Journal of Translational Gastroenterology **2023** vol. 1(1) | 22–29
DOI: 10.14218/JTG.2023.00027

Review Article

Utilizing the Gut Microbiome as a Therapeutic Target for Liver Disease – Narrative Review

Dharma Ayer[1], Alexa Trovato[2] and Micheal Tadros[3*]

[1]*Albany Medical College, Albany, NY, USA;* [2]*Internal Medicine, Boston Medical Center, Boston, MA, USA;* [3]*Department of Gastroenterology, Albany Medical Center, Albany, NY, USA*

Received: May 30, 2023 | **Revised:** August 31, 2023 | **Accepted:** September 13, 2023 | **Published online:** September 25, 2023

Abstract

The gut microbiome has been well-established in its role of regulating the onset of many gastrointestinal disorders. Recent evidence has shown a bidirectional relationship between the intestinal microbiota and the liver. The gut microbiome may affect liver disease progression through its bacterial composition, the metabolism of bile acids, and the translocation of bacterial products. Modulation of dysbiosis may be considered as a potential therapeutic target for liver disease regardless of the underlying cause. Continuing to identify parts of the gut-liver axis that are disordered in different etiologies of liver disease may offer insight into potential interventions to restore homeostasis. Thus, this review will focus on exploring some of the major gut microbiome targeted therapies for liver disease, including probiotics, prebiotics, and fecal microbiota transplantation.

Introduction

In the USA over 1.8% of adults are diagnosed with liver disease annually and liver disease is the cause of over 50,000 deaths each year.[1] Chronic liver disease is characterized by interference with normal liver functions for more than 6 months and involves a continual process of inflammation, destruction, and regeneration of liver tissue. There are several etiologies of chronic liver disease or cirrhosis including nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH), hepatitis, hepatotoxicity, and autoimmune disease.[2] The human intestinal microbiota plays a critical role in many parts of the digestive tract, and interactions between the gut and liver can contribute to translocation of this gut microbiota.[3] Thus, dysbiosis in the gut can contribute to the pathophysiology of liver disease.

The majority of the gut microbes are unable to cross the mucus barrier, preventing them from direct physical interaction with the epithelial lining.[4] Apart from microbes present at early stages of development, most are restricted to interaction with the epithelial cells in an indirect manner or via bacterial metabolic products.[5] Thus, the mucosal and epithelial barriers of the gut play an important role in immune defense, and dysbiosis of the gut microbiota may affect this barrier. Alterations in bacterial populations can result in increased permeability of the intestinal barriers and promote the influx of bacteria and their products to the liver.[6,7] Factors such as alcohol and high-fat diets may contribute to a leaky gut barrier and the dysbiotic microbiome. This can trigger inflammatory responses in the liver.[8] Likewise, bile acids (BAs) and secondary BAs are affected by the composition of bacteria present in the gut, while also modulating antibacterial and immune defenses.[9,10] An essential receptor in the signaling of secondary BAs is the farnesoid X receptor (FXR). When the gut microbiota is altered, regulation of BAs and homeostasis are impacted through downstream interactions with FXR. Thus, an altered microbiome may also contribute to liver disease progression by affecting the function of BAs.[11] The changes in the gut microbiome that contribute to transformation of a healthy liver to a cirrhotic liver are summarized in Figure 1.

Elements of the gut microbiome contributing to liver disease progression

In general, the composition of the human microbiome can be mediated by a variety of factors including diet, lifestyle, and antimi-

Keywords: Liver disease; Gut microbiota; Probiotics; Prebiotics; Fecal microbiota transplantation.

Abbreviations: ALT, alanine transaminase; AST, aspartate transferase; BA, bile acid; BF, Bacteroidetes and Firmicutes; ChREBP, carbohydrate-responsive element-binding protein; CTP, Child Turcotte Pugh; FMT, fecal microbiota transplantation; FXR, farnesoid X receptor; GI, gastrointestinal; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HDL, high-density lipoprotein; HE, hepatic encephalopathy; LDL, low-density lipoprotein; LPS, lipopolysaccharide; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; RCT, randomized controlled trial; SCFA, short- chain fatty acid; SREBP1v, sterol regulatory element-binding protein-1v; TJ, tight junction; TLR-4, toll-like receptor 4.

*Correspondence to: Micheal Tadros, Department of Gastroenterology, Albany Medical Center, 47 New Scotland Ave, Albany, NY 12208, USA. ORCID: https://orcid.org/0000-0003-3118-3893. Tel: +1-518-262-5276, Fax: +1-518-262-6470, E-mail: tadrosm1@amc.edu

How to cite this article: Ayer D, Trovato A, Tadros M. Utilizing the Gut Microbiome as a Therapeutic Target for Liver Disease – Narrative Review. *J Transl Gastroenterol* 2023;1(1):22–29. doi: 10.14218/JTG.2023.00027.

© 2023 The Author(s). This article has been published under the terms of Creative Commons Attribution-Noncommercial 4.0 International License (CC BY-NC 4.0), which permits noncommercial unrestricted use, distribution, and reproduction in any medium, provided that the following statement is provided. "This article has been published in *Journal of Translational Gastroenterology* at https://doi.org/10.14218/JTG.2023.00027 and can also be viewed on the Journal's website at https://www.xiahepublishing.com/journal/jtg".

