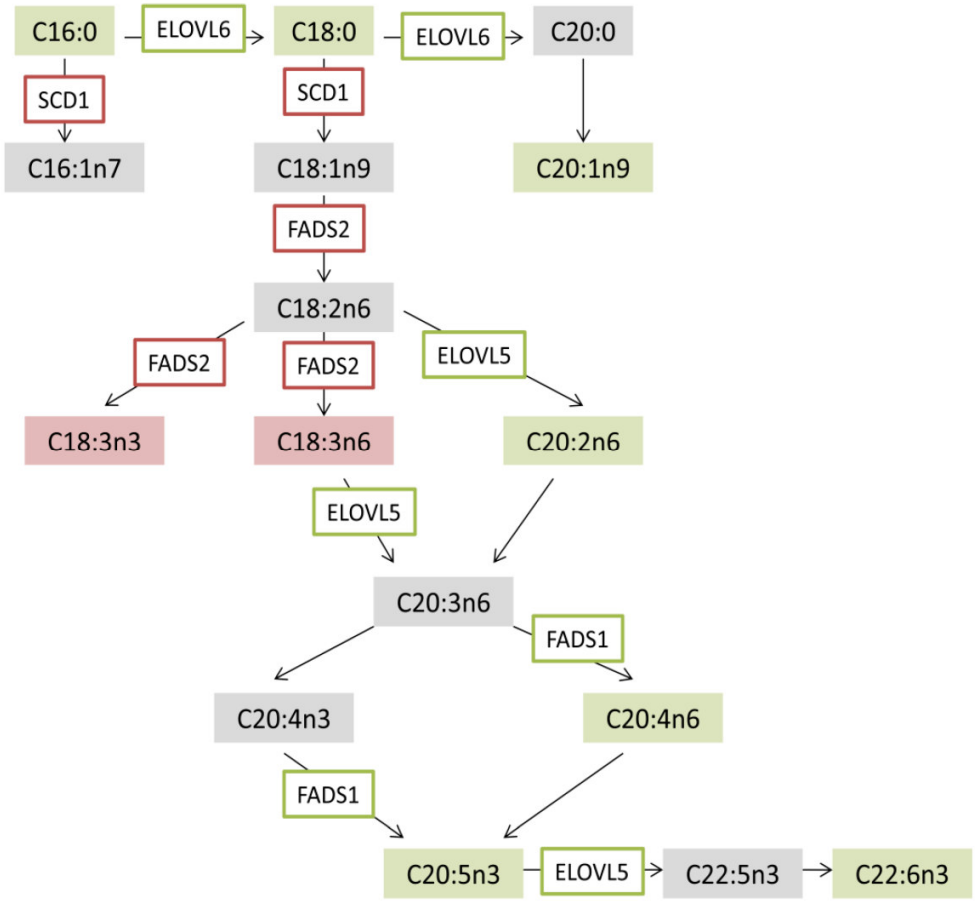

Figure 4. In steatotic hepatic cells (HepG2 cells), CeO2NPs altered the fatty acid metabolism. Fatty acids in red color represent the ones with increased content, and those in green color are the ones that decreased after the treatments. The activities of elongase and desaturase were also changed (red color represents an increase and green color represents a decrease) [138].

Among inorganic non-metallic NPs, silicon dioxide (SiO_2) NPs (SDNPs) are broadly applied to treat liver diseases. These NPs potentially lowered liver index and body weight gains, increased hematological indices (including white blood cells and platelets), and elevated concentration of ions (i.e., potassium, phosphorus, and iron). However, their probable toxicity caused a marked decrease in helatic enzymes (i.e., alkaline phosphatase, lactate dehydrogenase, AST, ALT) as well as low-density lipoprotein (LDL) [141]. For this purpose, mesoporous Santa Barbara amorphous-16 (SBA-16) silica NPs were produced using the facile sol-gel technique and appropriately functionalized to load atorvastatin, a bioactive molecule, on their surface. Then, parameters such as lipid profile, body weight, and activity of catalase, superoxide dismutase, ALT, AST, along with lipid peroxidation and liver histology, were monitored [142]. Besides reducing lipogenesis and increasing fatty acid catabolism, hollow mesoporous silica NPs (HMSNPs) were

