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Review

Hydrogel-Based Strategies for Liver Tissue Engineering

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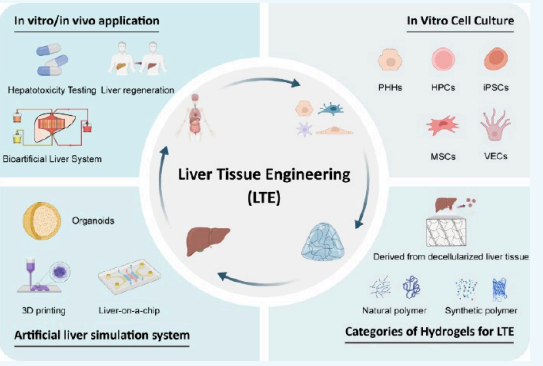
ABSTRACT: The liver's role in metabolism, detoxification, and immune regulation underscores the urgency of addressing liver diseases, which claim millions of lives annually. Due to donor shortages in liver transplantation, liver tissue engineering (LTE) offers a promising alternative. Hydrogels, with their biocompatibility and ability to mimic the liver's extracellular matrix (ECM), support cell survival and function in LTE. This review analyzes recent advances in hydrogel-based strategies for LTE, including decellularized liver tissue hydrogels, natural polymer-based hydrogels, and synthetic polymer-based hydrogels. These materials are ideal for in vitro cell culture and obtaining functional hepatocytes. Hydrogels' tunable properties facilitate creating artificial liver models, such as organoids, 3D bioprinting, and liver-on-a-chip technologies. These developments demonstrate hydrogels' versatility in advancing LTE's applications, including hepatotoxicity testing, liver tissue regeneration, and treating acute liver failure. This review highlights the transformative potential of hydrogels in LTE and their implications for future research and clinical practice.



KEYWORDS: *hydrogel, liver tissue engineering, cell culture, organoid, 3D bioprinting, liver-on-a-chip, hepatotoxicity testing, liver regeneration, acute liver failure*

1. INTRODUCTION

The liver, the largest and most complex organ in the human body, weighs about 1500 g in adults and plays a crucial role in metabolism, detoxification, and immune regulation.[1−4] Comprising around 30 billion cells, primarily hepatocytes, they have high regenerative capacity.[5−7] However, extensive injuries from drugs, toxins, viral infections, cancers, and metabolic dysfunctions can cause end-stage liver disease or acute liver failure, leading to millions of deaths annually.[8,9] Liver transplantation is currently the only effective treatment, but the severe shortage of donors leaves many patients without timely transplants.[10−13]

Liver tissue engineering has emerged to address liver disease by reconstructing liver models that mimic in vivo functions. LTE combines isolated liver cells with specific scaffold materials to create fully implantable, functional organs for transplantation. Bioengineered livers also serve as in vitro models for testing xenobiotics, conducting toxicological studies, and modeling diseases, bypassing ethical issues associated with human subjects.

Key challenges in LTE include obtaining functional hepatocytes, constructing tissue-specific ECM biomimetic scaffolds, and recreating the in vivo microenvironment, including microvascular and microbile duct networks and the immune barrier.[14−22] These elements are crucial for replicating the ECM's biochemical and mechanical environment, which significantly impacts liver function. Although the ECM is less than 3% of a normal liver section, it provides structural cohesion, induces cell polarization, and influences gene expression and differentiation.[23−26] Any alteration in the liver ECM affects liver structure and function, making its accurate replication essential in LTE.[27,28]

Hydrogels, with their unique properties, are promising materials to liver tissue engineering. Their high-water content and tissuelike consistency are ideal for mimicking the ECM environment, supporting cell survival, proliferation, and differentiation.[29,30] Hydrogels' adaptability allows precise control of biochemical and mechanical properties, making them ideal scaffolds for LTE.

Hydrogels in LTE are categorized into three types: decellularized liver tissue-derived, natural polymers, and

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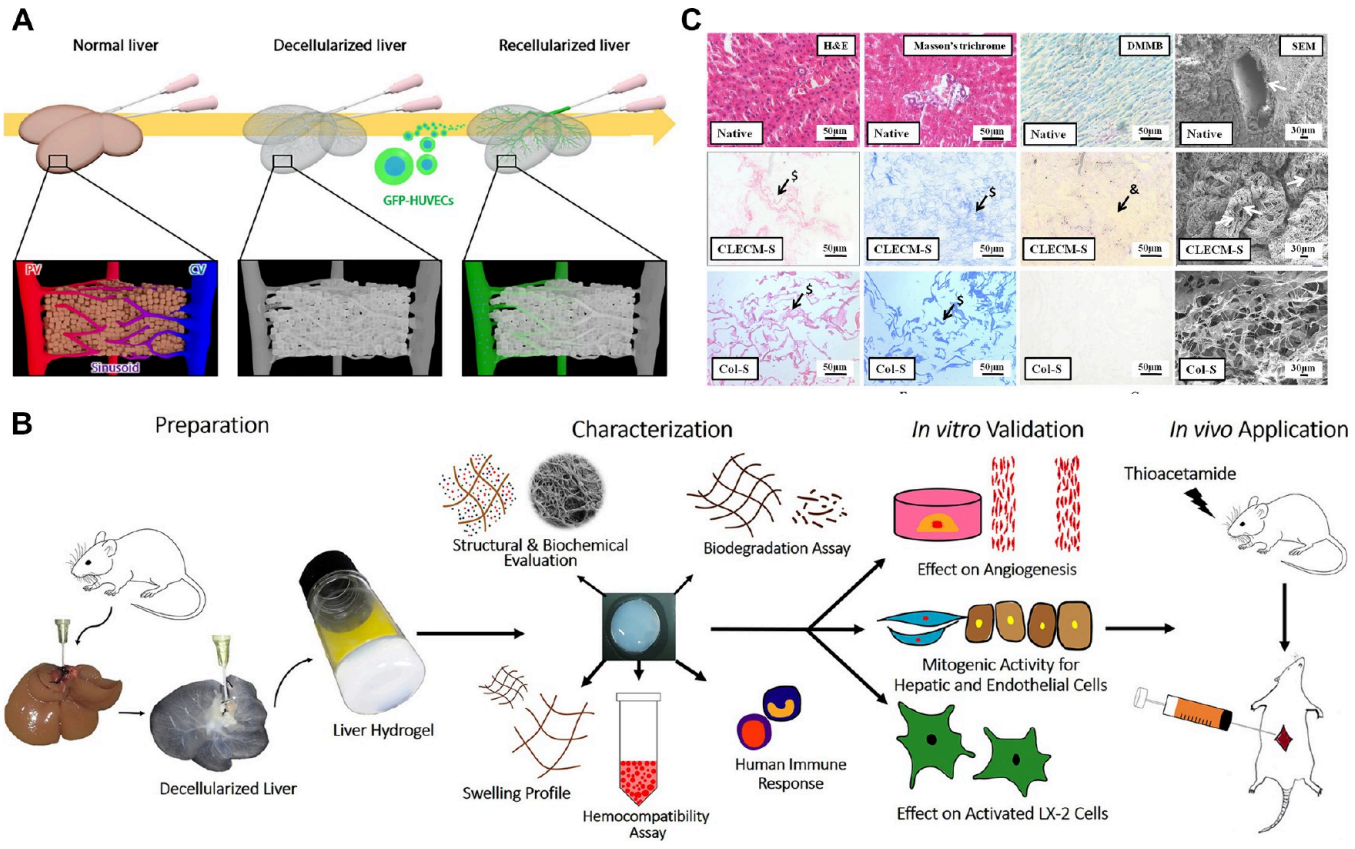

Figure 1. Hydrogels derived from decellularized liver tissue. (A) Diagrammatic representation of the experimental setup for liver decellularization and recellularization. Reproduced with permission from ref 42. Copyright 2019 Acta Materialia Inc. (B) Mouse livers were decellularized and converted into a hepatic hydrogel, which, when injected into the liver, significantly reduced fibrosis and facilitated recovery to an almost normal structure. Reproduced with permission from ref 50. Copyright 2020 Elsevier B.V. (C) Biochemical characterization of CLECM-S and Col-S scaffold compared to native caprine liver. Reproduced with permission from ref 51. Copyright 2019 Elsevier B.V.

synthetic polymers. Decellularized liver tissue hydrogels retain native biochemical cues, enhancing cell attachment, proliferation, and differentiation, thus promoting effective tissue regeneration. Natural polymer-based hydrogels, such as collagen, gelatin, alginate, and hyaluronic acid, offer excellent biocompatibility and bioactivity. These polymers inherently support cell attachment and proliferation, and their degradation products are nontoxic, suitable for in vivo applications. However, their mechanical properties may require enhancement. Synthetic polymer-based hydrogels are noted for their biocompatibility, hydrophilic nature, and minimal immune response. Their customizable degradation rates and mechanical properties make them versatile for various LTE applications.

Hydrogels provide a supportive environment for cultivating liver cells in vitro, aiding in producing sufficient hepatic parenchymal cells. Their 3D structure and high water content mimic the natural liver ECM, supporting cell growth and nutrient transport. Hydrogels' tunable properties are ideal for developing organoids, 3D bioprinted tissues, and liver-on-a-chip systems, which replicate liver architecture and functions more accurately than traditional methods. Organoids, miniaturized versions of organs created in vitro, exhibit similar microanatomy and functionality. Hydrogels support the growth and organization of liver cells into organoids, providing a powerful tool for studying liver biology and disease. 3D bioprinting with hydrogels allows precise placement of liver cells, creating detailed and functional liver tissues for drug testing and regenerative medicine. Liver-on-a-chip systems integrate liver cells within a microfluidic device, offering an accurate representation of liver functions in a controlled environment. Hydrogels in these systems provide a suitable matrix for cell growth and differentiation, enabling in vitro study of liver physiology and pathology.

Hydrogel-based artificial liver models have numerous applications both in vivo and in vitro, demonstrating their versatility and potential in advancing liver tissue engineering. One of the primary applications of hydrogel-based liver models is hepatotoxicity testing. These models provide a more physiologically relevant platform for assessing the liver toxicity of new drugs, reducing the reliance on animal testing and improving the predictability of human responses. Hydrogel-based organoids can be transplanted into damaged liver tissues to promote regeneration. The hydrogels support the survival and integration of the organoids within the host tissue, facilitating the repair and regeneration of liver function. Hydrogel-based artificial liver models are also being explored as potential treatments for acute liver failure (ALF). These models can provide temporary liver function support, buying critical time for patients awaiting liver transplants or for the liver to recover on its own.

This review will focus on recent advancements in hydrogel-based strategies for liver tissue engineering. We will explore various types of hydrogels, including those derived from decellularized liver tissue, natural polymers, and synthetic polymers, examining their unique properties and specific applications in liver tissue engineering. Additionally, the review

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will discuss the latest progress in developing artificial liver models, such as organoids, 3D bioprinted tissues, and liver-on-a-chip systems. These innovations hold significant potential for hepatotoxicity testing, tissue regeneration, and treatments for acute liver failure. By examining these developments, this review aims to provide a comprehensive overview of cutting-edge hydrogel-based approaches in liver tissue engineering and their implications for future research and clinical practice.

2. CATEGORIES OF HYDROGELS FOR LIVER TISSUE ENGINEERING

Cells receive mechanical signals from the ECM, such as Young's modulus, stress relaxation, toughness, pore size, and porosity. These cues profoundly impact their behaviors, including attachment, growth, proliferation, migration, and differentiation, by altering signaling pathways like Notch, Wnt, and BMP. Consequently, biomaterial scaffolds with specific mechanical properties are crucial in tissue engineering. They provide structural support, create a conducive environment for cell loading and tissue development, and ultimately facilitate liver regeneration.[31]

In tissue engineering, porous scaffolds are commonly employed for the fabrication of implantable natural or synthetic tissues.[32] For liver scaffolds, a variety of synthetic materials such as PCL, PCL−PLGA, and PU are used.[33−35] Among synthetic polymers, polyesters like PLLA and PLGA are frequently utilized to create porous constructs.[36,37] However, their hydrolytic degradation products can lead to the accumulation of acidic byproducts within the scaffold. This acidity has been shown to degrade peptides and trigger inflammation, potentially impairing hepatocyte function.[38] As a result, alternative approaches have been developed, focusing on the efficient and uniform encapsulation of hepatocytes within fully three-dimensional structures, rather than relying solely on macroporous scaffold systems. Hydrogels, which possess high water content and mechanical properties similar to native tissues, are particularly advantageous for liver tissue engineering scaffolds.

Hydrogels have emerged as a critical component in liver tissue engineering due to their ability to mimic the liver's ECM. Hydrogels derived from decellularized liver tissue, natural polymers, and synthetic polymers each offer unique benefits. The choice of hydrogel type significantly influences cell behavior, including adhesion, proliferation, and differentiation, which are crucial for engineering functional liver tissues. This section explores the various types of hydrogels utilized in liver tissue engineering, their properties, and their impact on liver tissue regeneration and functionality.

2.1. Hydrogels Derived from Decellularized Liver Tissue. Decellularized liver scaffolds (DLS) are proposed for their biochemical properties, which closely mimic the in vivo microenvironment (Figure 1A). This resemblance enhances cell attachment, proliferation, and differentiation, thereby promoting effective tissue regeneration. Hydrogels derived from decellularized liver tissue retain essential bioactive components, closely approximating the natural liver environment, making them highly promising for liver tissue engineering applications.[39−49]

Hydrogels prepared from decellularized livers retain essential components such as glycosaminoglycans, collagen, elastin, and growth factors, which promote hepatocyte and endothelial cell migration crucial for liver repair. A recent study demonstrates that mouse liver-derived hydrogels effectively inhibit hepatic stellate cell activation and reverse fibrotic changes both in vitro and in vivo.[50] By blocking the TGF-β1/Smad pathway, the hydrogel reduces the fibrotic process. When injected into the liver parenchyma of mice with thioacetamide-induced fibrosis, the hydrogel facilitates a significant reduction in fibrosis, thereby improving liver structure and function (Figure 1B). These injectable liver hydrogels offer ease of delivery and structural support, enabling host cell infiltration and enhanced tissue regeneration. This presents a promising therapeutic strategy for treating liver fibrosis and advancing liver tissue engineering.

The use of caprine liver tissue as a source for decellularization offers several advantages due to its structural similarity to human liver, biomimetic and pro-angiogenic properties, safety from transmissible spongiform encephalopathy, and availability in large quantities. Hydrogels derived from decellularized caprine liver tissue (CLECM-S) were developed and characterized for liver tissue engineering by Maiti et al.[51] The CLECM-S scaffolds retained essential extracellular matrix components and structural features similar to native liver tissue (Figure 1C). Compared to conventional glutaraldehyde cross-linked collagen scaffolds (Col-S), CLECM-S demonstrated superior performance in several key areas. It exhibited slow but sustained swelling and collagenase-mediated degradation, with mechanical stiffness approximating that of the native human liver. HepG2 cells cultured on CLECM-S showed enhanced expression of mature and functional hepatocyte markers, indicating a supportive environment for hepatocyte functionality. The pro-angiogenic properties of CLECM-S were confirmed using the chick chorioallantoic membrane (CAM) assay. Additionally, in vivo implantation in a mouse model showed no significant immunogenic response, underscoring the biocompatibility of the scaffold. The CLECM-S scaffold's potential to maintain the functional state of cultured human hepatocytes for long-term use, along with its pro-angiogenic capabilities, positions it as a promising material for liver tissue engineering applications.