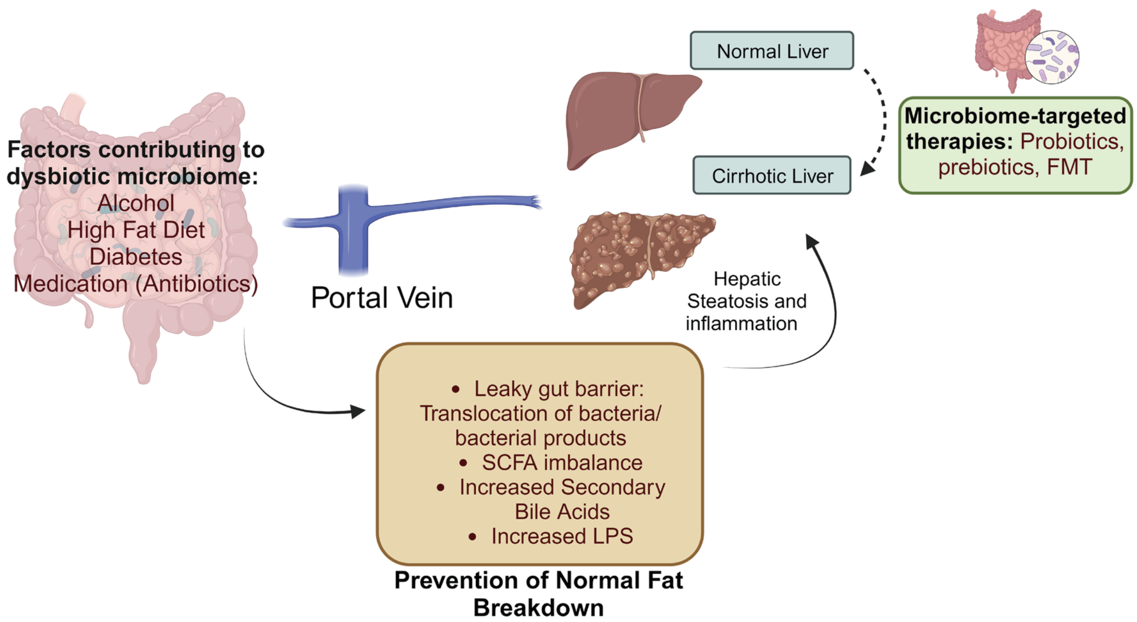

Fig. 1. Implication of gut dysbiosis in fibrosis of the liver. Figure created with www.BioRender.com. FMT, fecal microbiota transplantation; LPS, lipopolysaccharide; SCFA, short-chain fatty acid.

crobial agents. Shortly after birth, initial bacteria colonizing the newborn are usually facultative anaerobes followed by obligatory anaerobes such as *Bifidobacterium*, *Bacteroides,* and *Clostridium*. However, following introduction of solid foods and weaning from mothers' breast milk, diversity increases with actinobacteria and proteobacteria. By adulthood the main phyla dominating the gut include *Bacteroidetes* and *Firmicutes.* The short-chain fatty acids (SCFAs) propionate and acetate produced by *Bacteroides* and butyrate by *Firmicutes* are critical inflammation relievers which is why they may be an important target of restoration in microbiota focused treatments.[12] Finally, the gut microbiome continues to undergo changes as people age, and elderly individuals usually have reduced beneficial bacteria such as *Bifidobacteria* and increased pro-inflammatory microbes that may contribute to disease progression.[13] Exposure to components of the gut microbiome via the portal vein may influence the progression of liver disease and ultimately place those at an increased risk of hepatocellular carcinoma (HCC). Studies have shown that cirrhotic patients with or without HCC may have a higher abundance of certain bacterial genera such as *Lactobacillus* and *Bacteroides,* whereas healthier patients without liver disease have greater *Akkermansia* and *Methanobrevibacter* populations.[14] Variations in the gut microbiota are also present in cirrhosis patients as they often have colonic microbiota different from that of healthy control subjects, including significant increases in *Enterobacteriaceae* and *Enterococcus* or decreases in healthy microbial populations.[15] NAFLD patients tend to have lower proportions of *Bacteroidetes* and higher abundance of *Prevotella* and *Porphyromonas.* NASH, a severe form of NAFLD, may also be marked by bacteria that have increased ethanol production such as *Escherichia*.[16,17] These changes, however, may also be present differently in children. For example, *Bacteroidetes* and *Proteobacteria* have shown to be increased in children with NASH.[16] In hepatitis, specifically HBV-induced liver disease, beneficial taxa such as *Bifidobacterium* tend to be significantly decreased along with SCFA producers such as *Lachnospiraceae and Ruminococcaceae.* There is also a higher abundance of more harmful bacteria such as *Enterobacteriaceae.*[17] Most forms of cirrhosis are initially associated with decreased levels of *Bacteroidetes*, while progression may lead to decreases in *Bifidobacteria* as well as increase in *Streptococcus* and *Enterobacteriaceae*.[18] These clearly noted changes in microbial populations serve as an important marker of liver disease and are important considerations for microbiome-targeted therapy. It should also be mentioned that though there are accepted markers of a healthy microbiome constituting of a high microbial diversity, balanced ratio of Bacteroidetes and Firmicutes, high concentrations of fecal butyrate and low *C. albicans* numbers, there may still be several other variations between healthy individuals. Thus, this may serve as a challenge when optimizing microbiome-targeted therapies.[19]

Prebiotics and probiotics introduction

Probiotics, prebiotics, and synbiotics (i.e. combination of both), have important benefits for gut and liver health by regulating homeostasis of the microbiota. The use of probiotics often follows the guidelines of identification, functional characterization of the strain for safety and efficacy, validation of health benefits in clinical studies, and ensuring that the labeling is reflective of the efficacy. In creating ideal probiotics, factors to consider are nonpathogenic, genetically stable, acid and bile tolerant, as well as being able to survive processing conditions.[20] These criteria are similar for prebiotics. However, they vary in that they should be easily fermentable by healthy intestinal microbiota and should not be absorbed in the upper GI tract. Additionally, prebiotics may also help improve gut barrier function, host immunity, and reduce pathogenic subpopulations.[21]

Beneficial bacteria and nutrients promoted in probiotics and prebiotics have shown increases in proteins secreted, increases in SCFAs, as well as in bacteriocins which can improve the gut barrier. These changes include promoting mucus secretion by goblet cells and facilitating the expression of tight junction (TJ) proteins.[22] TJ proteins are important in the gut as they regulate paracellular transport across the intestinal epithelium.[23] For example, lipopolysaccharide (LPS), which is a component of gram-negative bacteria walls, is thought to alter the assembly of TJ proteins and can thus contribute to leaky gut. LPS may also cause further inflammatory responses by binding to Toll-like receptor 4 (TLR4). Thus, mechanisms to restore tight junctions via healthy bacteria in probiotics and prebiotics may help in preventing progression of liver disease.[24]

Probiotics and healthy microbial strains also produce what is known as bacteriocins, which are antimicrobial peptides that can prevent competing strains or pathogens and influence host immunity.[25] In animal studies including those of mice, pigs, and chickens, bacteriocins have resulted in changes in composition of the gut microbiota.[26] *In silico* identification of bacteriocin genes was done based on a reference database of the Human Microbiome Project, revealing 74 clusters of bacteriocin genes from members of the phyla Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, and Synergistetes.[27] Thus, further *in vitro* analysis of bacteriocin properties of gut microbial communities may allow for creation of probiotics that better regulate unfavorable bacterial populations, assisting in a healthier gut microbiome and preventing liver disease progression.