fabricated to yield a high and sustained hydrogen (H_2) release in the gut. With this aim, results displayed that functionalizing HMSNPs with monomethoxyl poly(ethylene glycol) (mPEG) (as described in Figure 5) via aminated intermediate efficiently increases drug loading efficiency of the SiO_2-based nano-carrier. Hence, drug loading efficiency was about three times superior to as-such HMSNPs, which also retained their porous nature after PEGylation. Moreover, HMSNP-mPEG allowed a more prolonged release without initial burst effect as compared to the HMSNP control. For instance, Jin and coworkers designed a hydrogen nanocarrier via loading ammonia borane into HMSNPs to study their therapeutic outcome in managing early-stage metabolic dysfunctions, including NAFLD and diabetes mellitus [143, 144].

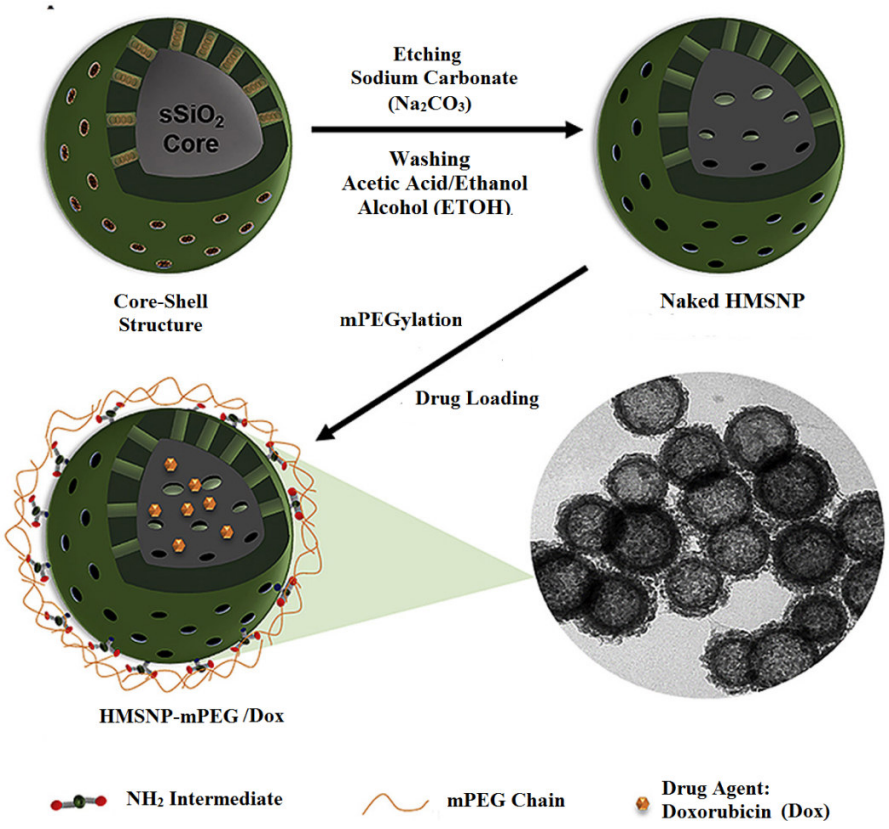

Figure 5. Synthesis Process of PEGylating HMSNP (HMSN-mPEG) [144].