Despite the promise of in vivo liver decellularization for liver engineering, long-term survival has been hindered by anatomical and technical difficulties. Recent research aimed to establish a survival model using rats,[52] comparing three decellularization protocols: 1% Triton X-100 followed by 1% SDS, 1% SDS alone, and 1% Triton X-100 alone. The optimal protocol, determined through macroscopic, histological, and DNA content evaluations, was 1% SDS. This protocol yielded an acellular scaffold with preserved structural integrity and key matrix proteins, including collagen IV, laminin, and elastin. To prevent abdominal contamination by the corrosive detergents, a combination of PVDC film and dry gauze with an aspiration tube was employed, resulting in a 100% survival rate over 7 days without severe postoperative complications. Upon reperfusion, the liver exhibited immediate blood flow without surface leakage, indicating effective physiological perfusion. This model addresses three major challenges: isolating the liver lobe without obstructing main vessels, limiting decellularization to 2 h, and preventing chemical injury from detergent exposure.

However, before acellular liver scaffolds can be clinically applied, several concerns must be addressed. These include the potential risks associated with xenogeneic and allogeneic livers, the long-term stability of liver grafts in vivo, and the bioengineering challenges of constructing a liver of physiologically relevant size.[53−56]

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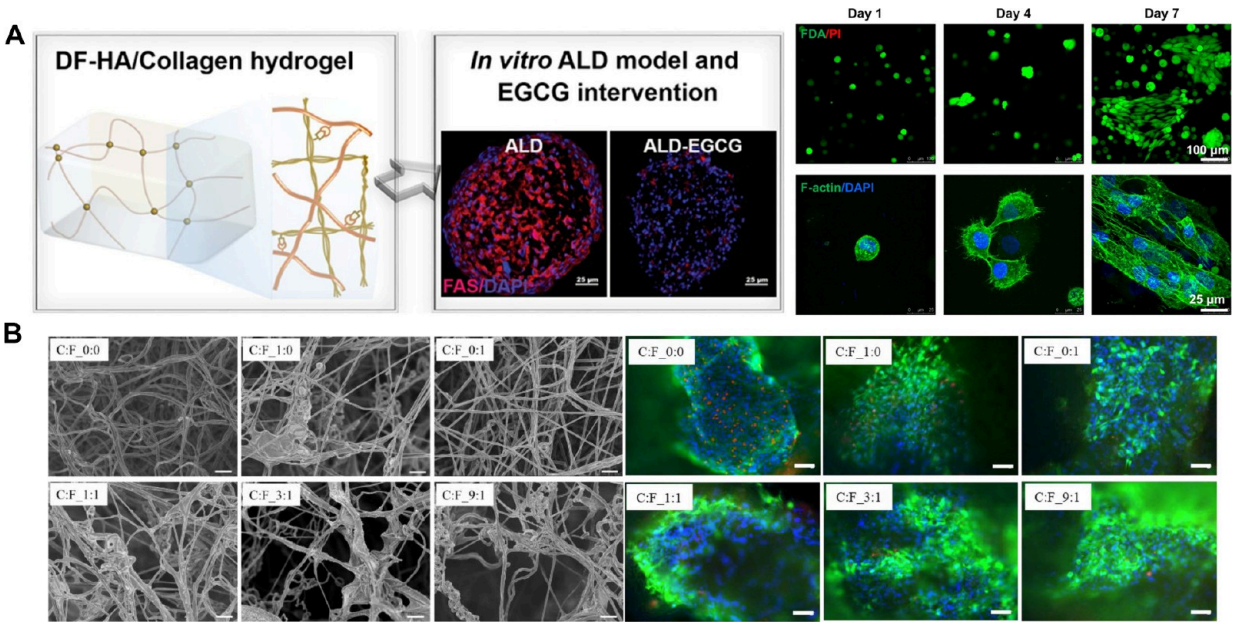

Figure 2. Natural polymer-based hydrogels for liver tissue engineering. (A) Artificial liver model has been created by embedding HepG2 cells in a hydrogel matrix made from bifunctional hyaluronan and collagen to study alcohol-induced alcoholic liver disease. Reproduced with permission from ref 58. Copyright 2021 Elsevier B.V. (B) Structure of hydrogel scaffolds incorporating different ratios of ECM proteins, alongside images showing live/dead staining of cells cultured on these scaffolds. Reproduced with permission from ref 57. Copyright 2020 Elsevier B.V.

2.2. Natural Polymers-Based Hydrogels. Natural hydrogels, derived from organisms and composed of polymers such as collagen,[57,58] fibronectin,[59] alginate,[60−64] chitosan,[65,66] and agarose,[67,68] offer excellent biocompatibility and bioactivity. These polymers inherently support cell attachment and proliferation, and their nontoxic degradation products make them suitable for in vivo applications, including cell culture, drug delivery, and tissue engineering.

Alginate is particularly popular in cell encapsulation research due to its biocompatibility and ability to form gels in the presence of divalent cations.[69,70] Bhatt et al. examined an innovative hydrogel system designed to enhance hepatic tissue engineering.[71] The study developed a three-dimensional scaffold incorporating fibrin into an injectable alginate dialdehyde (ADA) and gelatin (G) hydrogel matrix. ADA was synthesized by periodate oxidation of sodium alginate, and the hydrogel was formed by cross-linking ADA with gelatin and fibrin, catalyzed by thrombin. This ADA-G-FIB hydrogel was characterized using Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscopy (SEM), and microcomputed tomography (micro-CT), revealing a 3D porous structure with pores ranging from 100 to 200 μm. The hydrogel exhibited noncytotoxic properties, with over 80% cell viability in L929 cells. HepG2 liver cells cultured on the hydrogel showed significant cell adhesion, proliferation, and metabolic activity. Functional assays demonstrated effective indocyanine green uptake, albumin synthesis, cytochrome P450 expression, and ammonia clearance, indicating the hydrogel's potential to support liver-specific functions.

Hyaluronic acid-collagen hydrogels are emerging as a promising alternative to animal models due to their viscoelastic properties, which closely mimic real organs. A recent study developed an artificial liver model using viscoelastic hyaluronan-collagen hydrogel to reduce the reliance on animal models for alcoholic liver disease (ALD) research.[58] Researchers incorporated HepG2 cells into the hydrogel matrix and established an in vitro ALD model by introducing alcohol during the engineering process, successfully replicating the synthetic and metabolic functions of the liver with results comparable to those observed in ALD mice. The hydrogel matrix was created by difunctionalizing hyaluronan (DF-HA) with aldehyde groups and methacrylation, then integrating it into a collagen network to form imine cross-linking, resulting in a viscoelastic matrix system with native adhesion domains and a fibrillary architecture conducive to HepG2 cell growth and function. Additionally, the study investigated the intervention of epigallocatechin gallate (EGCG), a component of green tea, in mitigating liver damage within the ALD model, finding significant improvement and reversal of liver function, consistent with outcomes observed in alcohol-fed mice, thus demonstrating the potential of EGCG as a therapeutic agent against alcoholic fatty liver disease (Figure 2A).

The ECM proteins, such as collagen-I and fibronectin, play a crucial role in the liver's extracellular matrix, significantly influencing cellular behavior. A recent study investigated the use of three-dimensional electrospun poly(lactic-*co*-glycolic acid) (PLGA) scaffolds modified with collagen-I and fibronectin to improve hepatocyte culture.[57] These essential ECM proteins were incorporated into the scaffolds in varying ratios to optimize their synergistic effects (Figure 2B). The 3:1 ratio of collagen-I to fibronectin demonstrated the most effective chemisorption, resulting in the highest and most uniform protein grafting on the scaffold surfaces. This modification significantly enhanced cell seeding efficiency, proliferation, and upregulated liver-specific genes, including albumin and cytochrome P450 enzymes (CYP3A4 and CYP3A7). Compared to unmodified and single-protein modified scaffolds, the dual-protein modified scaffolds showed an 8- to 10-fold increase in albumin gene expression, a 5- to 7-fold increase in CYP3A4 gene expression, and a 4- to 4.5-fold increase in CYP3A7 gene expression after 28 days of culture. Additionally, albumin secretion improved 4-fold over un-

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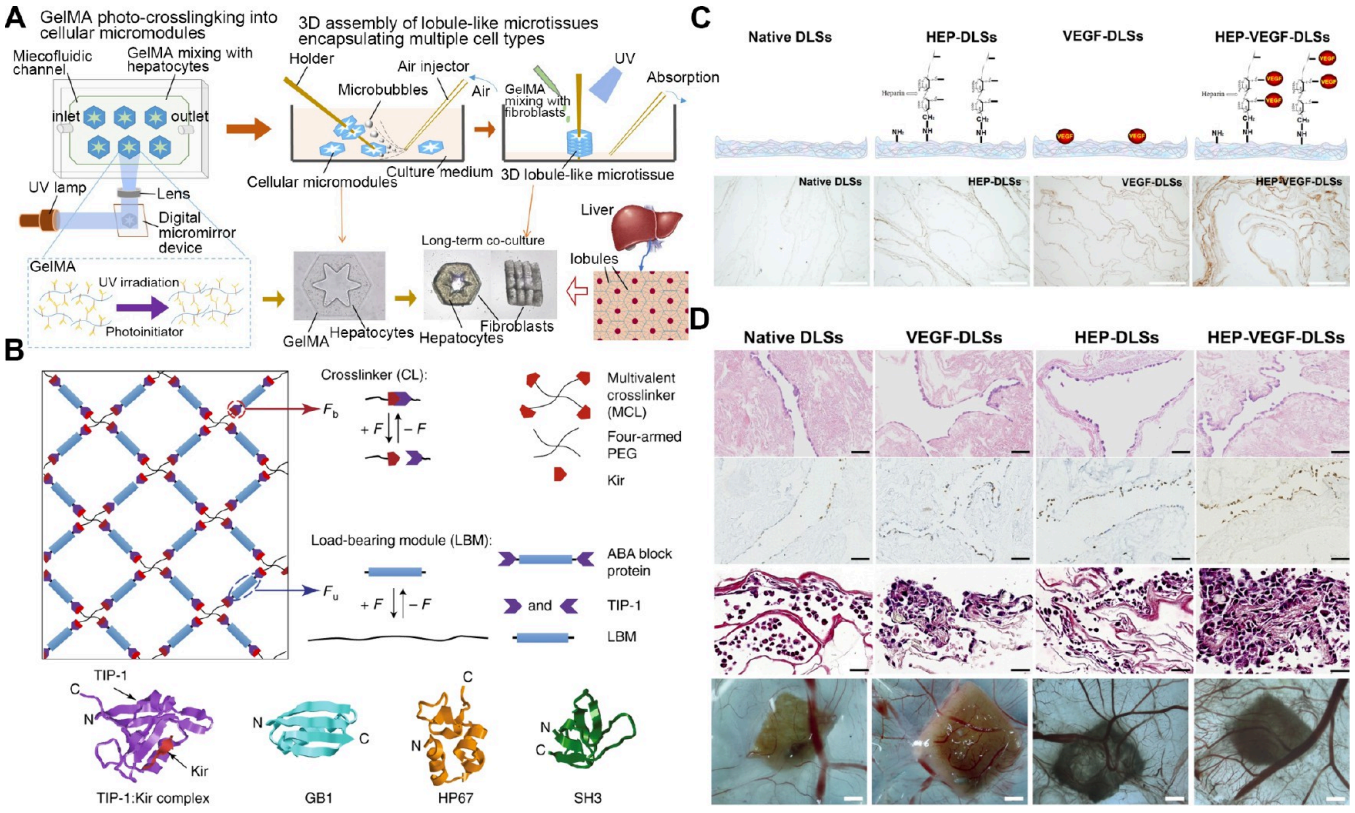

Figure 3. Synthetic polymers-based hydrogels for liver tissue engineering. (A) 3D gelatin methacryloyl hydrogels employed for liver tissue engineering through multicellular coculture. Reproduced with permission from ref 73. Copyright 2019 the author(s).) (B) Schematic of the protein hydrogel network design, highlighting the integration of cross-linkers (CLs) and load-bearing modules (LBMs), with detailed structures of the protein domains utilized in the hydrogel architecture. Reproduced with permission from ref 105. Copyright 2018 Springer Nature. (C) Schematics of various modified hydrogel structures and their VEGF distribution, and (D) the endothelial cells seeded on these hydrogels, alongside their performance in the chicken chorioallantoic membrane assay. Reproduced with permission from ref 111. Copyright 2016 Wiley Periodicals, Inc.

modified scaffolds. This study demonstrates that dual modification of 3D PLGA scaffolds with collagen-I and fibronectin significantly enhances hepatocyte viability and functionality, positioning it as a promising platform for liver tissue engineering.

However, the application range of natural polymers is somewhat restricted due to their limited capacity for mechanical property adjustment.[72]

2.3. Synthetic Polymers-Based Hydrogels. Biomaterial scaffolds made from synthetic polymers are highly valued for their biocompatibility, hydrophilicity, tolerance, nonimmunogenicity, and versatility, making them extensively used in areas such as cell transplantation and regenerative medicine.[73−86] Researchers have adopted a bottom-up rational method to design and create synthetic protein hydrogels that replicate the structure and mechanical properties of natural liver tissue.[87−89] By adjusting the relative strength of the cross-linking points and the load-bearing modules, the Young's modulus, ductility, toughness, and self-repairing ability of the hydrogel can be controlled. Using this strategy, researchers developed a hydrogel with exceptional toughness and quick recovery capabilities by designing dynamic cross-linking points using peptide cross-linking and metal coordination.[90] Tandem repeating proteins as cross-linkers and randomly coiled polymers as osmotic networks were also integrated into the design.[91] This configuration allows the multiprotein cross-linker to apply significant force only at the break area, thus preventing the spread of cracks. These durable hydrogel scaffolds provide stable physical support for in vitro liver tissue engineering simulation systems.

Gelatin methacryloyl (GelMA), renowned for its biocompatibility and biodegradability, is an ideal material for constructing biomaterial scaffold hydrogels in liver tissue engineering. Recent research developed a method to fabricate hepatocyte-encapsulated micromodules with a central radial-type hole, utilizing photo-cross-linking via a digital micromirror device-based microfluidic channel.[73] These micromodules were assembled through a noncontact pick-up strategy involving local fluid-based micromanipulation to prevent structural deformation. Subsequently, the micromodules were coated with fibroblast-laden GelMA and subjected to ultraviolet irradiation to achieve integration, forming 3D lobule-like microtissues encapsulating multiple cell types. The cocultured 3D microtissues maintained over 90% cell viability during long-term culture and demonstrated enhanced albumin secretion compared to microtissues containing only hepatocytes (Figure 3A). This study underscores that GelMA hydrogels effectively support the creation of 3D liver microtissues with high cell viability and improved liver functions, highlighting their potential for applications in liver tissue engineering and regenerative medicine.