Probiotics

Probiotics consist of live microorganisms that are nonpathogenic and may be administered to restore balance particularly in gut microbiome communities.[28] Traditionally clinicians have regulated the microbial environment in the gut with selective gut decontamination involving nonabsorbable disaccharides (Lactulose), which act by lowering the pH in the colonic lumen and excretion of ammonia. This has been used to reduce symptoms of hepatic encephalopathy (HE) which consists of neuropsychiatric disturbances that may accompany acute liver failure and cirrhosis.[29] However, there is now growing research on utilizing probiotics for HE and other liver disease states. A prospective clinical study evaluated 105 patients diagnosed with minimal HE (MHE).[30] Experimental groups received either a treatment of probiotic strains (*Enterococcus faecalis, Clostridium butyricum, Bacillus mesentricus,* and lactic acid bacillus) alone or probiotics with lactulose. Improvement of MHE was found in both treatment groups, suggesting probiotics are a useful supplement in treating this condition.[30]

Clinical trials have also been done to address effects on NAFLD. One recent prospective trial of 39 NAFLD patients assessed how lifestyle modifications supplemented with a multistrain probiotic improved NAFLD activity score (NAS), inflammation, and hepatocyte ballooning. It was found that the probiotic group had significant improvements in the NAS score, hepatocyte ballooning, and fibrosis when compared to the placebo group.[31] Additionally, a recent meta-analysis of 15 studies on patients with metabolic-associated fatty liver disease has shown that probiotic supplementation can reduce liver enzyme levels and regulate glucose metabolism. The most commonly used probiotics that tend to be effective include *Lactobacillus* and *Bifidobacterium*, along with *Lactococcus*, *Streptococcus*, *Enterococcus*, and *Bacillus*.[32] A combination of probiotics with Omega-3 has demonstrated decreases in hepatic steatosis in prospective human studies, as opposed to probiotic or Omega-3 treatment alone. Furthermore, Omega-3 treatment with probiotics has been shown to reduce hepatic de novo lipogenesis by inactivating sterol regulatory element-binding protein-1v (SREBP1v) and carbohydrate-responsive element-binding protein (ChREBP) activity and also reducing inflammation. Therefore, the utilization of supplements may serve more benefits along with the use of probiotics that also restore healthy populations of gut microbiome communities.[33,34]

Other liver related conditions also show changes in microbial populations. Hepatitis B virus (HBV) is a life-threatening liver infection that may put people at high risk of developing cirrhosis and death from liver disease or HCC. It has been noted that the gut microbiota may be altered as HBV progresses. Particularly, multiple patient-based studies have revealed there is a reduction in common microbes such as phylum Bacteroidetes and Firmicutes while there is a higher abundance of *E. coli* especially as it is associated with end-stage liver disease.[35] It is well known that medications such as rifaximin are used to reduce overgrown gut bacteria, however there may still be limitations including potential lowering in portal pressure and less long-term gut microbiome stability.[36] A supplementation of probiotics from donors may help in maintaining a stable microbiome by restoring populations of bacteria such as *Bacteroides.*[37] Hepatitis C virus (HCV) also infects the liver and can lead to chronic hepatitis, cirrhosis, and HCC.[38] The results from a study on the effect of the number of endotoxins in HBV/HCV patient blood showed that increased levels of *Bifidobacteria* and *Lactobacillus* could help alleviate endotoxemia.[39] Thus, probiotics can be important as part of initial treatments to prevent progression of these viruses to more serious liver disease.

Though probiotic supplementation is useful in restoring normal microbial populations and increasing immune functioning to improve liver function in cases such as cirrhosis, there are still limitations. Probiotics have been extensively used and safely incorporated in many food and dairy products commercially marketed, with suggested limited risks.[40] However, there have been individual reports of adverse experiences with probiotic usage, such as in immunocompromised patients.[41] As laid out by much ongoing research, it is essential to continue recording instances of adverse events to account for populations more susceptible to negative outcomes, and additionally further risk and quality assessment of various probiotics.[42] Furthermore, increased analysis of clinical probiotic efficacy is needed as there is much individual variability present in the composition of the gut flora. Diet-based and genetic factors can play a role in response to probiotic therapy, as seen in many patients with irritable bowel syndrome. Depending on the state of starting gut dysbiosis, such as an overrepresentation of species such as *Streptococcus* and *Dorea,* response to gut microbiota treatment through probiotics may not be as effective.[43] Additionally, genetic analyses done for human NAFLD samples showed that host genetic variation in certain risk-alleles, including rs738409 and rs58542926, may influence the liver microbial DNA and contribute to mechanisms of disease.[44] Given the current state of clinical trials and the suggested role of individual variability in response to treatment, there is a need for more studies that account for diet, genetic factors, and duration of treatment, to ensure precisely targeted therapy and long-term efficacy.

Prebiotics

Prebiotics are nondigestible food ingredients fermented in the gut that can modulate the microbiome in beneficial ways.[45]Common prebiotics that are utilized include galacto-oligosaccarides, fructo-oligosaccharides, and inulin which can feed and stimulate the growth of healthy bacteria.[46] Though certain foods such as asparagus, garlic, and soybean already contain natural prebiotics, they are being manufactured at larger scales to increase their effectiveness.[45] Existing studies have examined the effects of prebiotics on rodent liver disease models primarily of NAFLD and obesity-related steatosis. Supplementation of the prebiotic inulin in a NAFLD disease model in mice showed prevention of liver-steatosis even when fed a fat-enriched diet. Additionally, it revealed intestinal microbiota changes, especially in *Akkermansia muciniphila* numbers which increased by five times.[47] In high fat diet fed rats, supplementation of a nondigestible carbohydrate (prebiotic) was associated with increased *Bifidobacterium* levels which lowered endotoxin levels and inflammatory response.[48]

Prebiotics can also be important for stimulating the production of SCFAs and favoring the growth of indigenous bacteria. This can help in cases of liver disease as it assists in lowering plasma lipids and hepatic triglycerides. They may also impede the growth of pathogens as they help lower luminal pH, which assists in regulation of the microbiome prevention of liver disease.[49] Prebiotic treatment in combination with probiotics (synbiotic treatment) has also shown to help not only in establishment of diet derived-microbial communities, but also their survival.[50] A trial conducted on synbiotic supplementation of *Bifidobacterium* and fructo-oligosaccharides in 66 NASH patients showed significant differences in levels of inflammation, steatosis, and hepatic fat accumulation.[51] However, few other clinical trials have furthered these findings. Thus, while prebiotics may also be important to account for in treatment of liver disease symptoms and preventing progression, existing limitations in the lack of clinical trials must be addressed.

Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is the process of administering fecal matter from a screened donor to another patient with the goal of altering the recipient's microbiome to a healthier state. The donor is usually selected after examining the family history of diseases and potential pathogens. A filtered mixture of their feces can be administered in many ways including a nasojejunal tube, colonoscopy, or retention enema.[52] It is well known that FMT has been used to treat recurrent *Clostridioides difficile* infection. Risk factors for *C. difficile* include antibiotic use which reduces diversity of the natural microbiota, providing an ideal environment for the infection.[53] This infection also tends to be recurrent and difficult to treat with the standard antibiotic treatments of vancomycin or metronidazole.[36] Thus, FMT has been established as a relatively effective and safe method of treating recurrent *C. difficile* infection in adults.[52,54] Currently, there are many studies further exploring the use of FMT to treat other gut and liver related conditions. Many randomized controlled trials (RCTs) have been and continue to be conducted for cases of liver disorders such as NAFLD.[55] In one particular RCT focused on NAFLD patients, it was hypothesized that FMT from a healthy donor would improve insulin resistance, and hepatic protein density fat friction. However, there were no significant changes in insulin resistance in patients with either allogenic or autologous FMT, though reduced levels of gut permeability were found.[56] Another RCT with NAFLD patients had two groups, with the non-FMT group given oral probiotics and the FMT group randomized to receive FMT with donor stool via a colonoscopy followed by three enemas. In this study, there were no statistically significant differences between the FMT and non-FMT group. It was found, however, that within the FMT treatment group, patients had better Bacteroidetes-to-Firmicutes ratios and lower proportions of Proteobacteria, of which high numbers are associated with dysbiosis. Additionally, it was found that though FMT improved NAFLD in both lean and obese patients, there were more significant differences in clinical manifestations and gut microbial composition in lean patients.[57,58]

In patients with alcohol use disorder, a placebo vs. enema of feces from a donor enriched in *Lachnospiraceae* and *Ruminococcaceae* were compared. Both *Lachnospiraceae* and *Ruminococcaceae* are bacteria whose depletion is associated with fatty liver. Results of this study showed significant decreases in cravings in the FMT group vs. the placebo group on day 15.[59] Additionally, there were decreases in IL-6 and LPS protein, which are associated with inflammation and hepatic injury, in the FMT group. It has been found that microbial diversity also increases with higher levels of SCFAs from FMT. Thus, there is evidence of short term reductions in consequences of alcohol use disorder with the use of FMT.[59–61] FMT has also been used in attempts to help cognition in cases of HE, and though improvements have been seen, these results may vary depending on each donor and recipient. The results of the above FMT trials are summarized in Table 1.

There are still adverse events that may be of note, so despite some promising results, more clinical trials are required to help optimize FMT treatments to treat liver related conditions.[60] A major limitation of FMT is that is not representative of all bacterial communities and research in this area still has several unknowns, especially regarding the long-term efficacy and adversity of FMT.[19] Stool samples are primarily colonic luminal bacteria, and do not account for colonies embedded in the intestinal mucosa that may not shed as easily. Additionally, stool samples do not account for small intestinal bacterial communities that may also play a lesser known role in the onset of digestive and liver disorders.[62] As clinical studies persist, there will need to be clear documentation of post-treatment effects that are evaluated to gauge safety.

Diet

Though the focus of this review is on probiotics, prebiotics, and FMT, there are other methods of managing the gut microbiome. One of the most effective initial steps in managing liver disease is changes in diet and lifestyle factors that have an impact on gut microbiome communities.[63] As patients who are overweight or obese and struggling with liver disease, may be at increased risk of other complications, diet and exercise are important.[64] Diet can affect the SCFAs synthesized by gut microbiota, bacterial derived ethanol, choline metabolism by gut microbiota, LPS uptake and BAs.[65] Thus, interventions related to diet and exercise are important in managing gut dysbiosis as it relates to liver disease.

Other target therapies

Another novel strategy involves microbial engineering. Engineered microbiota aimed to maintain the survival of important bacteria based on intestinal gradients and/or metabolize toxic products is also a potential gut microbiome target therapy. One method is delivery via a multilayer hydrogel that forms a concentration gradient meeting needs of different intestinal bacterial communities.[66] Another approach that has been explored involves the use of CRISPR/Cas-based systems targeted to drug-resistant bacteria such as *Escherichia coli* to clear bad bacterial populations. Though microbial engineering therapies are still in early stages, they can be a useful strategy to eliminate microbes involved in gut dysbiosis associated with progressing liver disease.[63]