3.2. Metallic Nanocarriers

Metallic NPs such as gold and silver NPs possess great pharmaceutical properties associated with their physicochemical parameters and, hence, show promise for the NAFLD treatment. Combination of nano-metal with iron oxide (e.g. Au-Fe_3O_4 NPs) was also reported to be a valuable option for the accurate detection of NAFLD [33]; in this regard it is worth noting that both metallic and oxide NPs tend, on one

hand, to accumulate in the liver and be associated to a certain hepatic toxicity [145], and on the other hand can be exploited for early diagnostic of NAFLD and other liver diseases [139, 146, 147]. Dealing with therapeutic effects, some interesting studies deserve to be mentioned. Smilax glabra rhizome, an anti-diabetic plant, was encapsulated using Au-NPs to restore liver tissue internal membrane, nuclei, and cytoplasm. Thoroughly, these observations confirmed the anti-obesity and anti-diabetic effects of the Au NPs synthesized from China root [148]. In another experiment, Jia et al. [34] prepared Ag NPs with a safe dose that does not cause general toxicity in normal mice. Then, outcomes described that Ag^+ ions were condensed to Ag NPs in fatty livers. The cytotoxic activity of such nanoformulation was associated with the concentration of Ag NPs – and not Ag^+ ions – in the liver. As well, Ag NPs enhanced hepatic inflammation by activating Kupffer cells (liver macrophages) in the liver and suppressing fatty acid oxidation.

A highly sensitive target probe can be obtained using porous nanomaterials because of their excellent structural properties. There are three types of porous nanomaterials based on their size: microporous materials (2-nm pores), mesoporous materials (2-50 nm pores), and macroporous materials (pores > 50 nm). As a result, they have many excellent physicochemical characteristics, including large band gaps, large surface areas, adjustable pore sizes, non-toxicity, unique optical properties, outstanding chemical and thermal stabilities, and low preparation costs. Therefore, the fabrication technique of these nanomaterials are introduced as chemiluminescence (CL) method taking advantage of low detection limit (LOD), simplicity of instrumentation, as well as ultra-sensitivity [149, 150].

Albumin level in the blood can predict liver function [151]. In this regard, monitoring albumin secretion in HepG2 liver cells can help understand NAFLD pathophysiology and its possible treatments. Lopez-Muñoz and associates designed a direct and label-free albumin monitoring device through integrating nanotechnology and plasmonic biosensor. Gold-coated and functionalized nanoplasmonic surfaces in the template of nanogratings were fabricated considering the precise and large area of nanostructured arrays presented in commercial Blu-ray discs (Figure 6) [152]. Along with recognizing albumin level in a two-dimensional (2D) fatty sickness liver model, gold nanogratings noticed the optimal levels of fetal bovine serum (FBS) to maximize the accumulation of lipids in cells upon treatment. Above these data, the fat exposition encourages a reduction in hepatocytes viability and metabolism and a parallel intracellular lipid assembly.

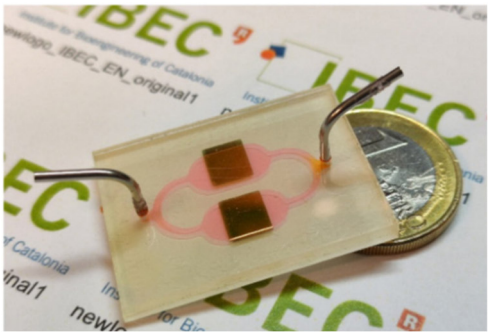

Figure 6. A image of the high reproducible gold nanogratings on a large scale [152].

3.3. Organic-based nanostructures

Biopolymers are widely proposed in the nanomedicine field to treat various diseases ranging from cardiovascular and neurological disorders to fatty liver diseases and deathful cancers, where drugs are loaded into the controlled degradable polymers. With the utilization of biopolymers and the aim of reaching more efficient outcomes in NAFLD treatment, the synthesis of bioactive compounds including curcumin (as an anti-inflammatory agent), carbon diselenide (CSe_2), and ZnO-RS are mainly considered. These nanosystems have also been able to suppress nucleotide-binding oligomerization domain (NOD), leucine-rich repeats (LRRs), inhibit the activation of pyrin domain-containing protein 3 (NLRP3) inflammasome, and enhance liver regeneration [139, 153, 154]. Furthermore, reduction of the possible side effects of bioactive substances can be managed by delivering them with a polymeric membrane [154].