In tissue engineering applications, an ideal cell scaffold should replicate the natural structure of the tissue it aims to replace while providing necessary structural support to the cells. Protein hydrogels are particularly promising as scaffolds for tissue engineering because they allow for precise control

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over various mechanical properties. For instance, the elastic moduli of tropoelastin-based hydrogels can be fine-tuned within a range of 8 to 20,000 kPa.[92−94] Hydrogels derived from human collagens offer sufficient mechanical strength to support tissues, making them promising candidates for bone tissue engineering applications.[95−97] Additionally, elastin-like polypeptides (ELP) facilitate a reversible sol−gel transition in response to temperature shifts or other external stimuli, making them ideal for cell encapsulation and controlled release.[98,99] They also offer inherent biological activities, such as RGD, heparin-binding domains, and matrix metalloproteinase (MMP)-sensitive domains, which are typically absent in inert polymers.[100−103]

Protein engineering enables the creation of protein hydrogels with specific and customizable biophysical properties tailored to particular physiological environments, achieved through precise control over structure and function (Figure 3B).[104,105] Recent research has developed protein hydrogels utilizing repetitive protein arrays that form covalent networks via the SpyTag/SpyCatcher system, which spontaneously cross-link upon mixing.[106] This method allows control over the hydrogels' viscoelastic properties and gelation speeds. The hydrogels supported cell attachment and encapsulation, maintaining cell viability, as demonstrated with HepG2 cells expressing GFP. Rheological studies indicated that the hydrogels exhibit mechanical properties comparable to decellularized liver tissue, with storage moduli of approximately 1 and 10 kPa, depending on the specific SpyTag/SpyCatcher ratio used. Microrheology revealed that gelation speed could be controlled by altering protein concentrations, with faster gelation at higher concentrations. The hydrogels remained stable in water and cell culture medium, retaining about 50% water content after 24 h and showing no signs of degradation. Fluorescence microscopy confirmed good cell encapsulation and attachment, with cells displaying healthy morphology and GFP expression. Additionally, the secretion of alpha-fetoprotein and albumin by the encapsulated cells indicated functional maintenance.

One significant application of protein hydrogels in tissue engineering is the immobilization of growth factors, which are essential for cell proliferation, differentiation, and tissue regeneration. Growth factors can be delivered exogenously via injection in solution or immobilized on scaffolds, with the latter method proving more effective in promoting desired cellular outcomes while preserving bioactivity and stability. This approach extends the duration of growth factor signaling and reduces the costs associated with growth factor therapies. Studies have shown that immobilizing vascular endothelial growth factor (VEGF) on collagen scaffolds significantly enhances endothelial cell proliferation compared to VEGF in solution.[107] Immobilized growth factors offer particular advantages for in vivo scaffold applications by confining their effects within the scaffold and preventing diffusion into surrounding tissues. This method also mirrors the physiological relevance of bound and free growth factors coexisting in vivo, where cells initially respond to soluble growth factors by migrating toward their source, then responding to higher concentrations of bound proteins at the source. For instance, covalently immobilizing VEGF on collagen scaffolds has been demonstrated to enhance angiogenesis in vivo, as evidenced by increased vascular density and greater endothelial cell infiltration and proliferation compared to control scaffolds without VEGF.[108]

In the context of LTE, biomaterial matrices can integrate growth factors such as VEGF and control their release through heparin, which interacts with various proteins possessing heparin-binding domains.[109,110] A recent study has developed heparinized decellularized liver scaffolds (HEP-DLSs) that bind VEGF to enhance angiogenesis for liver tissue engineering.[111] With the use of the end-point attachment (EPA) technique, heparin was immobilized on porcine decellularized liver scaffolds, facilitating the binding and gradual release of heparin-binding growth factors. The hypothesis that heparin functions as an antithrombotic agent and enhances VEGF-induced angiogenesis was tested. Human umbilical vein endothelial cells seeded on HEP-VEGF-DLSs remained bioactive, and these scaffolds significantly increased angiogenesis in the chicken chorioallantoic membrane assay compared to native DLSs (Figure 3C, D). When implanted in rats, HEP-VEGF-DLSs exhibited a substantial increase in blood vessel formation over time, demonstrating their potential to address the vascularization challenges of traditional matrices. The heparinization process preserved the structural integrity of heparin, enabling it to effectively stabilize and sustain the release of VEGF, thereby promoting endothelial cell proliferation and vessel formation.

3. HYDROGELS FOR IN VITRO CELL CULTURE IN LIVER TISSUE ENGINEERING

The in vitro culture of hepatocytes is fundamental to liver tissue engineering, aiming to produce a sufficient number of functional hepatocytes. Hydrogels play a pivotal role in this process by providing a supportive and biomimetic environment that facilitates cell growth and function. These hydrogels can be tailored to mimic the liver's ECM, offering the necessary biochemical and mechanical cues to sustain hepatocyte viability and functionality. By controlling properties such as stiffness, porosity, and bioactivity, hydrogels can significantly enhance hepatocyte proliferation and differentiation. This section delves into the use of hydrogels for in vitro cell culture, highlighting the types of hydrogels employed, their preparation methods, and their effectiveness in maintaining hepatocyte function over extended culture periods. The insights gained from these studies are crucial for advancing liver tissue engineering and developing reliable in vitro models for drug testing and disease modeling.

3.1. Hydrogels for In Vitro Cell Culture of Stem Cells.

The ability to culture mature hepatocytes in vitro is crucial for liver tissue engineering, emphasizing the importance of obtaining hepatocytes from stem cells in vitro.[112−120] Human hepatocyte-like cells (HLCs) have been generated through in vitro differentiation of human pluripotent stem cells or direct transdifferentiation of human somatic cells.[121−123] This method has successfully produced large numbers of functional HLCs, which have shown potential in rescuing liver failure in mice after transplantation.[124,125] However, the engraftment efficiency of human HLCs in livers has been limited.[126,127] The repopulation efficiency of HLCs is approximately 2%, which is significantly lower than the desired level. Considering that around 10% of liver cells need to be replaced for a therapeutic effect in human patients,[128] further optimization is necessary to improve the compatibility of these cells for clinical transplantation.

3.1.1. Primary Human Hepatocytes (PHHs). Primary human hepatocytes are highly valued in liver tissue engineering for their utility in applications such as drug metabolism

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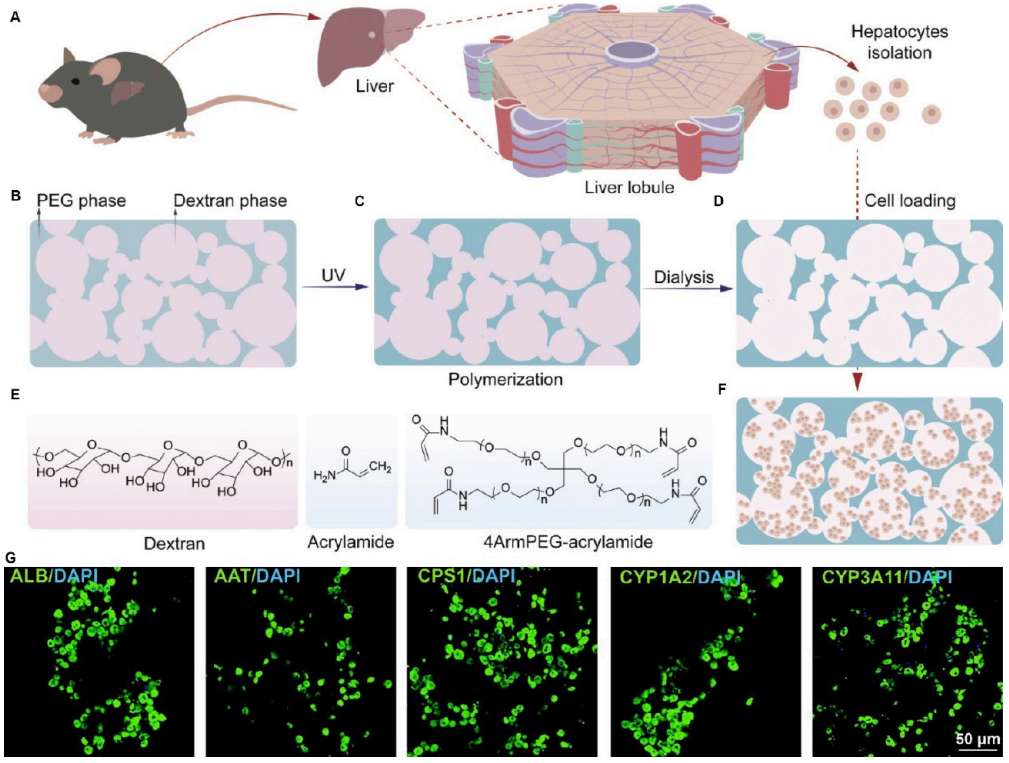

Figure 4. Hydrogel-based in vitro culture of PHHs for liver tissue engineering. Schematic of hepatocyte isolation, hydrogel fabrication, and immunofluorescence analysis. (A) Hepatic lobules are the functional units of the liver, mainly composed of hepatocytes. (B) Phase separation was observed by mixing PEG and dextran. (C) UV-triggered polymerization stabilized the hydrogel, followed by removal of dextran and unpolymerized monomers via dialysis. (D) Isolated hepatocytes were encapsulated in the hydrogel. (E) Chemical structures of dextran, acrylamide, and 4ArmPEG-acrylamide. (F) Hepatocytes aggregated in the hydrogel. (G) Immunofluorescence analysis showed functional markers (ALB, AAT, CPS1, CYP1A2, CYP3A11) on day 14. Reproduced with permission from ref 137. Copyright 2024 Springer Nature.

evaluation, modeling liver infectious diseases, and liver regeneration.[129−131] Michailidis et al. demonstrated that the alkaloid retrorsine significantly enhances the humanization of murine livers, enabling the routine production of highly humanized mice and high-quality mouse-passaged PHHs.[130] They also showed that PHH cultures could be genetically modified and retransplanted, resulting in highly humanized mice with genetically altered grafts.

Long-term culture of PHHs in vitro has been a focal point in liver research for an extended period. For instance, Hui et al. developed a definitive medium that significantly expands primary human hepatocytes.[132] These proliferating hepatocytes display characteristics of both hepatocytes and progenitor cells, capable of reverting to mature phenotypes in organoid culture and efficiently repopulating mouse livers. However, PHHs have limited proliferation capacity and are in a state of terminal differentiation.[133,134] Differences between donors due to genotypes and environmental factors can lead to immune rejection after transplantation.[135,136] Moreover, prolonged in vitro culture can diminish their inherent liver functions.[132]

Gu et al. tackle a crucial challenge in liver tissue engineering: maintaining hepatocyte functionality in vitro.[137] Inspired by the natural macroporous structure of liver tissues, the researchers developed synthetic hydrogel scaffolds through phase separation between polyethylene glycol (PEG) and polysaccharides (Figure 4). These hydrogels, featuring interconnected macroporous structures and optimal mechanical properties, create a favorable environment for hepatocyte adhesion and the formation of large cell aggregates. Hepatocytes cultured in these hydrogels exhibited improved functionalities compared to traditional two-dimensional cultures over 14 days. Quantitative reverse-transcription−polymerase chain reactions (qRT-PCR), immunofluorescence, and enzyme-linked immunosorbent assay (ELISA) analyses highlighted enhanced metabolic and functional characteristics. Additionally, protein sequencing data indicated robust cell−cell interactions within the hydrogels, with hepatocytes maintaining a protein expression profile akin to freshly isolated cells, particularly in the Notch and tumor necrosis factor (TNF) signaling pathways.

3.1.2. Hepatic Progenitor Cells (HPCs). Hepatic progenitor cells are a heterogeneous population with bidirectional differentiation potential.[138−141] Fifteen years ago it was found that Foxl1, a forkhead transcription factor, could be designated as a definitive marker for hepatic progenitor cells.[142] With the use of Foxl1-Cre transgenic mice, the study employs genetic lineage tracing to show that Foxl1-expressing cells, located near the portal triad postinjury, differentiate into both hepatocytes and cholangiocytes. Recent studies have shown that mouse or human hepatocytes can be induced to differentiate into scalable liver progenitor cells (iHepLPCs) in vitro, which exhibit at least a 40-fold efficient expansion with stable proliferative capacity.[143]

Macroporous hydrogels, particularly those composed of poly(ethylene oxide) (PEO) and gelatin (GEL), exhibit significant potential for in vitro cell culture of hepatic progenitor cells in liver tissue engineering, due to their favorable mechanical properties and excellent cell compatibility. Recent studies have explored the development of hydrogels formed via photopolymerization of water-in-water

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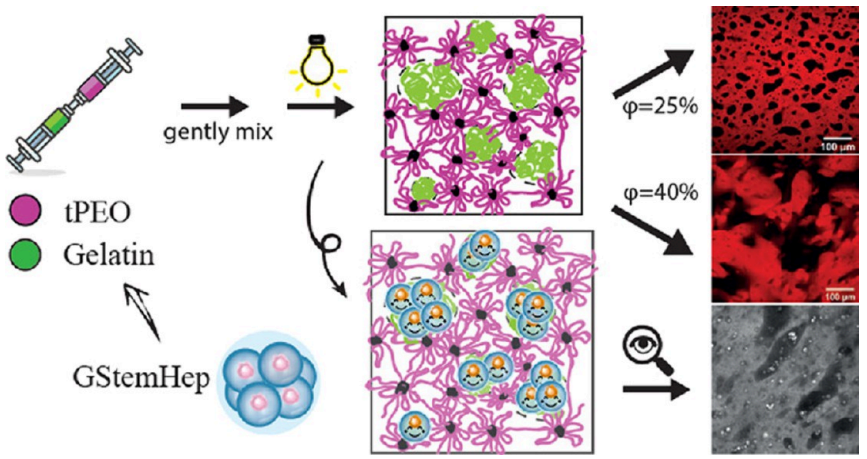

Figure 5. Hydrogel-based in vitro culture of HPCs for liver tissue engineering. Schematic of the macroporous hydrogel composed of PEO and gelatin, encapsulating hepatic progenitor cells (GStemHep). Reproduced with permission from ref 144. Copyright 2023 American Chemical Society.