Conclusions

Liver disease is a prominent issue affecting much of the population

Table 1. Overview of probiotic, prebiotic, and FMT RCTs

Summary of Probiotic, Prebiotic, and Fecal Microbiota transplantation (FMT) RCTs			
Study	**Condition**	**Subjects, Treatments, Methods**	**Major Results**
Probiotics and Prebiotics			
Sharma *et al*. (2008)[30]	Cirrhosis/ Minimal Hepatic Encephalopathy (MHE)	Patients with Cirrhosis were tested for MHE through psychometric testing and P300 auditory event-related potential (P300ERP) of which 105 were diagnosed with MHE; patients were randomized into three groups including lactulose (n = 35), probiotics (n = 35), and lactulose plus probiotics (n = 35) which received treatment for one month; treatment included probiotic strains (*Enterococcus faecalis, Clostridium butyricum, Bacillus mesentericus*, and lactic acid bacillus) alone or probiotics with lactulose; P300ERP values, CTP scores, and MHE were evaluated after one month of treatment	Treatment with lactulose, probiotics, and a combination of lactulose plus probiotic were equally effective and led to an improvement in MHE in 51–56% of the patients; 38.8% of patients in the lactulose group, 40% of patients in the probiotics group, and 38% in the combination group showed improvement in CTP class
Duseja *et al*. (2019)[31]	Nonalcoholic Fatty Liver Disease (NAFLD)	39 patients with liver biopsy-proven NAFLD were randomized to either lifestyle modifications and an oral multistrain probiotic (n = 19) or identical placebo (n = 20); lifestyle modifications included regular exercise and dietary restrictions; improvements in the NALD activity score (NAS) and improvement in ALT and cytokine profiles were measured at 1-year follow up	30 out of 39 patients with NAFLD completed the study with 1 year follow-up; repeat biopsy was done in 10 patients in the probiotic group and 5 in the placebo; hepatocyte ballooning, lobular inflammation, NAS score, and fibrosis, improved significantly in the probiotic group; a significant improvement was also observed in ALT levels in the probiotic compared to the placebo
Kobyliak *et al*. (2018)[34]	Nonalcoholic Fatty Liver Disease (NAFLD)	48 patients were randomly assigned to receive a combination of probiotics combined with flax and wheat germ oil (250 mg each, omega-3 fatty acid 1–5%) or a placebo for 8 weeks; primary measured outcomes included change fatty liver index (FLI) and liver stiffness (LS) measured by Shear Wave Elastography; secondary outcomes included cholesterol levels and cytokine markers	In the group receiving the probiotic-omega-3 treatment, FLI decreased significantly; changes of LS in both groups were insignificant; analysis of secondary outcomes showed significant reduction in total cholesterol of the probiotic-omega-3 group and decrease in chronic systemic inflammatory markers including IL-1β ($P = 0.029$), TNF-α ($P < 0.001$), IL-8 ($P = 0.029$), IL-6 ($P = 0.003$), and INF-γ ($P = 0.016$)
Malaguarnera *et al*. (2011)[51]	Nonalcoholic Steatohepatits (NASH)	A total of 66 patients were divided into groups either receiving a probiotic plus prebiotic treatment of *Bifidobacterium longum* with fructo-oligosaccharides (Fos) and lifestyle modifications or just lifestyle modifications; variables assessed at various time points up to 24 weeks included AST, ALT, HDL and LDL cholesterol, fasting glucose, and (TNF)-α; liver biopsies were performed at entry and repeated at 24 weeks	The probiotic plus prebiotic treatment group with lifestyle modifications vs. the lifestyle modification alone group showed significant decrease in many measures including the AST, LDL, TNF-α, steatosis and the NASH activity index (activity index revealed at least 2 point decrease in all patients)
Fecal Microbiota Transplantation			
Craven *et al*. (2020)[56]	Nonalcoholic Fatty Liver Disease (NAFLD)	21 patients with NAFLD recruited and randomized to allogenic (n = 15) or autologous (n = 6) FMT; insulin resistance (IR) measured via HOMA-IR, hepatic proton density fat fraction (PDFF) measured via MRI, and permeability of intestines measured with urine test	FMT did not show improvements in IR or PDFF but did show reduced small intestine permeability in patients with NAFLD
Xue *et al*. (2022)[58]	Nonalcoholic Fatty Liver Disease (NAFLD)	75 patients were recruited and divided into FMT (n = 47) and non-FMT group (n = 28), FMT group further divided into lean (n = 15) and obese (n = 32); FMT group patients received donor stool via colonoscopy followed by 3 enmenas over the course of 3 days	FMT improved BF ratios and dereased Proteobacteria numbers; FMT improved therapeutic effects on NAFLD patients and clinical efficacy was higher in lean NAFLD patients
Bajaj *et al*. (2021)[59]	Cirrhosis/ Alcohol Use Disorder (AUD)	20 cirrhosis patients diagnosed with AUD after screening randomized to FMT (n = 10) or no FMT (placebo, n = 10) groups; 90 mL of FMT material from donor administered via enema to FMT group and 90 mL of placebo to non-FMT group; selection of donor FMT was aimed to maximize *Lachnospiraceae* and *Ruminococcaeceae*	Shannon diversity increased in post-FMT group measured at day 15; increases in SCFAs (butyrate, isobutyrate, and isovalerate) in FMT group at day 15; reduction in serum IL-6 and LPS protein; more adverse events in placebo vs. FMT group; reduction in AUD-related events over 6 months in FMT group
Bloom *et al*. (2022)[60]	Cirrhosis/Hepatic Encephalopathy (HE)	10 patients enrolled; FMT capsules administered 5 times over 3 weeks; primary outcomes were changes in psychometric HE score (PHES) and serious adverse events (SAEs)	Mean improvement of 3.1 points in the PHES 4 weeks after the last FMT dose; FMT led to SAE in one patient (pertaining to *E. coli*); subtle or proximal changes in microbial composition

ALT, alanine transaminase; AST, aspartate transferase; BF, Bacteroidetes and Firmicutes; CTP, Child Turcotte Pugh; FMT, fecal microbiota transplantation; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LPS, lipopoly-saccharide; SCFA, short-chain fatty acids.

in various forms worldwide. Dysbiosis of the gut microbiome has an established role in contributing to the progression of liver disease manifestations such as NAFLD. The use of probiotics, prebiotics, FMT, and other targeted therapies has shown to be beneficial in treating different etiologies of liver disease and preventing liver disease progression in animal models and early clinical trials. However, there are still many limitations that need to be addressed including managing risks, ensuring preciseness, and evaluating the quality of the various microbiome-targeted therapies. Additionally, the lack of longitudinal studies examining the effects of prebiotics, probiotics, synbiotics, and FMT needs to be addressed in clinical trials as current studies may only be showcasing transient beneficial impacts rather than long-term effects. Better understanding of the gut-brain and gut-liver axes is also essential to better understanding the specific effects of bacterial metabolites on the host. As the mechanisms of prebiotics, probiotics, and FMT continue to be elucidated, expanding on clinical studies to better the efficacy of these microbiome-targeted therapies and individualize them based on patient needs may help improve the management of liver disease, especially in patients who do not respond well to traditional therapies alone.