3.3.1. Polymeric nanocarriers

The controlled release of nano-drugs, which have excellent biocompatibility and degradability in a physiological environment, can be accomplished using a polymeric shell with high encapsulation efficiency (EE%). Different types of synthetic and natural polymers, such as polyesters (e.g. poly(D,L-lactic-co-glycolic acid) (PLGA) [155], PEG, polymeric nanomicelles, and chitosan (CS) [156] were approved by US Food and Drug Administration (FDA) for NAFLD treatment. Meanwhile, functionalizing bioactive agents and polymeric shells with metallic NPs can efficiently intensify the therapeutic effect of metallic NP-based treatments. For example, in Zhou et al.'s experiment, citrate acid (CA), CS, and PEG-functionalized Au NPs were injected intravenously into the liver of mice in order to study the NAFLD-related drug metabolism. The increase in the activity of the drug-metabolizing enzymes was correlated with the increased cytotoxicity of Au NPs. Likewise, accumulated Au NPs in the liver interfered with the expression of enzymes involved in drug metabolism, including drug efflux and uptake transporters [157]. Therefore, both surface chemistry and nanomaterial interactions with the liver must be considered before deciding whether Au NPs can be helpful in NAFLD treatment.

3.3.1.1. Natural Polymer-based nanocarriers

In order to overcome bioactive substance or drug delivery limitations and enhance their hepatoprotective activities, the necessity of loading them into NPs has been proposed. For example, a study on incorporating nicotinamide or ascorbic acid within NPs of CS, a carbohydrate from a natural source, proved the amelioration in the insulin-resistant status [156]. On the other side, extracts or purified compounds derived from various natural sources, termed polyphenols, can be incorporated into NPs to surpass some key drawbacks, including insolubility in water and low systemic availability when orally administrated, thereby making such nanosystems effective upon targeting a particular diseased location [158]. In a similar study, hepatic-targeted oxidized starch-lysozyme (OSL) nanocarriers covalently conjugated with galactose (Gal), which is detected by highly expressed asialoglycoprotein receptors in hepatocytes, were fabricated and used to deliver RSV to liver tissue to maximize its therapeutic efficiency [159].

3.3.1.2. Synthetic Polymer-based nanocarriers

The low toxicity, clinical potential, and simplicity of production of polymeric nanocarriers make them ideal nanotools to target specific cell types, tissues, and organs when conjugated with a specific ligand or antibody. For instance, a water-soluble cationic polymer was synthesized with the capability of forming compact complexes by electrostatic interaction with deoxyribonucleic acid (DNA) named poly[2-(dimethylamino) ethyl methacrylate] (PDMAEMA) [156]. Another group of synthetic polymers, i.e. polyurethanes (PUs) that are formed by a reaction between isocyanates and polyols, have been used to synthesize NPs for delivering drugs to the liver, specifically when encapsulating fenofibrate (FNB) to prevent NAFLD. Although the FNB drug itself decreased the triglyceride levels in HepG2 cells, incorporating FNB into PU was shown to increase the absorption of FNB to liver cells [160]. Between all types of manipulated polymers, polyesters, lipids, hybrid polymers, and their blends have received more attention for the aim of beneficial NAFLD treatment.

Poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), poly-β-hydroxybutyric acid (PHB), and other polyester-based compounds have vast biomedical applications owing to their high-safety profile, especially in drug delivery assays. With this knowledge and biodegradability features of these materials, nanotechnology prevented premature release and facilitated sustained and controlled delivery of bioactive substances/drugs [161]. In this regard, different nanoformulations of

polyester-based nanocarriers, which were more efficient in NAFLD treatment, are shortly discussed in this section.