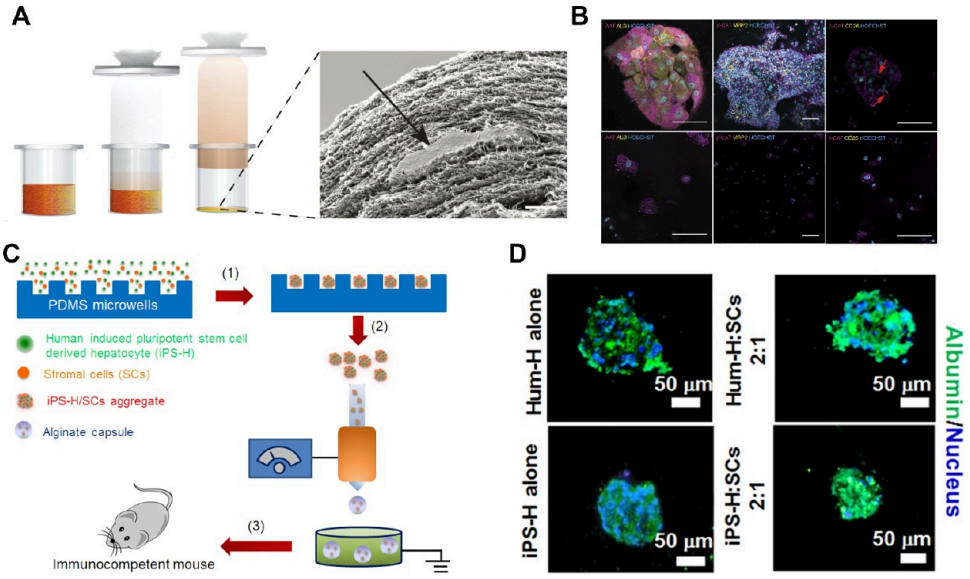

Figure 6. Hydrogel-based in vitro culture of iPSCs for liver tissue engineering. (A) Schematic of the RAFT process for iPSC-Hep maturation and scanning electron micrograph of 3D clump culture, as well as (B) confocal micrographs illustrating iPSC-Hep 3D cultures. Reproduced with permission from ref 166. Copyright 2014 Public Library of Science. (C) Generation of size-controllable iPS-H/SCs cell aggregates using PDMS microwells and their encapsulation in alginate capsules, alongside (D) immunostaining images depicting albumin secretion after 8 days of culture. Reproduced with permission from ref 167. Copyright 2015 Springer Nature.

emulsions, creating a matrix with macropores essential for cell proliferation and viability.[144] The PEO-based triblock copolymer provides mechanical strength, while gelatin enhances cell adhesion through RGD peptide sequences (Figure 5). Studies have shown that hepatic progenitor cells embedded in these macroporous hydrogels demonstrate improved viability compared to those in monophasic hydrogels. This makes macroporous hydrogels a promising scaffold for liver tissue engineering, potentially aiding in cell therapy and regenerative medicine. Furthermore, the successful in vivo functionality of PEO/GEL hydrogels, evidenced by the detection of human alpha-fetoprotein in mouse serum, underscores their application in liver cell culture and therapy.

To develop hepatocyte culture models that more closely mimic physiological conditions, it is essential to utilize three-dimensional (3D) formats, as traditional two-dimensional (2D) cultures cannot fully replicate in vivo liver morphology. In their study, Urtti et al. investigated the use of wood-derived nanofibrillar cellulose (NFC) and hyaluronan-gelatin (HG) hydrogels for culturing HepaRG liver progenitor cells.[145] The 3D cultures in NFC and HG hydrogels promoted the formation of multicellular spheroids with apicobasal polarity and functional bile canaliculi-like structures. These structures supported the expression and localization of hepatic markers such as albumin and CYP3A4, maintained metabolic activity, and facilitated the vectorial transport of substances. In contrast, predifferentiated HepaRG cells in 3D cultures exhibited decreased expression and activity of hepatic markers. Therefore, NFC and HG hydrogels significantly enhance the hepatic differentiation of HepaRG cells compared to standard 2D cultures, presenting promising materials for liver tissue engineering and drug development.

3.1.3. Induced Pluripotent Stem Cells (iPSCs). Induced pluripotent stem cells represent another potential strategy for

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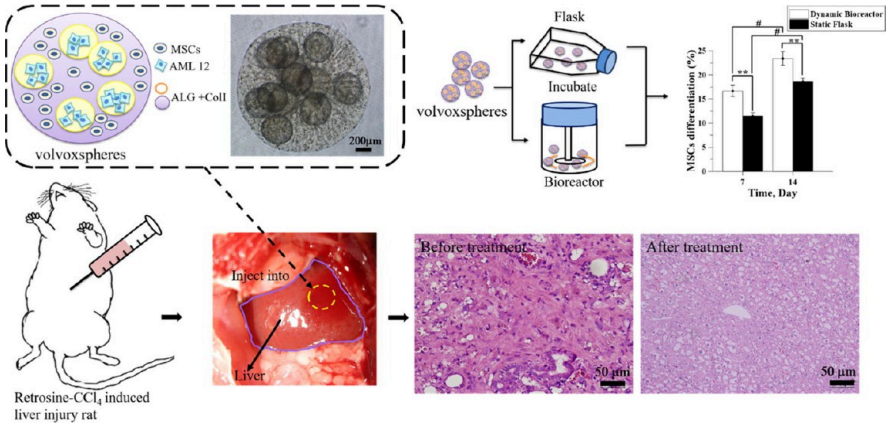

Figure 7. Hydrogel-based coculture of MSCs for liver tissue engineering. Coculturing mesenchymal stem cells and AML12 cells to generate hepatocyte-like cells, followed by implantation into rats for liver repair. Reproduced with permission from ref 186. Copyright 2017 Acta Materialia Inc.

providing a cell source for liver tissue engineering.[146−154] Since human iPSCs were generated in 2007,[155,156] building on earlier findings that mouse somatic cells can be reprogrammed into iPSCs using four transcription factors, Oct3/4, Sox2, Klf4, and c-Myc,[157] iPSCs have been an excellent source for generating functional cell types, due to their ability to stably and perpetually self-renew in vivo.[155,158−165]

Conventional 2D culture methods often result in induced pluripotent stem cell-derived hepatocytes (iPSC-Heps) exhibiting fetal-like characteristics and suboptimal functionality. To address these limitations, researchers investigated the maturation of iPSC-Heps using a three-dimensional collagen matrix culture method.[166] They employed the real architecture for 3D tissues (RAFT) system, which involves encapsulating cells within a type-I collagen hydrogel and using a biocompatible absorber to create physiological collagen densities, enabling reproducible, high-throughput 3D cultures (Figure 6A). Utilizing the RAFT system, the researchers observed significant improvements in the maturation and functional longevity of iPSC-Heps, closely resembling adult primary hepatocytes (Figure 6B). Three iPSC lines were differentiated toward the hepatic lineage using a 2D protocol, then further cultured under three conditions: traditional 2D, 3D with intact cell−cell junctions, and 3D with disrupted cell−cell junctions. The study concluded that the 3D culture system, particularly with intact cell−cell junctions, significantly enhanced the functional maturation of iPSC-Heps, promoting adult hepatocyte-like characteristics, such as polarized structures essential for drug metabolism and prolonged functional longevity.

3D cultures have been shown to support cell survival and function better than 2D cultures; however, they face challenges such as nonuniform hepatic-inducing signals. Recent studies have demonstrated the successful engraftment of iPSC-derived hepatocyte-like cells (iPS-H) in immunocompetent mice using a novel approach that combines 3D coaggregation with stromal cells (SCs) and encapsulation in biocompatible hydrogel capsules.[167] The process involved two key steps: first, iPS-H were derived using a 2D monolayer culture method and then coaggregated with SCs in a microwell platform to form uniform 3D cell aggregates. This platform allowed precise control over aggregate size, mitigating issues related to mass transfer and growth factor gradients. Subsequently, the aggregates were encapsulated in alginate hydrogel capsules and transplanted into the intraperitoneal cavity of C57BL/6 mice (Figure 6C). The encapsulated iPS-H/SC aggregates demonstrated survival and functionality in vivo, evidenced by levels of human albumin and α1-antitrypsin (A1AT) in mouse sera comparable to those of primary human hepatocyte (Hum-H) controls. Immunohistochemistry confirmed the presence and functionality of iPS-H in the retrieved cell aggregates (Figure 6D). This encapsulation strategy provided immune protection and enhanced hepatic maturation and function of iPS-H, offering a robust method for improving the engraftment of iPSC-derived hepatocytes in liver tissue engineering.

Despite significant advancements, several challenges persist in the field of iPSCs research.[158] One major issue is the potential for tumor formation, as iPSCs can proliferate indefinitely, leading to a risk if they continue to grow uncontrollably after transplantation.[168] Immune rejection also remains a critical hurdle. Although iPSCs derived from a patient's own cells could theoretically facilitate autologous transplantation, the immunogenicity of these cells is still under debate.[169] Furthermore, iPSCs exhibit considerable heterogeneity, with each cell line varying in morphology, growth rate, gene expression, and differentiation potential, thereby complicating their practical applications.[170,171]

3.2. Hydrogels for Coculture of Multiple Cells. The liver primarily consists of parenchymal and nonparenchymal cells.[172] The parenchymal cells, mainly hepatocytes, carry out the liver's primary functions, while the nonparenchymal cells, including Kupffer cells, hepatic endothelial cells, and hepatic stellate cells, provide support and connectivity to the parenchymal cells.[173] These diverse cell types collaborate to maintain liver function, homeostasis, and coordinate responses to injury and disease progression, highlighting the importance of their combined presence for the liver's metabolic and synthetic functions.[174−176]

3.2.1. Mesenchymal Stem Cells (MSCs). Mesenchymal stem cells have attracted significant attention for their role in supporting hepatocyte culture.[177−184] In a rat model with 90% partial hepatectomy, MSC transplantation markedly improved survival rates, glucose metabolism, and liver regeneration.[178] The observed benefits were attributed to the activation of the AKT/glycogen synthase kinase-3β (GSK-3β)/β-catenin pathway, which is crucial for hepatocyte proliferation. MSCs enhance liver metabolism, thereby providing the energy required for efficient liver regeneration. Additionally, it was

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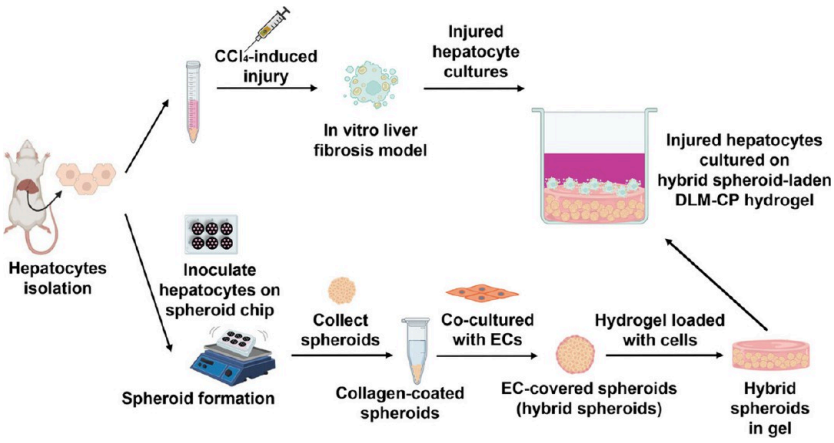

Figure 8. Hydrogel-based coculture of VECs for liver tissue engineering. Schematic of a self-healing hydrogel composed of chitosan-phenol integrated with decellularized liver matrix. The DLM, sourced from pigs, is combined with CP hydrogel and incorporates endothelial cell-covered hepatocyte spheroids. Reproduced with permission from ref 197. Copyright 2024 Wiley-VCH.

demonstrated that MSCs secrete cytokines such as IL-6 and TNF-α, which regulate hepatocyte proliferation and mitigate inflammatory damage, further promoting liver regeneration.

Compared to hepatocyte-like cells, primary hepatocytes exhibit high functionality but struggle to maintain this functionality in vitro. Recent studies have developed a method to create hepatocyte organoids by coculturing primary rat hepatocytes with human mesenchymal stem cells on a porcine liver extracellular matrix (PLECM) hydrogel.[185] The PLECM gel was prepared through perfusion and enzymatic hydrolysis, retaining key extracellular matrix components such as collagen types I and IV, fibronectin, and laminin. Rat hepatocytes and human MSCs were mixed and plated on the presolidified PLECM gel in a 48-well plate, forming organoids within 48 h. These organoids were subsequently analyzed using hematoxylin and eosin staining, periodic acid-Schiff staining, immunohistological and immunofluorescence techniques, and quantitative PCR for liver-specific genes like alb, CYP450 markers, and urea cycle genes. The results demonstrated that hepatocytes and MSCs interacted with the liver-derived ECM to form 3D structures, maintaining high expression levels of critical genes and stable functionality, as evidenced by consistent albumin and urea production in vitro over 20 days.

Several researchers have explored the use of customizable hydrogels for tissue engineering. Kuo and colleagues designed Volvox spheres to mimic the natural Volvox structure, creating a three-dimensional environment conducive to cell encapsulation and differentiation.[186] In their study, rat mesenchymal stem cells and AML12 hepatocytes were cocultured within these Volvox spheres for liver tissue engineering applications (Figure 7). The results demonstrated that MSCs encapsulated in Volvox spheres and cultured in a dynamic bioreactor exhibited a 2-fold increase in albumin expression and a 2.5-fold increase in cytokeratin 18 expression compared to static cultures. Furthermore, the therapeutic potential of these encapsulated cells was assessed in a rat model of retrorsine-exposed CCl4-induced liver injury. The implantation of Volvox spheres containing both MSCs and AML12 hepatocytes led to significant reductions in AST and ALT levels, and histopathological analysis revealed liver repair and new tissue formation. These findings suggest that Volvox spheres are an effective scaffold for coculturing MSCs and hepatocytes, enhancing cell differentiation and promoting liver tissue regeneration both in vitro and in vivo.

3.2.2. Vascular Endothelial Cells (VECs). Vascular endothelial cells are often cocultured with hepatocytes to promote angiogenesis because of their advantages in regulating liver function and regeneration.[187−195]

The 3D cellular architecture can lead to the hypoxic death of central cells due to inadequate internal vascularization. To address this, researchers have developed a perfusable 3D liver cell cultivation system using cell-laden hydrogel microfibers, aiming to replicate the hepatic lobule's structure.[196] By coculturing HepG2 cells with vascular endothelial cells, they form vascular networklike conduits that ensure efficient oxygen and nutrient delivery. Utilizing microfluidic devices, HepG2 cells are encapsulated within anisotropic hydrogel microfibers, creating a sandwich-type structure. These microfibers are then bundled and packed into a perfusion chamber for cultivation. This system allows dynamic control of the culture environment by adjusting perfusion flow rates, ensuring uniform oxygen and nutrient delivery. The researchers evaluated liver cell viability and functions, monitored oxygen consumption, and observed vascularized tissue formation within the hydrogel fibers. This method effectively replicates the in vivo environment of liver tissues, providing a versatile model for further biomedical research.