Acknowledgments

None.

Funding

None.

Conflict of interest

There are no conflict of interests related to this publication.

Author contributions

Contributed to review concept and design (DA, MT), drafting of the manuscript (DA, AT), critical revision of the manuscript (DA, AT, MT), and supervision (MT).

References

[1] Sharma A, Nagalli S. Chronic Liver Disease. 2023 Jul 3. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023. PMID: 32119484.
[2] Wiegand J, Berg T. The etiology, diagnosis and prevention of liver cirrhosis: part 1 of a series on liver cirrhosis. Dtsch Arztebl Int 2013;110(6):85–91. doi:10.3238/arztebl.2013.0085, PMID:23451000.
[3] Schwenger KJ, Clermont-Dejean N, Allard JP. The role of the gut microbiome in chronic liver disease: the clinical evidence revised. JHEP Rep 2019;1(3):214–226. doi:10.1016/j.jhepr.2019.04.004, PMID:320 39372.
[4] Ma J, Piao X, Mahfuz S, Long S, Wang J. The interaction among gut microbes, the intestinal barrier and short chain fatty acids. Anim Nutr 2022;9:159–174. doi:10.1016/j.aninu.2021.09.012, PMID:35573092.
[5] Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: Pathophysiological basis for therapy. J Hepatol 2020;72(3):558–577. doi:10.1016/J.JHEP.2019.10.003, PMID:31622696.
[6] Plaza-Díaz J, Solís-Urra P, Rodríguez-Rodríguez F, Olivares-Arancibia J, Navarro-Oliveros M, Abadía-Molina F, *et al*. The Gut Barrier, Intestinal Microbiota, and Liver Disease: Molecular Mechanisms and Strategies to Manage. Int J Mol Sci 2020;21(21):E8351. doi:10.3390/ijms21218351, PMID:33171747.
[7] Tilg H, Adolph TE, Trauner M. Gut-liver axis: Pathophysiological concepts and clinical implications. Cell Metab 2022;34(11):1700–1718. doi:10.1016/j.cmet.2022.09.017, PMID:36208625.
[8] Schnabl B. The Microbiome and the Liver. Gastroenterol Hepatol (N Y) 2014;10(8):519–521. PMID:28845144.
[9] Ramírez-Pérez O, Cruz-Ramón V, Chinchilla-López P, Méndez-Sánchez N. The Role of the Gut Microbiota in Bile Acid Metabolism. Ann Hepatol 2017;16(Suppl 1):s15–s20. doi:10.5604/01.3001.0010.5494, PMID:29080339.
[10] Shao JW, Ge TT, Chen SZ, Wang G, Yang Q, Huang CH, *et al*. Role of bile acids in liver diseases mediated by the gut microbiome. World J Gastroenterol 2021;27(22):3010–3021. doi:10.3748/wjg.v27.i22.3010, PMID:34168404.
[11] Sayin SI, Wahlström A, Felin J, Jäntti S, Marschall HU, Bamberg K, *et al*. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab 2013;17(2):225–235. doi:10.1016/j.cmet.2013.01.003, PMID:23395169.
[12] Carpi RZ, Barbalho SM, Sloan KP, Laurindo LF, Gonzaga HF, Grippa PC, *et al*. The Effects of Probiotics, Prebiotics and Synbiotics in Non-Alcoholic Fat Liver Disease (NAFLD) and Non-Alcoholic Steatohepatitis (NASH): A Systematic Review. Int J Mol Sci 2022;23(15):8805. doi:10.3390/ijms23158805, PMID:35955942.
[13] Xu C, Zhu H, Qiu P. Aging progression of human gut microbiota. BMC Microbiol 2019;19(1):236. doi:10.1186/s12866-019-1616-2, PMID:31660868.
[14] Zhang C, Yang M, Ericsson AC. The Potential Gut Microbiota-Mediated Treatment Options for Liver Cancer. Front Oncol 2020;10:524205. doi:10.3389/fonc.2020.524205, PMID:33163393.
[15] Liu J, Wu D, Ahmed A, Li X, Ma Y, Tang L, *et al*. Comparison of the gut microbe profiles and numbers between patients with liver cirrhosis and healthy individuals. Curr Microbiol 2012;65(1):7–13. doi:10.1007/s00284-012-0105-8, PMID:22484797.
[16] Albhaisi SAM, Bajaj JS. The Influence of the Microbiome on NAFLD and NASH. Clin Liver Dis (Hoboken) 2021;17(1):15–18. doi:10.1002/cld.1010, PMID:33552480.
[17] Tsay CJ, Lim JK. NASH and the Gut Microbiome: Implications for New Therapies. Clin Liver Dis (Hoboken) 2022;19(3):97–100. doi:10.1002/cld.1170, PMID:35355843.
[18] Minemura M, Shimizu Y. Gut microbiota and liver diseases. World J Gastroenterol 2015;21(6):1691–1702. doi:10.3748/wjg.v21.i6.1691, PMID:25684933.
[19] Sung JJY, Wong SH. What is unknown in using microbiota as a therapeutic? J Gastroenterol Hepatol 2022;37(1):39–44. doi:10.1111/jgh.15716, PMID:34668228.
[20] Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and synbiotics- a review. J Food Sci Technol 2015;52(12):7577–7587. doi:10.1007/s13197-015-1921-1, PMID:26604335.
[21] Slavin J. Fiber and prebiotics: mechanisms and health benefits. Nutrients 2013;5(4):1417–1435. doi:10.3390/nu5041417, PMID:23609775.
[22] Xu S, Zhao M, Wang Q, Xu Z, Pan B, Xue Y, *et al*. Effectiveness of Probiotics and Prebiotics Against Acute Liver Injury: A Meta-Analysis. Front Med (Lausanne) 2021;8:739337. doi:10.3389/fmed.2021.739337, PMID:34621765.
[23] Choi W, Yeruva S, Turner JR. Contributions of intestinal epithelial barriers to health and disease. Exp Cell Res 2017;358(1):71–77. doi:10.1016/j.yexcr.2017.03.036, PMID:28342899.
[24] Lee B, Moon KM, Kim CY. Tight Junction in the Intestinal Epithelium: Its Association with Diseases and Regulation by Phytochemicals. J Immunol Res 2018;2018:2645465. doi:10.1155/2018/2645465, PMID:30648119.
[25] Dobson A, Cotter PD, Ross RP, Hill C. Bacteriocin production: a probiotic trait? Appl Environ Microbiol 2012;78(1):1–6. doi:10.1128/AEM.05576-11, PMID:22038602.
[26] Anjana, Tiwari SK. Bacteriocin-Producing Probiotic Lactic Acid Bacteria in Controlling Dysbiosis of the Gut Microbiota. Front Cell Infect Microbiol 2022;12:851140. doi:10.3389/fcimb.2022.851140, PMID:35651753.
[27] Walsh CJ, Guinane CM, Hill C, Ross RP, O'Toole PW, Cotter PD. In silico identification of bacteriocin gene clusters in the gastrointestinal tract, based on the Human Microbiome Project's reference genome database. BMC Microbiol 2015;15:183. doi:10.1186/s12866-015-