In the attempt of preventing the major limitations caused by free curcumin in liver disorders, poly(lactic acid)−poly(ethylene glycol) (PLA−PEG) copolymer NPs were designed to encapsulate it through a simple nano-emulsification technique and became stable via cationic surfactant. Five groups of rats were considered in the in vivo investigation of El-Naggar ME et al. [147], comprised of untreated rats (as the control group), rats administrated with streptozotocin (STZ) to induce diabetes, diabetic rats treated with standard curcumin, diabetic rats treated with PLA-PEG NPs, and finally, diabetic rats administrated with curcumin-encapsulated PLA-PEG NPs. As a result, histopathological investigations demonstrated a noticeable reduction in inflammation when rats were administrated with curcumin loaded into PLA-PEG NPs as compared to the control rats. Those researchers also found some effects on CD98, a heterodimer glycoprotein comprising SLC3A2 and SLC7A5, which can constitute a large neutral amino acid transporter that is abundantly expressed in the liver, triggering NAFLD and even liver cancer. As a result, a reduction of *CD98* expression was observed, and this finding was correlated with attenuation of liver disease markers by CD98 small interfering RNA (siRNA)-loaded PLA NPs [162].

PLGA is known for its well-controlled cargo release, bioactivity, improving cargo stability, and desirable solubility in water [33]. PLGA NPs encapsulating Vitamin E (VE) may show promising results in reducing side effects induced by glucocorticoids, which are regarded as a strong treatment for many pathologies that cause inflammation, such as NAFLD. This nanostructure may well reduce visceral fat and cholesterolemia, preventing hypercholesterolemia and cortisol-induced visceral fat accumulation [163]. In order to elucidate this importance, Wan and colleagues [164] loaded RSV into PLGA NPs (RSV-PLGA-NPs), fabricated as an oil-in-water (O/W) nanoemulsion, to treat NAFLD using HepG2 cells and an *in-vitro* model. In an acidic environment, sustained release of RSV improved the inhibitory activity of RSV-PLGA-NPs on the growth of HepG2 cells and buildup of lipids. Hence, it is expected the mechanism of NAFLD can be an efficient way for treatment this sickness by reducing inflammation, activating Sirtuin 1 (*SIRT1*, a protein-coding gene [165]), and mimicking the influences of caloric restriction. Overall, RSV-PLGA-NPs improved the physicochemical features of RSV (i.e., bioactivity, stability, and water solubility) and was considered a beneficial polyphenolic compound for NAFLD therapy. In addition, chitosan as a natural polymer has been effectively applied for designing polymer hybrid NPs. For instance, silymarin (a potent hepatoprotective agent) was encapsulated into hybrid lipid NPs containing chitosan (CS-LPNs) in the study of Liang et al. [166] to deliver it to the liver and increase its lipid-lowering impact. After that, silymarin bioavailability and its lipid-lowering effect were evaluated, resulting in better oral bioavailability and higher aqueous solubility and membrane permeability.

A solvent evaporation technique was employed to prepare dexamethasone (DXM)-loaded PCL degradable NPs. At the same time, for the treatment of symptomatic inflammatory diseases, other biocompounds (e.g., indomethacin), have shown significant anti-inflammatory effects [167, 168]. In this regard, the prepared PCL-based NPs showed good EE% and sustained drug release both *in vitro* and *in vivo* with an enhancement in the pharmacokinetic properties of the drug and offered a practical approach for drug targeting to the liver [167]. Also, this biocompatible polymer was used to synthesize NPs in combination with PEG to deliver botanical triterpene celastrol drugs. Regarding celastrol-loaded nanomicelles, the expression of IL-6, IL-1β, and TNF-α as inflammatory cytokines and macrophage M1 biomarkers was reduced dose-dependently, whereas the macrophage M2 biomarkers (i.e., Arg-1, IL-10) were dramatically upregulated. Overall, celastrol-loaded PCL-PEG NPs can represent a clinic-translatable therapeutic opportunity deserving further investigation [169]. Table 2 summarizes a selection of recent studies on polymeric-based nanocarriers for NAFLD treatment.