Although coculturing vascular endothelial cells with various cell types promotes angiogenesis and enhances liver-specific functions, the random distribution of VECs within hydrogels often results in disorganized and inadequate blood vessel development. Recent research has developed a self-healing chitosan-phenol (CP) hydrogel with decellularized liver matrix (DLM), embedded with endothelial cell-covered hepatocyte spheroids, by extracting DLM from pigs and mixing it with CP hydrogel.[197] The incorporation of ECs in hepatocyte spheroids within the DLM-CP hydrogel enhances liver-specific functions and promotes better vascularization, addressing the limitations of traditional hepatocyte spheroid treatments. These spheroids, fabricated using a customized method, were cocultured with injured hepatocytes exposed to carbon tetrachloride (CCl4) to model toxin-mediated liver fibrosis (Figure 8). The study found that this coculture significantly improved hepatic function and reduced damage in injured hepatocytes, evidenced by a 91% restoration in urea synthesis and a 49%

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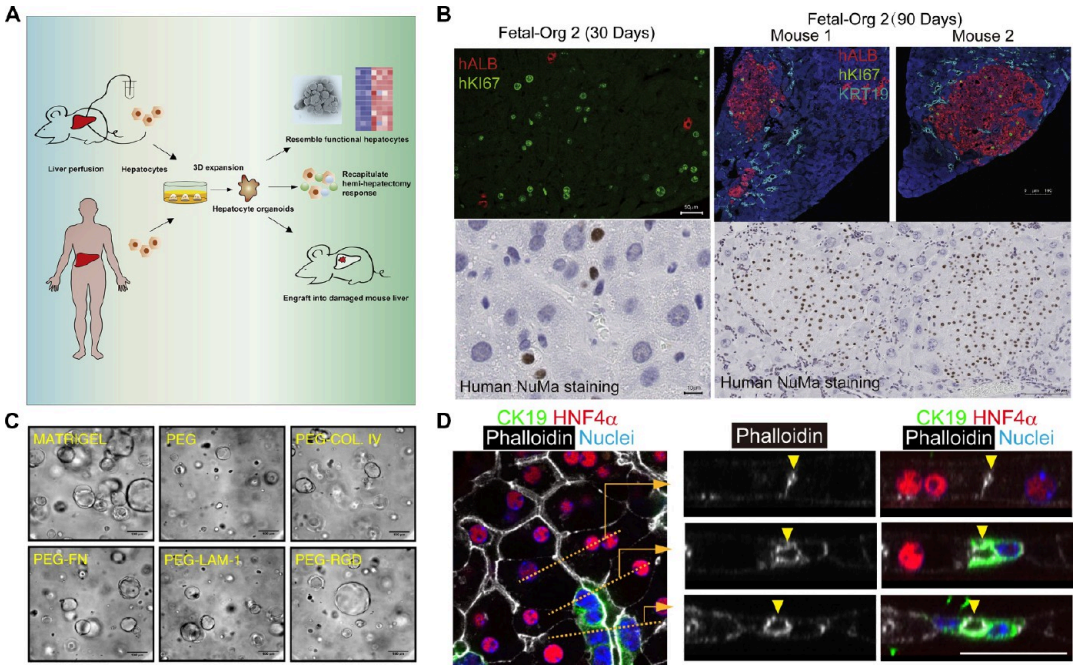

Figure 9. Hydrogel-based liver organoids for liver tissue engineering. (A) Establishment of a long-term 3D organoid culture system for mouse and human primary hepatocytes that (B) replicates the proliferative damage response of hepatocytes after organoid engraftment. Reproduced with permission from ref 204. Copyright 2018 Elsevier Inc. (C) Cultivation of mouse liver progenitor cells in diverse chemically defined and mechano-modulatory 3D culture systems. Reproduced with permission from ref 206. Copyright 2020 The Author(s). (D) Formation of hepatobiliary connections observed in cocultured hepatobiliary tubular organoids. Reproduced with permission from ref 208. Copyright 2021 The Author(s).

decrease in lactate dehydrogenase activity after 7 days. This innovative approach of using a self-healing, injectable hydrogel combined with DLM and EC-covered hepatocyte spheroids offers a promising therapeutic strategy for liver tissue engineering, providing a more physiologically relevant microenvironment and enhancing cell delivery and functionality, thereby offering potential for more effective treatment of liver fibrosis and cirrhosis.

The advancement of 3D printing technology introduces a novel research approach that integrates 3D printing with stimuli-responsive hydrogels to fabricate multifurcated vessels and complex vascular networks within heterogeneous porous scaffolds.[198] This innovative technique leverages the sol−gel transition properties of temperature-responsive gelatin and the physical cross-linking capabilities of pH-responsive chitosan to create stable hydrogel tubes. These hydrogels facilitate the formation of biomimetic vessels with physiological stability, mechanical strength, semipermeability, and hemocompatibility. The precision of 3D printing enables the creation of intricate, interconnected networks, resulting in artificial blood vessels composed of gelatin and chitosan that exhibit excellent biocompatibility and low inflammatory response when cocultured with hepatocytes and human umbilical vein endothelial cells, thereby effectively modeling liver tissue. This approach provides a robust solution for fabricating free-standing multifurcated blood vessels and prevascularized scaffolds, significantly advancing vascular tissue engineering and regenerative medicine by addressing the challenge of replicating natural blood vessel structures and constructing functional vascular networks within tissue scaffolds.

4. HYDROGELS FOR ARTIFICIAL LIVER MODELS IN LIVER TISSUE ENGINEERING

Reconstructing the in vivo microenvironment is a major goal of liver tissue engineering, particularly for developing artificial liver models. These models aim to replicate the liver's complex architecture, including microvascular and microbile duct networks, and the immune barrier, which are essential for normal liver function. There are three primary types of artificial liver models in liver tissue engineering: organoids, 3D bioprinting, and liver-on-a-chip. Hydrogels serve as an ideal scaffold material for these models due to their tunable properties, allowing the creation of structures that closely mimic the liver's natural microenvironment. This section examines the application of hydrogels in building these artificial liver models, focusing on the strategies used to incorporate vascularization, bile duct formation, and immune components.

4.1. Organoid. Liver organoids are miniature liver tissues created using in vitro three-dimensional culture techniques, capable of self-renewal and performing specific liver functions after culture or transplantation.[199] As advanced in vitro models, they are highly applicable in research and treatment of liver diseases.[200−203] Organoids made from cell scaffolds and biomaterials are extensively used in areas like disease modeling, drug screening, and transplantation of artificial liver organoids.

Recent studies have delved into constructing three-dimensional organoids from long-term cultured mature liver cells sourced from mice and humans.[204] The transcriptional profiles of organoids derived from single hepatocytes closely resemble those of proliferating hepatocytes following partial hepatectomy, while maintaining their original morphology, function, and gene expression characteristics (Figure 9A, B). Human hepatocellular organoids, when transplanted into mice, displayed proliferation, mirroring the liver's response to

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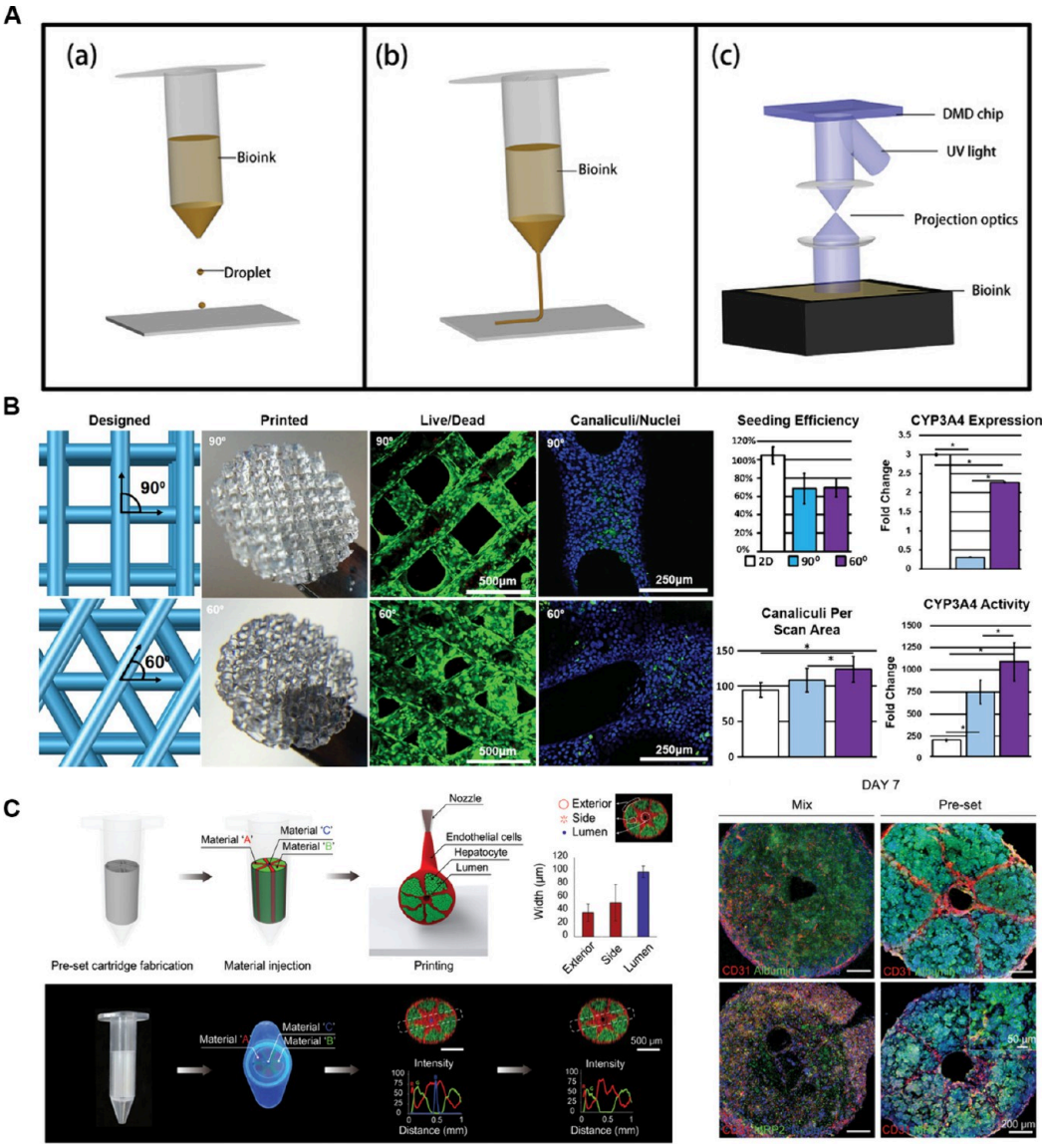

Figure 10. 3D bioprinting liver structure for hydrogel-based liver tissue engineering. (A) 3D bioprinting techniques for scaffold construction: inkjet-based, extrusion-based, and photocuring-based bioprinting methods. Reproduced with permission from ref 219. Copyright 2020 Wiley-VCH. (B) Differential gene expression and function in a proliferative human hepatocyte model cell line seeded in 3D-printed gelatin scaffolds with varying geometries. Reproduced with permission from ref 238. Copyright 2018 Acta Materialia Inc. (C) Preset extrusion 3D bioprinting technique is used to create a hepatic lobule array by simultaneously producing heterogeneous, multicellular, and multimaterial structures. Reproduced with permission from ref 241. Copyright 2020 Wiley-VCH.

damage. The capability to culture mature human liver cells for prolonged periods is crucial for developing in vitro toxicological methods and studying liver-specific infections, such as malaria and hepatitis viruses, as well as various inherited and metabolic liver diseases. Understanding the mechanisms behind mature hepatocyte expansion will significantly contribute to designing new therapeutic strategies for regenerative liver therapy.

Co-culturing human primary hepatocytes and Kupffer cells can partially replicate the effects of activated Kupffer cells on hepatocyte functions. However, these models are limited by the scarcity and variability of primary cells and the challenges in maintaining their function in vitro. Human induced pluripotent stem cell-derived liver organoids (hiPSC-LOs) serve as potent models for studying human liver diseases due to their in vivo-like cellular interactions in 3D structures while maintaining long-term hepatic functions. However, earlier versions of these organoids either lacked hematopoietic activity or contained insufficient ratios of Kupffer cells due to undefined differentiation processes. Integrating Kupffer cells into human iPSC-derived liver organoids (LOs) marks a significant advancement in modeling liver dysfunction.[205] Researchers have generated liver organoids containing Kupffer cells (KuLOs) by replicating fetal liver hematopoiesis using hiPSC-derived erythro-myeloid progenitors (EMPs). Within the liver organoid environment, these EMPs differentiated into myeloid and erythroid lineages, which are essential for creating functional KuLOs. The study identified macrophage colony-stimulating factor (M-CSF) as crucial for maintaining the hematopoietic cell population within the organoids. When KuLOs were exposed to endotoxins mimicking septic conditions, they exhibited significant dysfunction similar to that seen in human septic liver. Additionally, the study showed that KuLOs could self-recover from endotoxin-induced

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damage, a process expedited by a toll-like receptor-4-directed antagonist. This research provides a detailed protocol for generating EMPs from hiPSCs and coculturing them with liver organoids to sustain long-term hematopoietic activity and promote Kupffer cell differentiation, thereby creating a robust model for investigating liver dysfunction.

Traditional three-dimensional cultures often rely on ill-defined, potentially immunogenic matrices, hindering their practical application. Schoonjans et al. presented chemically defined hydrogels that provide a consistent and controlled environment for efficiently deriving mouse and human hepatic organoids.[206] These organoids exhibit growth sensitivity to matrix stiffness, regulated by the activation of Src family kinases (SFKs) and yes-associated protein 1 (YAP), rather than acto-myosin contractility.[207] Incorrect matrix stiffness negatively impacts proliferative capacity. By tuning the synthetic microenvironment's mechanical properties to match the physiological stiffness of the mouse liver, researchers optimized liver organoid derivation efficiency (Figure 9C). They also modeled the stiffness of fibrotic liver tissue, demonstrating that such aberrant mechanics hinder liver progenitor proliferation. Notably, this study achieves biopsy-derived human liver organoids without animal components, paving the way for clinical-grade applications. This approach offers a reproducible and defined method for growing liver organoids, potentially enhancing their use in basic research and regenerative medicine.

Hydrogels not only support the generation of functional liver organoids but also simulate the bile canalicular network of hepatocytes. Recent advancements have enabled the creation of hepatobiliary tubular organoids (HBTOs) by combining mouse hepatocyte progenitors, cholangiocytes, and various epithelial cell types within a hydrogel matrix.[208] These HBTOs successfully replicate the functional bile canalicular network, where hepatocytes secrete metabolites into the canaliculi, which are then transported through the biliary tubular structure formed by cholangiocytes (Figure 9D). The study demonstrates that hepatocytes in HBTOs acquire and maintain essential metabolic functions, such as albumin secretion and cytochrome P450 activities, over the long-term. Within the HBTOs, hepatocyte-derived small hepatocytes (SHs) and epithelial cell adhesion molecule-positive (EpCAM +) cholangiocytes create an interconnected network, facilitating the absorption, excretion, and accumulation of substances like bilirubin and fluorescein-labeled bile acid. This setup effectively simulates the in vivo connection between hepatocytes and cholangiocytes, allowing for detailed study of metabolite transport within liver tissue. The hydrogel matrix supports the growth, division, and organization of these cells into a structure that mimics liver tissue, establishing a functional bile drainage system ex vivo. This offers a novel platform for investigating liver functions and the interactions between different liver cell types.