0515-4, PMID:26377179.
[28] Williams NT. Probiotics. Am J Health Syst Pharm 2010;67(6):449–458. doi:10.2146/ajhp090168, PMID:20208051.
[29] Sharma V, Garg S, Aggarwal S. Probiotics and liver disease. Perm J 2013;17(4):62–67. doi:10.7812/TPP/12-144, PMID:24361022.
[30] Sharma P, Sharma BC, Puri V, Sarin SK. An open-label randomized controlled trial of lactulose and probiotics in the treatment of minimal hepatic encephalopathy. Eur J Gastroenterol Hepatol 2008;20(6):506–511. doi:10.1097/MEG.0b013e3282f3e6f5, PMID:18467909.
[31] Duseja A, Acharya SK, Mehta M, Chhabra S, Rana S, Das A, *et al*. High potency multistrain probiotic improves liver histology in non-alcoholic fatty liver disease (NAFLD): a randomised, double-blind, proof of concept study. BMJ Open Gastroenterol 2019;6(1):e000315. doi:10.1136/bmjgast-2019-000315, PMID:31423319.
[32] Wang Q, Wang Z, Pang B, Zheng H, Cao Z, Feng C, *et al*. Probiotics for the improvement of metabolic profiles in patients with metabolic-associated fatty liver disease: A systematic review and meta-analysis of randomized controlled trials. Front Endocrinol (Lausanne) 2022;13:1014670. doi:10.3389/fendo.2022.1014670, PMID:36407321.
[33] Kobyliak N, Abenavoli L, Falalyeyeva T, Mykhalchyshyn G, Boccuto L, Kononenko L, *et al*. Beneficial effects of probiotic combination with omega-3 fatty acids in NAFLD: a randomized clinical study. Minerva Med 2018;109(6):418–428. doi:10.23736/S0026-4806.18.05845-7, PMID:30221912.
[34] Kobyliak N, Falalyeyeva T, Bodnar P, Beregova T. Probiotics Supplemented with Omega-3 Fatty Acids are More Effective for Hepatic Steatosis Reduction in an Animal Model of Obesity. Probiotics Antimicrob Proteins 2017;9(2):123–130. doi:10.1007/s12602-016-9230-1, PMID:27660157.
[35] Li YG, Yu ZJ, Li A, Ren ZG. Gut microbiota alteration and modulation in hepatitis B virus-related fibrosis and complications: Molecular mechanisms and therapeutic inventions. World J Gastroenterol 2022;28(28):3555–3572. doi:10.3748/wjg.v28.i28.3555, PMID:36161048.
[36] Gatta L, Scarpignato C. Systematic review with meta-analysis: rifaximin is effective and safe for the treatment of small intestine bacterial overgrowth. Aliment Pharmacol Ther 2017;45(5):604–616. doi:10.1111/apt.13928, PMID:28078798.
[37] Caraceni P, Vargas V, Solà E, Alessandria C, de Wit K, Trebicka J, *et al*. The Use of Rifaximin in Patients With Cirrhosis. Hepatology 2021;74(3):1660–1673. doi:10.1002/hep.31708, PMID:33421158.
[38] Szabó E, Páska C, Kaposi Novák P, Schaff Z, Kiss A. Similarities and differences in hepatitis B and C virus induced hepatocarcinogenesis. Pathol Oncol Res 2004;10(1):5–11. doi:10.1007/BF02893401, PMID:15029254.
[39] Imani Fooladi AA, Mahmoodzadeh Hosseini H, Nourani MR, Khani S, Alavian SM. Probiotic as a novel treatment strategy against liver disease. Hepat Mon 2013;13(2):e7521. doi:10.5812/hepatmon.7521, PMID:23610585.
[40] Kechagia M, Basoulis D, Konstantopoulou S, Dimitriadi D, Gyftopoulou K, Skarmoutsou N, *et al*. Health benefits of probiotics: a review. ISRN Nutr 2013;2013:481651. doi:10.5402/2013/481651, PMID:24959545.
[41] Doron S, Snydman DR. Risk and safety of probiotics. Clin Infect Dis 2015;60(Suppl 2):S129–S134. doi:10.1093/cid/civ085, PMID:25922398.
[42] Merenstein D, Pot B, Leyer G, Ouwehand AC, Preidis GA, Elkins CA, *et al*. Emerging issues in probiotic safety: 2023 perspectives. Gut Microbes 2023;15(1):2185034. doi:10.1080/19490976.2023.2185034, PMID:36919522.
[43] Ng QX, Yau CE, Yaow CYL, Chong RIH, Chong NZ, Teoh SE, *et al*. What Has Longitudinal 'Omics' Studies Taught Us about Irritable Bowel Syndrome? A Systematic Review. Metabolites 2023;13(4):484. doi:10.3390/metabo13040484, PMID:37110143.
[44] Pirola CJ, Salatino A, Quintanilla MF, Castaño GO, Garaycoechea M, Sookoian S. The influence of host genetics on liver microbiome composition in patients with NAFLD. EBioMedicine 2022;76:103858. doi:10.1016/j.ebiom.2022.103858, PMID:35092912.
[45] Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, *et al*. Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. Foods 2019;8(3):92. doi:10.3390/foods8030092, PMID:30857316.
[46] Guarino MPL, Altomare A, Emerenziani S, Di Rosa C, Ribolsi M, Balestrieri P, *et al*. Mechanisms of Action of Prebiotics and Their Effects on Gastro-Intestinal Disorders in Adults. Nutrients 2020;12(4):1037. doi:10.3390/nu12041037, PMID:32283802.
[47] Pérez-Monter C, Álvarez-Arce A, Nuño-Lambarri N, Escalona-Nández I, Juárez-Hernández E, Chávez-Tapia NC, *et al*. Inulin Improves Diet-Induced Hepatic Steatosis and Increases Intestinal Akkermansia Genus Level. Int J Mol Sci 2022;23(2):991. doi:10.3390/ijms23020991, PMID:35055177.