3.3.1.3. Polymer-Hybrid Nanocarriers

Researches indicate a unique superiority in endosomal escape capacity, satisfactory biocompatibility, and predominant liver accumulation using polymer-hybrid NPs. Most importantly, these structures distinctly improve hepatic steatosis, revitalize insulin sensitivity, and upgrade metabolic syndrome. For instance, Kececiler-Emir et al. encapsulated *trans*-RSV within hybrid NPs comprised of silica and G4 polyamidoamine dendrimers (PAMAM), which inhibited inducible nitric oxide synthase avoiding instability of RSV [170]. Furthermore, forming stable nanocomplexes in gene therapy with interleukin-22 (IL-22), which has been recognized as a promising agent for alleviating NAFLD, was reported via novel polymetformin in the research of Zai et al. [171], who achieved a targeted and sustained expression of IL-22 in the liver. This carrier was designed by conjugating biguanide to chitosan, termed chitosan-metformin (CM), that could exert advanced gene delivery efficiency and trigger intrinsic therapeutic efficacy from metformin for NAFLD.

Table 2. Literature review (selection) of researches on polymeric-based nanocarriers with the aim of NAFLD treatment**.**

Polymer Type	Bioactive Substances	Effects on NAFLD Treatment	Reference
Natural Polymer: CS	Nicotinamide	Reducing nitrosative and/or oxidative stresses while markedly increasing the hepatocellular energy	[156]
Natural Polymer: OSL-Conjugated Gal	RSV	Recovering hepatic insulin sensitivity in a mice model of NAFLD	[159]
Synthetic Polymer: PDMAEMA	Lac/microRNA 146b	Alleviating the hepatic steatosis in NAFLD	[156]
Synthetic Polymer: PU	FNB	Improvement of the inhibitory effects of FNB on NAFLD	[160]
Synthetic Polymer: PLA-PEG	DG, AD, and IKA under the category of TFEB Regulator	Ameliorate metabolic syndrome of steatosis	[172]
Synthetic Polymer: PLA	CD98-siRNA	Decrease hepatic steatosis	[162]
Synthetic Polymer: PLGA	RSV	Reducing the inflammation with activating SIRT1	[164]
Synthetic Polymer: PCL	DXM	Prevention of carbon tetrachloride-induced liver toxicity	[167]

GDM: Gestational diabetes mellitus, RSV: Resveratrol (3, 4′, 5-trihydroxy-*trans*-stilbene), FNB: Fenofibrate, OSL: Starch-lysozyme, Gal: Galactose, SIRT1: Sirtuin 1 (Protein Coding gene), DXM: Dextromethorphan.

3.3.2. Lipid-based nanostructures and nanoemulsions

Stable nucleic acid-lipid NPs, liposomes, solid-lipid NPs, nanostructured lipid carriers, self-nanoemulsifying drug delivery platforms, and steroid-based lipid NPs are discussed in this section. Strategies explored for targeting hepatic stellate cells can be pursued in view of elucidating the complex

molecular events connecting effects of el[173]evated free fatty acid levels, insulin resistance, and diabetes through the pathogenesis of liver fibrosis.

Colloid chemistry and nanotechnology have been developed in response to the increased demand for the effective delivery of active chemicals. Recent advancements in creating a wide variety of soft nanostructures, such as single or multiple nano-sized emulsions, have led to the investigation of various new formulations with excellent medicinal potential. Nanoemulsions are typically regarded as transparent/translucent heterogeneous systems comprising a continuous phase and a droplet-like scattered phase. In contrast, due to their small size and thermodynamic stability, they are unfavorably impacted by variations in temperature and composition [174, 175]. For example, Agame-Lagunes et al. [176] decided to investigate the therapeutic impacts of curcumin (*Curcuma longa*)-loaded nanoemulsions by using medium-chain fatty acids (in the form of mono and di-acylglycerides) as stabilizers. As a result, this nanosystem improved curcumin bioactivity without requiring high drug concentrations and prevented potential side effects.