4.2. 3D Bioprinting. 3D bioprinting is a promising technology for tissue and organ construction, offering precise control over the spatial arrangement of cells and their microenvironment.[209−215] This technique enables the creation of tissuelike 3D structures by strategically placing cells, biomaterials, and growth factors, thereby supporting the cells' in vivo functions (Figure 10A).[216−221] A key benefit of 3D bioprinting is its capability to fabricate complex organs, such as the liver, with multiple cell types organized in intricate patterns.[222−236]

4.2.1. 3D-Printed Liver Scaffolds with Varied Geometry. With the use of 3D bioprinting technology, various shapes and sizes of in vitro liver tissue models have been developed to mimic liver functions for different research objectives. Lewis et al. demonstrated that 3D-printed gelatin scaffolds with varied pore geometries and sizes markedly influence hepatocyte performance, viability, and gene expression.[237,238] They used porcine gelatin to print scaffolds with strut orientations of 0° and 90° (0−90) and 0°, 60°, and 120° (60 AA), which were cross-linked and seeded with HUH7 human hepatocytes (Figure 10B). The study found that hepatocytes on more interconnected 3D gelatin scaffolds exhibited higher viability, proliferation, and specific liver functions, such as albumin secretion, cytochrome P450 enzyme activity, and bile transport, compared to those on less interconnected scaffolds and traditional 2D cultures. The 60 AA scaffolds, characterized by tortuous pore geometries, enhanced initial cell seeding efficiency and influenced cell aggregation, though cell numbers remained similar across scaffold types over time. Notably, scaffolds with 60° pore geometry resulted in greater albumin secretion and CYP enzyme activity than those with 90° geometry. Although gene expression for certain proteins was higher in 2D cultures, the functional activity of these proteins was significantly enhanced in the 3D scaffolds, highlighting the importance of 3D culture environments in promoting functional hepatocyte behavior. By day 7, significant changes in gene expression, particularly in drug metabolism genes CYP3A4, CYP3A7, and CYP2C9, were observed between different scaffold geometries. This is hypothesized to result from the tight pore geometries mimicking the liver's pericentral environment, influencing cell packing.

Recent research indicates that hepatocyte growth is more favorable on hexagonal scaffolds compared to square ones. A study involving HepG2 cells, as opposed to HUH7 cells, supported these findings.[239] Researchers investigated the influence of scaffold architecture on HepG2 cells using 3D-printed poly lactic acid (PLA) combined with gelatin (Gel) coatings. Two scaffold designs, a simple square and a bioinspired hexagonal cross-section, were fabricated using fused deposition modeling (FDM). Gelatin coatings at concentrations of 1% and 2% were applied to enhance wettability and surface properties. Biological assays demonstrated that the hexagonal design significantly improved cell adhesion, viability, and specific liver functions. Notably, albumin and urea production were 1.22 times higher with the hexagonal design compared to the square design over 3 days.

4.2.2. Bioprinting of Hepatic Lobules. Subsequent advancements in bioprinting technology have significantly influenced the field of liver tissue engineering. Researchers have transitioned from merely replicating the liver's morphological attributes to emulating its complex functionalities within a laboratory setting. The liver lobule, the fundamental unit of the liver, is distinguished by its intricate vascular architecture. Hepatic sinusoids are arranged from the center to the periphery of the lobules, forming a hexagonal vascular network. Blood enters through the hepatic artery and portal vein and exits via the central hepatic vein. The liver lobule plays a crucial role in essential liver functions such as albumin secretion, urea production, and cytochrome P450 (CYP) enzyme activity, which are critical for the body's metabolic and detoxification processes. Recent technological strides in coaxial printing, sacrificial printing, and preset extrusion methods have

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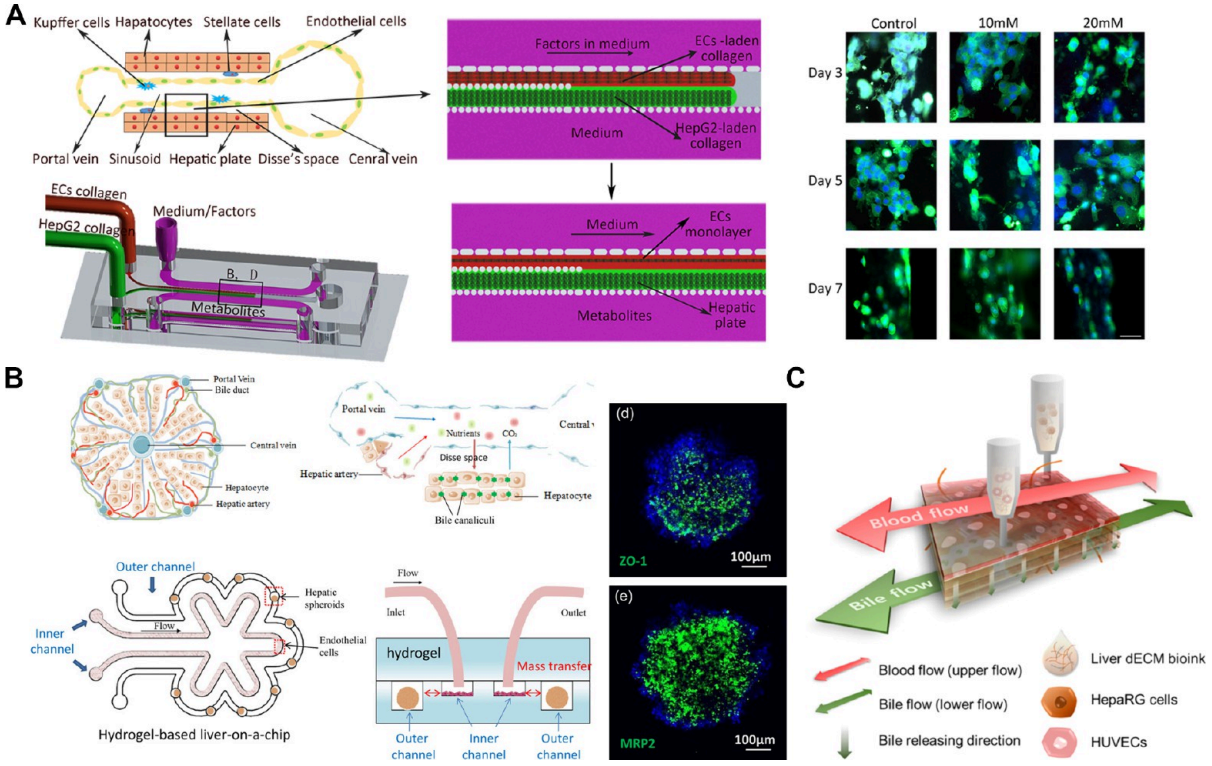

Figure 11. Liver-on-a-chip for hydrogel-based liver tissue engineering. (A) Schematic depiction of a biomimetic liver sinusoid formation on a chip, illustrating functional testing of liver-specific activities within the sinusoid. Reproduced with permission from ref 252. Copyright 2018 IOP Publishing Ltd. (B) Schematic representation of a biomimetic liver-on-a-chip enabling nutrient diffusion from endothelial cell monolayers to hepatic cells, mimicking in vivo mass transfer processes while protecting hepatic cells from harmful shear stress. Reproduced with permission from ref 256. Copyright 2020 Wiley Periodicals LLC. (C) Schematic illustration of 3D cell-printing for liver-on-a-chip, incorporating vascular and biliary systems. Reproduced with permission from ref 257. Copyright 2019 IOP Publishing Ltd.

facilitated the integration of vascular systems into 3D bioprinted liver models. These advancements are critical for creating more physiologically relevant tissues that offer improved nutrient and oxygen diffusion, which are essential for maintaining cellular viability and function in vitro.

The preset extrusion bioprinting technique, which utilizes a precursor cartridge to stably preserve multimaterials in a predefined configuration, mimics human tissues with high-resolution and simultaneous printing of various bioinks, overcoming the limitations of tradition 3D bioprinting like the lack of vascular structure, low resolution, and difficulty in handling heterogeneous cells.[240] In their study, Jin et al. utilized this technique to develop multiscale hepatic lobules, successfully creating complex, vascularized liver tissue (Figure 10C).[241] The engineered lobules were composed of hepatocytes and endothelial cells, precisely arranged around a central lumen, closely mimicking the natural architecture of the liver. Gelatin served as a key sacrificial material in the bioprinting process due to its biocompatibility and structural support during fabrication. Its use allowed for precise control over the architecture, facilitating the formation of interconnected vascular networks that significantly improved cell viability and function. The bioprinted liver constructs demonstrated enhanced albumin secretion, urea production, and enzymatic activity compared to traditional hepatocyte-endothelial cell cocultures. These results emphasize the potential of hydrogel-based bioprinting in replicating the complex architecture of liver tissues while maintaining high cell viability and functionality.

The printed tissue, despite having endothelialized lumens, faces challenges in oxygen and nutrient supplementation and waste metabolite clearance due to its large size. Consequently, small-diameter cell spheroids, suitable for direct injection, have garnered increasing attention in regenerative medicine in recent years. These spheroids have a higher surface area-to-volume ratio compared to tissue blocks, enabling better survival, viability, and engraftment after injection. Researchers have advanced this technique by integrating the preset extrusion bioprinting with a microfluidic emulsification system to produce uniform cell-laden microtissue spheroids at a rate of 45 spheroids per minute.[242] These spheroids incorporate hepatic and endothelial cells to mimic liver lobule structures, facilitating long-term culture with high cell viability and structural integrity. The method utilizes thermal gelation instead of cross-linking agents to stabilize the structures. Results show that structured spheroids have higher expression levels of liver-specific markers MRP2, albumin, and CD31 compared to nonstructured spheroids. Moreover, in vivo studies reveal stable engraftment of these structured spheroids. The resulting microtissue spheroids demonstrate improved protein secretion, enzyme expression, and overall liver function, highlighting the method's effectiveness in creating biomimetic liver tissue constructs.

3D printed biodegradable scaffolds offer significant potential for developing complex structures for hepatic lobules. The selection of biodegradable materials, essential for their biocompatibility and degradation properties, becomes even more challenging when considering their compatibility with 3D printing technologies. These scaffolds must possess physical

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properties, such as mechanical strength and contact angle, that closely mimic natural liver tissue to facilitate effective liver regeneration. To address these challenges, researchers have developed photocurable, biodegradable scaffolds using digital light processing-additive manufacturing (DLP-AM) specifically for hepatic lobule regeneration.[232] By employing innovative biodegradable polymers like poly(glycerol sebacate) acrylate (PGSA) and poly(ethylene glycol) diacrylate (PEGDA), they have created hexagonal scaffolds that enhance hepatocyte proliferation and metabolic activity. Among these, the high-diffusion staircase (HDS) variant is particularly noteworthy, optimized for cell growth and exhibiting superior cell viability and structural integrity in long-term cultures. By fine-tuning the ratios of PGSA and PEGDA, the mechanical properties of the scaffolds have been adjusted to closely match those of natural liver tissue, thereby improving cell attachment and growth. This integration of advanced bioprinting techniques and customized hydrogels holds substantial promise for supporting hepatocyte growth and promoting liver-specific functions.

4.3. Liver-On-a-Chip. The liver chip is a dynamic microfluidic-based biochip model for culturing liver parenchymal cells in vitro. Unlike static platforms, dynamic microfluidics-based liver systems enable precise control over the microenvironment for liver parenchymal cell culture, including temperature, pH, cell shear stress, oxygen levels, nutrient supply, and waste removal.[243−251]

4.3.1. Precise Control of Liver Parenchymal Cell Culture Microenvironment in Liver Chip Systems. The liver sinusoid, the fundamental functional unit of the liver, has been extensively studied to replicate liver functions in vivo. A functional biomimetic liver sinusoid necessitates an endothelial cell layer, which is crucial for its operation. Recent advancements have successfully recreated the liver sinusoid's biomimetic microenvironment by utilizing laminar flow and the self-assembly of endothelial cells within a naturally degradable gel, specifically rat tail collagen-I, to develop a liver sinusoid on a chip.[252] By concurrently injecting collagen containing HepG2 and HUVEC cells, distinct cell layers with controlled and uniform endothelial cell distribution were achieved through self-assembly and growth factor stimulation (Figure 11A). This liver-on-chip model demonstrated sustained bioactivity and functionality, as evidenced by tests for cell viability, albumin secretion, and urea synthesis. Evaluations conducted on days 3, 5, and 7 postcollagen injection and acetaminophen treatment at concentrations of 0, 10, and 20 mM confirmed that the liver sinusoid maintained its bioactivity and function for at least 7 days.

Recent studies have increasingly recognized that the composition and topography of the ECM significantly influence cell behavior and function.[253] To mimic the natural ECM accurately, it is essential to select hydrogel materials, cross-linking methods, and conjugable biomolecules with care. One such study has developed an advanced model system that not only structurally mimics human liver tissue but also modulates its ECM physiological microenvironment.[254] This research introduces an advanced liver-on-a-chip model engineered to replicate the microenvironment of liver parenchymal cells, focusing specifically on liver fibrosis. Gelatin-methacryloyl hydrogels with adjustable cross-linking degrees, combined with transforming growth factor-beta (TGF-β) induction, recreate the early and late stages of liver fibrosis. This system employs a three-dimensional mixed-culture approach, enabling the construction of a liver sinusoid model that accurately represents physiological conditions. The chip incorporates an endothelial cell layer on a polyethylene terephthalate (PET) membrane, a 3D porous GelMA hydrogel encapsulating stellate cells, and hepatocyte spheroids arranged in the bottom layer. By fine-tuning the GelMA cross-linking and incorporating TGF-β, the researchers successfully recreated the microenvironment of liver fibrosis, demonstrating different cellular responses to drug treatments at various fibrosis stages. The model accurately mimics liver fibrosis progression, including hepatic stellate cell activation, abnormal protein synthesis, and cytokine secretion associated with fibrosis.

Precise control of spatial distribution and temporal assessment of cell behavior, along with regulation of the microenvironment, are crucial in cellular studies. Researchers have developed a liver-on-a-chip platform for the long-term culture of 3D human HepG2/C3A hepatic spheroids encapsulated in photo-cross-linkable gelatin methacryloyl hydrogel.[255] This platform features a bioreactor that enables continuous perfusion and in situ monitoring, maintaining a controlled microenvironment that supports cellular viability and functionality for over 30 days. The bioreactor's design allows direct access to hepatic constructs during experiments without compromising operational integrity. Integrated with a bioprinter, the system fabricates precise 3D hepatic constructs, closely mimicking the architecture of in vivo liver tissue. Functional assessments revealed consistent secretion of albumin, alpha-1 antitrypsin, transferrin, and ceruloplasmin, along with positive immunostaining for hepatocyte markers such as cytokeratin 18, MRP2 bile canalicular protein, and ZO-1 tight junction protein. The liver-on-a-chip's response to 15 mM acetaminophen exposure reflected known toxicological outcomes, demonstrating its efficacy in drug toxicity assessment.

4.3.2. Hydrogel Microfluidic-Based Liver-On-a-Chip Mimic the Structure and Function of Hepatic Sinuses. A variety of biochips were constructed on the basis of simulating the microstructure and function of hepatic sinuses. The hepatic lobules are composed of several hepatic sinuses and four types of cells (hepatic endothelial cells, kupffer cells, mesenchymal stem cells, and hepatic parenchymal cells). The researchers cocultured the four types of cells on a double-layer microfluidic biochip.[247] The liver endothelial cells, Kupffer cells, and mesenchymal stem cells were attached to the porous PE membrane, and the liver parenchymal cells were cultured in the inferior cavity to simulate the structure of hepatic sinuses.

Unlike traditional liver models that expose hepatocytes directly to perfusion culture medium and harmful shear stress, the hydrogel in the liver-on-a-chip creates a barrier that facilitates nutrient diffusion similar to natural liver processes, thereby enhancing cell viability and liver-specific functions. The permeable hydrogel ensures effective mass transfer, avoiding the limitations of convection-dominated transfer found in conventional materials like PDMS. This accurate mimicry of the hepatic lobule's nutrient exchange dynamics positions the liver-on-a-chip as a valuable tool for various applications, including bioartificial livers and drug toxicity screening, by providing a physiologically relevant environment for hepatocyte culture.