[48] Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, *et al*. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 2007;50(11):2374–2383. doi:10.1007/s00125-007-0791-0, PMID:17823788.
[49] Parnell JA, Raman M, Rioux KP, Reimer RA. The potential role of prebiotic fibre for treatment and management of non-alcoholic fatty liver disease and associated obesity and insulin resistance. Liver Int 2012;32(5):701–711. doi:10.1111/j.1478-3231.2011.02730.x, PMID:22221818.
[50] Safari Z, Gérard P. The links between the gut microbiome and non-alcoholic fatty liver disease (NAFLD). Cell Mol Life Sci 2019;76(8):1541–1558. doi:10.1007/s00018-019-03011-w, PMID:30683985.
[51] Malaguarnera M, Vacante M, Antic T, Giordano M, Chisari G, Acquaviva R, *et al*. Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. Dig Dis Sci 2012;57(2):545–553. doi:10.1007/s10620-011-1887-4, PMID:21901256.
[52] Gupta S, Allen-Vercoe E, Petrof EO. Fecal microbiota transplantation: in perspective. Therap Adv Gastroenterol 2016;9(2):229–239. doi:10.1177/1756283X15607414, PMID:26929784.
[53] Liubakka A, Vaughn BP. Clostridium difficile Infection and Fecal Microbiota Transplant. AACN Adv Crit Care 2016;27(3):324–337. doi:10.4037/aacnacc2016703, PMID:27959316.
[54] Nicholson MR, Mitchell PD, Alexander E, Ballal S, Bartlett M, Becker P, *et al*. Efficacy of Fecal Microbiota Transplantation for Clostridium difficile Infection in Children. Clin Gastroenterol Hepatol 2020;18(3):612–619.e1. doi:10.1016/j.cgh.2019.04.037, PMID:31009795.
[55] Stols-Gonçalves D, Mak AL, Madsen MS, van der Vossen EWJ, Bruinstroop E, Henneman P, *et al*. Faecal Microbiota transplantation affects liver DNA methylation in Non-alcoholic fatty liver disease: a multi-omics approach. Gut Microbes 2023;15(1):2223330. doi:10.1080/19490976.2023.2223330, PMID:37317027.
[56] Craven L, Rahman A, Nair Parvathy S, Beaton M, Silverman J, Qumosani K, *et al*. Allogenic Fecal Microbiota Transplantation in Patients with Nonalcoholic Fatty Liver Disease Improves Abnormal Small Intestinal Permeability: A Randomized Control Trial. Am J Gastroenterol 2020;115(7):1055–1065. doi:10.14309/ajg.0000000000000661, PMID:32618656.
[57] Moon CD, Young W, Maclean PH, Cookson AL, Bermingham EN. Metagenomic insights into the roles of Proteobacteria in the gastrointestinal microbiomes of healthy dogs and cats. Microbiologyopen 2018;7(5):e00677. doi:10.1002/mbo3.677, PMID:29911322.
[58] Xue L, Deng Z, Luo W, He X, Chen Y. Effect of Fecal Microbiota Transplantation on Non-Alcoholic Fatty Liver Disease: A Randomized Clinical Trial. Front Cell Infect Microbiol 2022;12:759306. doi:10.3389/fcimb.2022.759306, PMID:35860380.
[59] Bajaj JS, Gavis EA, Fagan A, Wade JB, Thacker LR, Fuchs M, *et al*. A Randomized Clinical Trial of Fecal Microbiota Transplant for Alcohol Use Disorder. Hepatology 2021;73(5):1688–1700. doi:10.1002/hep.31496, PMID:32750174.
[60] Bloom PP, Donlan J, Torres Soto M, Daidone M, Hohmann E, Chung RT. Fecal microbiota transplant improves cognition in hepatic encephalopathy and its effect varies by donor and recipient. Hepatol Commun 2022;6(8):2079–2089. doi:10.1002/hep4.1950, PMID:35384391.
[61] Wang L, Wan YY. The role of gut microbiota in liver disease development and treatment. Liver Res 2019;3(1):3–18. doi:10.1016/j.livres.2019.02.001, PMID:32461811.
[62] Nigam M, Panwar AS, Singh RK. Orchestrating the fecal microbiota transplantation: Current technological advancements and potential biomedical application. Front Med Technol 2022;4:961569.

doi:10.3389/fmedt.2022.961569, PMID:36212607.
[63] Kirundi J, Moghadamrad S, Urbaniak C. Microbiome-liver crosstalk: A multihit therapeutic target for liver disease. World J Gastroenterol 2023;29(11):1651–1668. doi:10.3748/wjg.v29.i11.1651, PMID:37077519.
[64] Nobili V, Carter-Kent C, Feldstein AE. The role of lifestyle changes in the management of chronic liver disease. BMC Med 2011;9:70. doi:10.1186/1741-7015-9-70, PMID:21645344.
[65] Jennison E, Byrne CD. The role of the gut microbiome and diet in the pathogenesis of non-alcoholic fatty liver disease. Clin Mol Hepatol 2021;27(1):22–43. doi:10.3350/cmh.2020.0129, PMID:33291863.
[66] Zheng DW, Qiao JY, Ma JC, An JX, Yang CH, Zhang Y, *et al*. A Microbial Community Cultured in Gradient Hydrogel for Investigating Gut Microbiome-Drug Interaction and Guiding Therapeutic Decisions. Adv Mater 2023;35(22):e2300977. doi:10.1002/adma.202300977, PMID:37029611.