Incorporating bioactive natural substances, such as pumpkin seed oil in nanoemulsion formulation, can be helpful to suppress the progression of fatty liver to steatohepatitis or to yield the desirable physicochemical parameters [177]. Furthermore, lutein as a non–provitamin A and an oxygenated carotenoid can protect hepatic cells against steatosis. In this regard, adipose tissue cholesterol can be utilized in nanoemulsions and alter lipoprotein metabolism, oxidative stress, and inflammation. These outcomes suggest that the metabolic effects of this specific nanoemulsion might not be protective in all tissues [178]. Moreover, lipid-based nanocarriers were structured according to deliver naringenin (NGN) for investigating their inhibitory effects on NAFLD. Finally, the enhanced rate of drug release, improved transepithelial transport, and intestinal absorption, along with the increased oral bioavailability with liver NGN distribution, demonstrated that the NGN-loaded nanostructured lipid carrier exhibited potent inhibitory effects against NAFLD [179].

4. Conclusions

NAFLD has become the deadliest disease associated with hyperlipidemia, obesity, and T2DM, which is caused by the fatty liver (steatosis), inflammatory liver failure due to extra fat in liver cells, without the existence of alcohol. So, its diagnosis and treatment require more attention to be in the most efficient direction. As a result, many researchers tried to find the best therapeutic way against steatosis with bioactive/drug agents loaded into nanomaterials and then figured out that these nanosystems could act notably better than simple injection of bioactive species. Herein, we reviewed the current state of the art about the utilization of nanoformulations and nanobiosensing technologies based on biocompatible

materials with great potential for application in early diagnosis, treatment, and prognosis of this progressive liver disease. We found that the efficacy of many of these nanostructures can be potentiated by functionalization with target ligands or substances that lead them to the liver tissues.

To sum up, among various nano-based drug delivery platforms, liposomes, nanomicelles, and inorganic NPs have been extensively examined, mainly because of their unique properties to deliver biocompounds and other therapeutic moieties. The main challenges behind the design of these nanosystems include the proper selection of the inorganic or polymeric NPs as well as polymeric coatings, their dosage, the way to avoid instability and agglomeration, and the choice of the most suitable preparation method for a given application and drug incorporation. Moreover, inorganic NPs modified with polymers, bimetallic NPs, superparamagnetic and ultra-small superparamagnetic NPs have been used to design sensitive nanosensors for NAFLD diagnosis. However, certain limitations are associated with their use as diagnostic agents. It seems necessary to better elucidate the nanomaterial-liver interactions and manipulate the NP surface chemistry prior to biomedical applications. Inorganic NPs based on iron oxides, like SPIONs, although being already marketed and used in clinics, suffer from moderate toxicity that may become an important concern in the treatment of NAFLD. The risk of toxicity to healthy cells and tissues as well as genotoxicity is a common limitation to all insoluble nanomaterials that persist in the human body: hence, the actual feasibility of any theranostic approach should be corroborated by careful in vivo investigation, also in the long term.

5. Future Perspectives

Among all nanostructures widely used for NAFLD treatment, nanoemulsions perhaps show better promise and are currently under discussion owing to their influences on NAFLD as regards liver fatty degeneration, liver lipids, histopathological alterations, and fibroblasts activation. For this reason, using the low energy spontaneous emulsification technique is recommended.

Hypovitaminosis D often coexists with NAFLD, and recent studies have outlined the potential advantages of vitamin D to combat NAFLD. Despite good features, the low water solubility of this lipid-soluble vitamin D as well as its poor oral bioavailability cause limitations that require solutions based on designing valid complexes that trigger better hepatoprotective effects. On the other point of view, sustainable building blocks are available for the assembly of polyurethanes, which was drawn out by the recent introduction of polyethercarbonate polyols. This structure combines excellent biocompatibility with smart applications such as “shuttles” for molecular components, especially in NAFLD curing techniques. Therefore, more investigations are required to improve the design and tailoring of nanoemulsions and polyurethanes as nanocarriers for bioactive compounds to more efficiently target NAFLD. Further investigations should be

carried out on developing nanocarriers that offer more stable pharmaceutical dosage forms with enhanced release patterns, increased pharmacological activity, and higher solubility that could pass cellular barriers to deliver the loaded drugs to the liver tissue. Finally, the advantages and disadvantages of the current nanomedicine-based approaches, specifically in developing point-of-care devices meeting the international guidelines for NAFLD diagnosis, should be considered.

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