For instance, a recent study introduces a novel approach to creating a liver-on-a-chip model using diacrylated Pluronic F127 (F127-DA) hydrogel to better replicate the structure and

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function of hepatic sinuses.[256] Traditional liver models face challenges due to the direct exposure of hepatocytes to perfusion culture mediums, which reduces cell viability and liver-specific functions because of shear stress (Figure 11B). To address these issues, the study designs a microfluidic chip incorporating features of hepatic lobules. The chip features hepatocellular carcinoma HepG2 cells and human hepatic stellate cell LX-2 cultured in microwells along an outer channel, while endothelial cells line an inner channel. This setup allows for the diffusion of nutrients from the endothelial cell monolayers to the hepatic cells, simulating the in vivo mass transfer process without exposing the hepatic cells to harmful shear stress. The liver-on-a-chip demonstrated improved cell viability and liver-specific functions, with hepatic spheroids forming within 1 day and maintaining enhanced functionality for up to 8 days. This model shows potential for advanced applications in bioartificial livers and drug toxicity testing by providing a more accurate in vitro representation of liver structure and function.

Simulating biliary fluidic channels is as crucial as simulating blood flow to accurately replicate the liver's natural processes. A recent study introduced a 3D liver-on-a-chip model developed using cell-printing technology, designed to mimic the liver's complex microenvironment and biliary system.[257] This model employs liver decellularized extracellular matrix (dECM) bioink, providing a realistic 3D microenvironment that supports the coculture of multiple liver cell types (Figure 11C). The incorporation of vascular and biliary fluidic channels enhances liver-specific functions within the model. Comparative analyses demonstrate that the 3D liver-on-a-chip with a biliary system exhibits superior liver functionalities and gene expression compared to models without a biliary system and traditional 2D/3D cultures. Testing with acetaminophen indicated a responsive drug interaction, underscoring its potential for drug testing. The integration of dECM hydrogels in the chip ensures the presence of essential ECM components, promoting hepatocyte function and biliary system formation, both crucial for bile acid removal and overall liver health.

In addition, the use of extracellular matrix mimetic hydrogels supports hepatocyte viability and functionality within a perfused liver-on-a-chip device. A modular hydrogel system based on hyaluronan and poly(ethylene glycol) (HA-PEG), modified with cyclooctyne moieties for bioorthogonal strain-promoted alkyne−azide cycloaddition (SPAAC), was developed and characterized.[75] The HA-PEG hydrogel demonstrated superior support for hepatocyte viability and functionality when compared to agarose and alginate hydrogels in a perfused liver-on-a-chip device. Hepatoma cells (HepG2) formed viable spheroids in all hydrogels, with the highest albumin and urea expression in alginate hydrogels. Notably, HA-PEG hydrogels modified with cyclic RGD peptides enhanced the viability and functionality of human-induced pluripotent stem cell-derived hepatocytes (hiPS-HEPs), promoting 3D migration, growth, and increased albumin production. This flexible SPAAC cross-linked hydrogel system enabled the creation of perfused 3D cell cultures, highlighting its potential for developing robust and physiologically relevant liver-on-a-chip models. These advancements support the optimization of liver models for drug testing and tissue engineering applications, showcasing the HA-PEG hydrogel's ability to mimic the supportive functions of the liver's extracellular matrix.

5. IN VITRO/IN VIVO APPLICATION OF HYDROGEL-BASED LIVER TISSUE ENGINEERING

Hydrogel-based applications in liver tissue engineering span both in vitro and in vivo contexts, each with its unique challenges and advantages. In vitro applications primarily focus on creating reliable and functional liver models for drug screening, disease modeling, and studying liver biology. In contrast, in vivo applications aim to develop implantable liver constructs that can support liver function and promote regeneration in living organisms. Hydrogels, with their versatile and tunable properties, are at the forefront of these applications, providing scaffolds that support cell growth, differentiation, and tissue integration. This section explores the dual role of hydrogels in liver tissue engineering, detailing their use in both laboratory settings and clinical applications. It highlights the progress made in translating in vitro findings to in vivo applications and discusses the potential of hydrogels to bridge the gap between experimental research and therapeutic implementation.

Liver tissue engineering is revolutionizing hepatotoxicity testing,[258−260] liver regeneration,[261−263] and bioartificial liver systems (BALS)[264−267] for acute liver failure.[268] In vitro hepatotoxicity testing with engineered liver tissues offers a more human-relevant alternative to animal models, enhancing drug safety evaluations. Liver organoid transplantation, derived from stem cells or primary hepatocytes, presents a promising avenue for liver tissue regeneration, potentially alleviating the donor organ shortage and enabling personalized treatments. Furthermore, BALS are being developed as temporary support for ALF patients, utilizing engineered tissues to perform critical liver functions and bridge the gap to transplantation or natural recovery. These applications highlight the transformative potential of liver tissue engineering in medical research and treatment.

5.1. Hepatotoxicity Testing by Hydrogel-Based Liver Tissue Engineering. Drug-induced liver injury (DILI) remains a significant obstacle in drug development.[269−271] From 1975 to 2007, hepatotoxicity was the second leading cause of drug withdrawals in the United States, accounting for 15 out of 47 cases.[272] Accurate prediction of DILI during the preclinical phase could potentially reduce both the time and cost of drug development while increasing the success rate.[273] However, animal models, which are heavily relied upon in preclinical evaluations, often exhibit only modest correlations with clinical outcomes due to differences in metabolism between animals and humans.[272,274] Consequently, there is an increasing need for alternative and more accurate test methods to replace animal models.

The liver contains a variety of oxidative and conjugative enzymes crucial for metabolizing the myriad compounds in today's pharmaceuticals. The differential expression of these drug-metabolizing enzymes in the human liver can lead to variations in pharmacokinetic profiles, resulting in interindividual variability in drug toxicity and efficacy. Traditional in vitro methods often fail to replicate the complexity and variability of human liver metabolism. To address this limitation, the "transfected enzyme and metabolism chip" (TeamChip) was developed.[258] TeamChip can simulate the genetic polymorphisms present in different population subgroups, providing a more accurate prediction of drug toxicity and efficacy. This system incorporates multiple drug-metabolizing enzymes into 3D cellular microarrays, enabling

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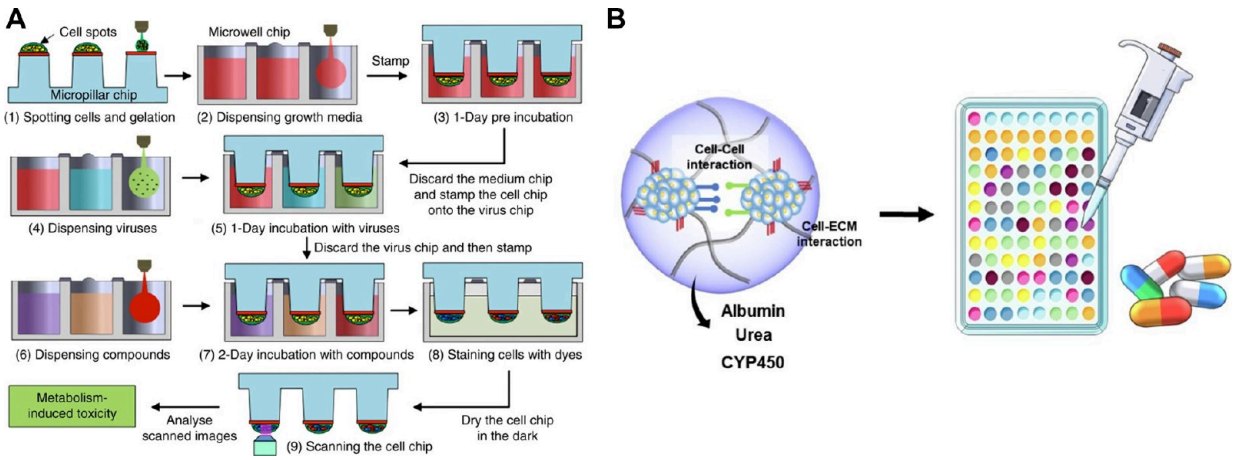

Figure 12. Hydrogel-based liver tissue engineering for hepatotoxicity testing. (A) Experimental procedure using the TeamChip to predict potential drug-induced toxicity through drug metabolism. Reproduced with permission from ref 258. Copyright 2014 Springer Nature Limited. (B) 3D spheroid-hydrogel platform embedding HepG2 cells within gelatin hydrogel matrices for evaluating hepatotoxicity. Reproduced with permission from ref 278. Copyright 2024 Elsevier B.V.

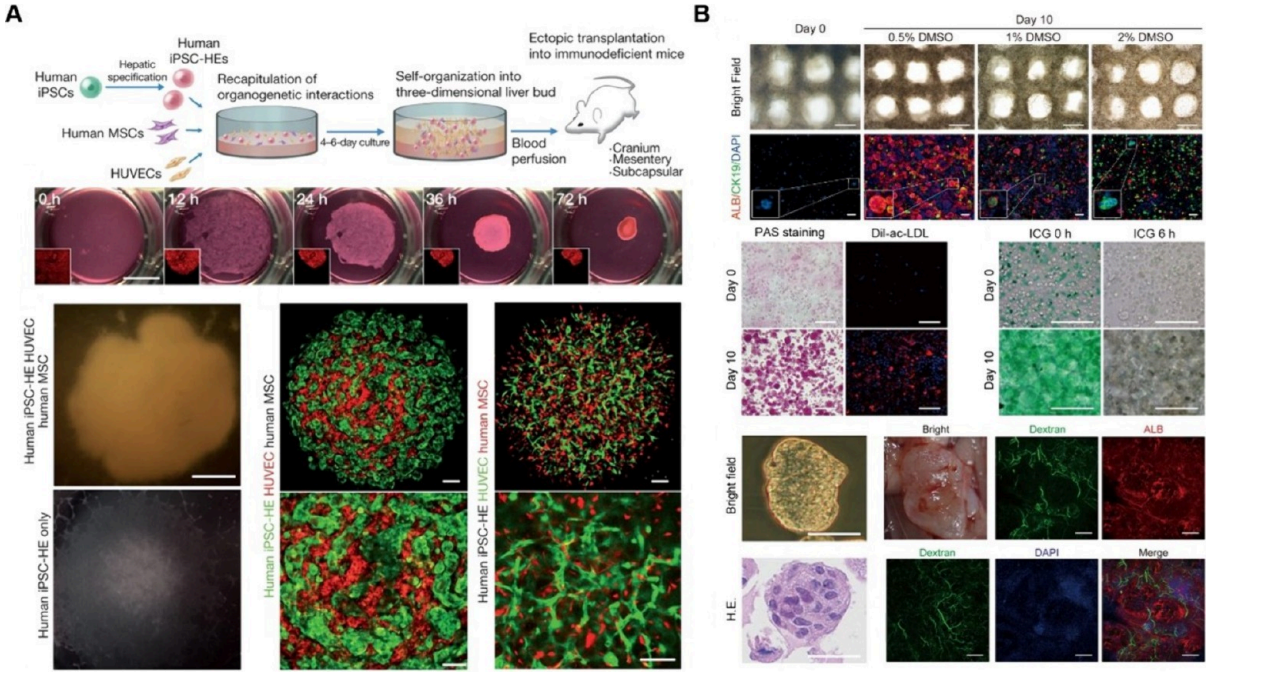

Figure 13. Liver tissue regeneration by organoid transplantation. (A) Creation of vascularized and functional human liver from human induced pluripotent stem cells through the transplantation of in vitro-generated liver buds. Reproduced with permission from ref 282. Copyright 2013 Springer Nature Limited. (B) 3D bioprinted hepatorganoids made from HepaRG cells and bioink showed increased liver-specific protein synthesis after mouse transplantation. Reproduced with permission from ref 285. Copyright 2021 The Author(s).

high-throughput screening of drug-induced liver toxicity (Figure 12A). These microarrays are created using recombinant adenoviruses to transfect THLE-2 liver cells encapsulated in a hydrogel matrix, allowing controlled expression of enzymes such as CYP3A4, CYP2D6, and UGT1A4. The platform's ability to analyze 84 combinations of enzyme expressions on a single chip offers mechanistic insights into drug detoxification processes, facilitating the identification of toxic metabolites. By using small quantities of cells, viruses, and test compounds, TeamChip minimizes resource usage while maximizing throughput and reproducibility. This system's precision in controlling enzyme expression levels and its compatibility with in vivo-like 3D cell cultures position it as a versatile tool for early stage drug discovery and personalized medicine.

Conventional 2D cell culture frequently results in a rapid loss of hepatocyte phenotype, decreased cytochrome P450 (CYP) expression, and loss of characteristic apical-basal polarity, leading to reduced efficiency in drug toxicity evaluation.[275−277] In contrast, 3D hydrogel spheroid models more closely resemble the behavior of hepatocytes in normal tissues compared to 2D cell cultures and have emerged as a viable alternative to overcome the limitations of 2D culture. An innovative 3D spheroid-hydrogel platform encapsulating HepG2 cells in gelatin hydrogel matrices, providing a more physiologically relevant environment, was introduced to enhance hepatotoxicity assessment.[278] These encapsulated

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spheroids maintain liver-specific functionality, exhibit enhanced expression of drug-metabolizing enzymes, and show increased drug sensitivity (Figure 12B). Notably, IC50 values for acetaminophen and other drugs indicate significant differences in drug response between 2D and 3D cultures, with variations ranging from 1.3-fold to over 13-fold. The mechanical properties of the hydrogels, influenced by different concentrations of GelMA, affect hepatocyte behavior and enzyme expression, underscoring the role of extracellular matrix stiffness in modulating liver function.

3D bioprinting offers a significant advantage in generating spheroids for robust liver toxicity testing due to its high flexibility, speed, and cost-effectiveness.[279,280] With the use of a 3D bioprinter, a HepG2 liver spheroid model was developed within gelatin-alginate hydrogels for in situ quantitative evaluation of drug-induced hepatotoxicity.[281] This method allows precise control of cell placement and spheroid size, measuring various hepatotoxic effects, including mitochondrial permeability transition (MPT), cytosolic calcium levels, and apoptosis. Fluorescence intensities of apoptotic and cell death markers were assessed for both in-focus and out-of-focus spheroids, enabling accurate determination of EC50 values, which indicate the concentration of a drug required to induce a 50% maximal response. Notably, HepG2 spheroids exhibited greater resistance to nefazodone-induced MPT compared to 2D cultures, suggesting enhanced protection within the 3D hydrogel environment. The gelatin-alginate hydrogel system supported the retention of spheroid morphology and cell viability, facilitated intercellular interactions, and prevented nutrient transfer limitations. Consistent monitoring of mitochondrial dysfunction, cytosolic calcium increase, and caspase-3/7 activity provided comprehensive insights into drug-induced hepatotoxicity. This platform allowed simultaneous observation of multiple biomarkers and integration of high-content data from numerous spheroids, demonstrating its effectiveness in quantitatively evaluating hepatotoxicity in a controlled and reproducible manner.

5.2. Liver Tissue Regeneration by Organoid Transplantation. The scarcity of donor organs for treating end-stage organ failure has led to the exploration of generating organs in vitro.

Since the discovery of embryonic stem cells in 1981, decades of laboratory research faced challenges in generating a complex vascularized organ from pluripotent stem cells until Taniguchi and his team successfully created the first vascularized and functional human liver.[282] The researchers began by differentiating hepatic endoderm cells from induced pluripotent stem cells and cocultivating them with human umbilical vein endothelial cells (HUVECs) and human mesenchymal stem cells on hydrogel (Figure 13A). This coculture process led to the self-organization of hepatic cells into three-dimensional liver buds (iPSC-LBs) within 48 h. When transplanted into animal models, these liver buds rapidly integrated with the host's vasculature, forming functional blood vessels that promoted the maturation of the liver buds. These buds began performing essential liver functions, including protein production and human-specific drug metabolism. The transplanted liver buds exhibited significant proliferation and maturation, forming structures similar to adult liver tissue. Gene expression analysis revealed upregulated pathways crucial for liver development and function in these iPSC-LBs. Over 60 days, the liver buds continued to mature, achieving a high level of functional similarity to human liver tissue and metabolizing drugs in a human-specific manner, demonstrating their functional competence. Furthermore, mesenteric transplantation of iPSC-LBs improved survival in a mouse model of drug-induced liver failure, highlighting the therapeutic potential of these organoids.

The HepaRG cell, known for its efficient proliferation and differentiation interplay leading to hepatocyte-like cells, offers another promising approach for creating liver organoids.[259,283] Utilizing pullulan-dextran hydrogels, functional liver tissues were developed, facilitating the self-assembly of HepaRG cells into three-dimensional liver microtissues.[284] These microtissues maintained essential liver functions, including detoxification, glycogen storage, and bile canaliculi formation. The engineered liver constructs demonstrated sustained liver polarity and functionality for 21 days. When implanted in mice, the hydrogels containing HepaRG 3D structures successfully engrafted onto liver lobes without inducing liver toxicity. In a model of acetaminophen-induced liver failure, mice treated with these engineered liver constructs exhibited significantly higher survival rates compared to untreated controls.

In recent years, 3D bioprinting has emerged as a promising and innovative technology for creating functional physiological tissues and organs. This includes advancements in printing bones, hearts, kidneys, blood vessels, livers, and various other tissues.[234,235] In a study investigating 3D bioprinted hepatorganoids (3DP-HOs) using HepaRG cells and hydrogel-based bioink, these organoids were cultivated for 7 days to develop essential liver functions such as ALBUMIN secretion, drug metabolism, and glycogen storage.[285] Following differentiation, the organoids were transplanted into the abdominal cavity of Fah-deficient mice, a model for liver injury. Post-transplantation, the 3DP-HOs further matured, exhibiting increased synthesis of liver-specific proteins and human-specific drug metabolism activities (Figure 13B). The hydrogel-based 3DP-HOs developed functional vascular systems, enhancing material transport and overall liver functionality. This transplantation significantly improved the survival of the mice, demonstrating that the 3DP-HOs possessed in vivo hepatic functions and effectively alleviated liver failure.

A decellularized liver scaffold-based engineering approach, which preserves a 3D matrix architecture that closely mimics the native liver and retains a plethora of matrix-associated proteins as well as endogenous growth factors, holds promise for developing functional liver surrogates. A recent study involved perfusing immortalized endothelial cells into decellularized rat liver scaffolds using gelatin hydrogels, significantly improving cell retention and vascular lumen coverage compared to traditional media-based perfusion.[286] The endothelial cells aligned along the scaffold vasculature, proliferating actively and enhancing the blood retention ability of the scaffolds. Doppler ultrasound detected active blood flow in the re-endothelialized liver scaffolds 8 days post-transplantation, demonstrating effective revascularization. The study also showed that gelatin-based cell infusion resulted in more and larger endothelial cell aggregates, potentially facilitating better re-endothelialization. These findings suggest that the re-endothelialized scaffolds better retained perfused blood and displayed a higher vascular lumen coverage ratio, indicating a successful reconstitution of vascular networks within the grafts.

5.3. Hydrogels-Based Bioartificial Liver Systems for Acute Liver Failure. BALS offer an alternative to traditional liver transplantation for treating patients with acute liver

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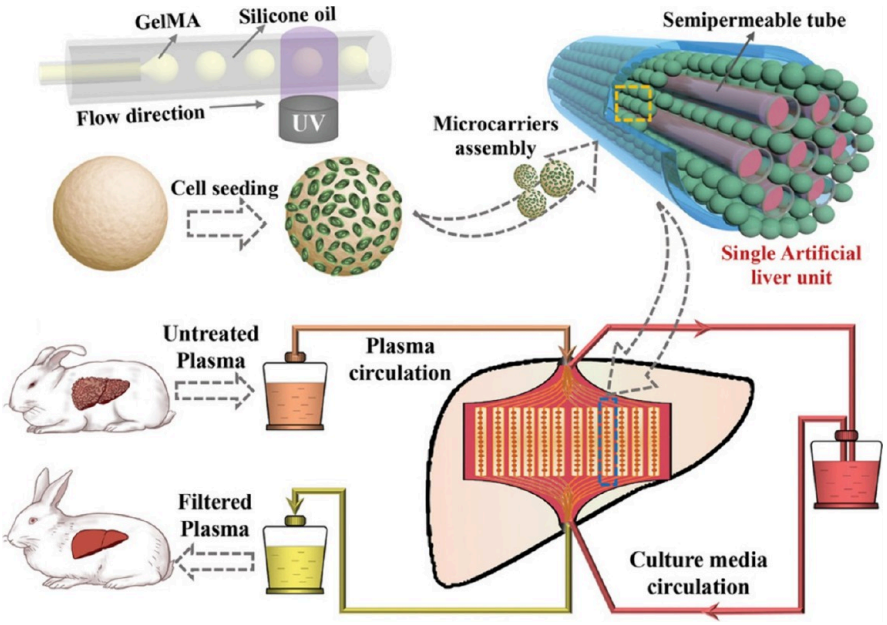

Figure 14. Hydrogel-based BALS for ALF. Illustrated diagram detailing the assembly of the core unit and the operational protocol of the bioinspired artificial liver system intended for treating patients with ALF. Reproduced with permission from ref 292. Copyright 2021 Wiley-VCH.

failure.[287−290] The primary goal of BALS is to create a biomimetic scaffold that supports the culture of hepatocytes and related cells to replicate liver function in vitro.

Scaffolds derived from commonly used bulk or porous materials have significantly advanced the development of bioartificial liver (BAL) studies by effectively increasing cell density. However, the uncontrollability of the porosity and structures of these materials has hindered cell growth and the further regeneration of liver function.[261,291] Therefore, there is a need for novel BALS that can replicate real liver functions, including dynamic substance exchange and cell−cell or cell-environment interactions, as observed in living organs. To address this, a biomimetic bioartificial liver system (BBALS) was developed.[292] This system integrates hiPSC-Heps-laden microparticles with semipermeable microtubes, inspired by the natural microstructure of hepatic lobules, to treat acute liver failure. The microparticles, made from gelatin methacryloyl hydrogel, provide an extracellular matrixlike environment that supports cell proliferation and the formation of functional cell aggregates. These microparticles are produced through single-emulsion droplet microfluidics and combined with ethylene vinyl alcohol copolymer (EVOH) semipermeable microtubes, enabling effective substance exchange and circulation (Figure 14). The BBALS mimics the radial arrangement of hepatocytes around a central vein, typical of hepatic lobules. In vitro studies demonstrated the functionality of a 3D liver chip with multiple parallel BBALS units in filtering the plasma of rabbits with acute liver failure. In vivo tests showed that the integrated BBALS effectively treated acute liver failure in rabbits by supporting liver function, facilitating serum protein generation, and reducing inflammation.

6. SUMMARY

6.1. Conclusion. The future of liver tissue engineering hinges on an interdisciplinary approach that integrates cell biology, biophysical materials science, bioengineering, life sciences, computer artificial intelligence, and clinical hepatology. One approach to this vision is the development and utilization of hydrogels, which play a crucial role in mimicking the hepatocyte microenvironment. By designing composite 2D/3D hydrogel cell culture scaffolds, researchers can closely replicate the biomechanical and physicochemical properties necessary to preserve and enhance the function of liver parenchymal cells. Hydrogels enable precise control over the mechanical properties of biological scaffolds, facilitating the introduction of biomolecular assembly structures and various growth factors. These factors are essential for the efficient expansion and differentiation of 3D cultured stem cells into hepatocytes. Moreover, innovative printable hydrogel designs that replicate liver tissue mechanics, coupled with interface fusion printing technology for heterogeneous materials, are leading to the creation of 3D printed functional liver organoid scaffolds. These advancements are paving the way for diverse applications, including in vitro screening of hepatitis and liver cancer drugs, liver tissue regeneration, and bioartificial liver therapy.

In conclusion, liver tissue engineering, with hydrogels at its core, presents a promising avenue for addressing liver disease challenges and transplantation shortages. Innovations in 3D bioprinting, bioartificial liver systems, and organoid technology are driving the creation of functional liver tissues in vitro. The successful generation of vascularized and functional liver tissues from iPSCs and the development of large-scale bioreactors for acute liver failure treatment in animal models highlight the potential of these technologies for clinical applications. However, further research is required to optimize these systems for human use, ensure long-term functionality, and address regulatory and ethical considerations. As we continue to explore liver tissue engineering possibilities, hydrogels will remain instrumental in revolutionizing liver disease treatment and improving patient outcomes.

6.2. Challenges. While significant progress has been made in the development of hydrogel-based in vitro liver simulation systems, further research is essential to achieve artificial liver transplantation and address the critical shortage of liver sources in clinical settings. Several key issues need to be addressed to

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overcome these challenges: (1) Immune rejection: despite their biocompatibility, hydrogel materials can still elicit an immune response, potentially leading to graft rejection. Therefore, it is crucial to identify strategies to mitigate immune rejection, such as employing more biocompatible scaffolds and cell sources. (2) Integration of growth factors: incorporating growth factors into hydrogels in a controlled manner remains challenging. Ensuring the stability, bioactivity, and sustained release of these molecules, while coordinating the spatial and temporal interactions of various growth factors with different cell types, is crucial for promoting cell behavior and tissue development. (3) Despite significant advancements in hydrogels as smart materials with notable biological and physicochemical properties, there remains a need for a hydrogel that can be temporally and spatially modulated, is robust enough to endure physiological stresses, flexible enough to support cell migration, proliferation, and differentiation, and can adequately mimic liver ECM to provide the appropriate biochemical and physical cues. (4) Designing hydrogels with controlled degradation rates is essential to match the pace of tissue regeneration and remodeling. The degradation products must be nontoxic and cleared by the body without eliciting adverse reactions, which is critical for maintaining the integrity and function of the engineered liver tissue over time.

6.3. Outlook. To address these challenges, various innovative directions in hydrogel development offer promising solutions for liver tissue engineering. Hydrogels, with their highly tunable properties, provide a versatile platform to create environments that closely mimic the natural liver extracellular matrix. They can be engineered to support cell viability, proliferation, and differentiation, making them ideal for constructing functional liver tissue. The following are some specific directions that highlight the potential of hydrogels in overcoming the challenges and advancing liver tissue engineering. (1) Protein hydrogels: engineered recombinant protein hydrogels offer several advantages in liver tissue engineering: they allow for the incorporation of precisely defined chemical components; the chemical and mechanical properties of the hydrogels can be independently altered; they exhibit low polydispersity; and their degradation rate can be programmed by including recognition sites for appropriate MMP-degrading enzymes. By incorporating biological structural units recognized by encapsulated cells, such as peptides mimicking integrin-binding regions and enzymatic cleavage sites within native ECM proteins, synthetic protein hydrogels can be finely tuned to produce three-dimensional matrices with variable density ECM signals that match the signal density in native tissues. (2) Injectable hydrogels: injectable hydrogels represent a promising solution in liver tissue engineering due to their minimally invasive delivery and ability to conform to the defect site. These hydrogels can gel in situ under physiological conditions, providing an optimal environment for cell growth and tissue regeneration. They can be loaded with cells and bioactive molecules, further enhancing their potential to repair and regenerate liver tissue. The versatility and adaptability of injectable hydrogels make them a vital component in the advancement of liver tissue engineering. (3) Antibacterial hydrogels: antibacterial hydrogels are essential in liver tissue engineering to prevent infections during implantation. These hydrogels can release antibacterial agents in a controlled manner or inherently possess antimicrobial properties through the incorporation of antimicrobial peptides or silver nanoparticles. By ensuring sterility and preventing infection, antibacterial hydrogels can significantly enhance the success rates of liver tissue engineering applications, ensuring the safety and longevity of the implanted scaffolds. (4) Growth factor-immobilized hydrogels: growth factors play a critical role in cell proliferation, differentiation, and tissue regeneration and can be supplied exogenously either by injection in solution or by immobilization on scaffolds. The latter method is often more effective in promoting desired cellular outcomes while maintaining bioactivity and stability, thereby extending growth factor signaling and reducing the costs associated with growth factor therapies. Previous studies have shown that growth factors immobilized on scaffolds, such as VEGF on collagen scaffolds, can promote greater endothelial cell proliferation compared to soluble VEGF treatments. Immobilized growth factors are particularly beneficial for in vivo scaffold applications, providing the advantage of confining growth factor effects within the scaffold rather than allowing them to diffuse freely into surrounding tissues. This physiological relevance is significant, as it mimics the natural existence of both bound and free growth factors within the body, enhancing cell response and function. (5) Biomimetic ECM-structured hydrogels: biomimetic ECM-structured hydrogels aim to replicate the complex architecture and functionality of the native extracellular matrix in the liver. These hydrogels can be designed to include multiple ECM components, providing a more accurate microenvironment for liver cells. By mimicking the physical and biochemical cues of the natural ECM, these hydrogels can improve cell adhesion, migration, and function, facilitating the formation of functional liver tissue. This approach ensures that the artificial environment closely resembles the native tissue, promoting better integration and performance of the engineered liver constructs. (6) Organoids: incorporating vascularization within liver organoids using hydrogels is a significant advancement in tissue engineering. Hydrogels can support the formation of blood vessel networks within organoids by providing a scaffold that promotes angiogenesis. By ensuring adequate nutrient supply and oxygen diffusion, these hydrogels help develop high-quality vascular systems within liver organoids, enhancing their viability and functionality. This approach is critical for creating more physiologically relevant liver models and improving the success of organoid transplantation and integration. (7) 3D/4D Printed Hydrogels: 3D and 4D printing technologies offer innovative approaches to fabricating complex hydrogel structures for liver tissue engineering. 3D printing allows for the precise placement of cells and materials to create intricate tissue architectures, while 4D printing introduces the concept of time-dependent changes, enabling dynamic adjustments in response to environmental stimuli. These advanced printing techniques can produce customized and functional liver tissue constructs, opening new possibilities for regenerative medicine and personalized therapies. The ability to create patient-specific constructs tailored to individual needs represents a significant leap forward in the field of liver tissue engineering.

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Notes

The authors declare no competing financial interest.

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