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Exosomes in Hepatocyte Repair and Liver Regeneration: Biological Mechanisms, Autologous Strategies, and Translational Evidence

Dr. Leroy Rebello[1], Dr. Sreesudha Chepyala[2], Sahithi Polineni[3*], Dr. Rani Jangala[4]

Department of Research and Development, Biotex Life Solutions Pvt. Ltd., Secunderabad, Telangana, India

*Corresponding author: **Sahithi Polineni**
E-mail: sahithi.biotexlife@gmail.com

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Abstract

Background: Exosomes and related small extracellular vesicles (EVs) participate in intercellular signalling during liver injury and regeneration. Selected EV preparations can influence hepatocyte survival and proliferation, oxidative and mitochondrial stress, inflammatory responses, and hepatic stellate-cell activation. Autologous blood-derived EV strategies are attractive because collection is patient-specific, but disease state and pre-analytical handling may alter vesicle composition and biological activity.

Objective: To critically synthesise evidence on exosome- and EV-mediated hepatocyte repair and liver regeneration, with particular emphasis on autologous approaches and the translational requirements of the Biotex Autologous Exosome Isolation Kit (manufacturer designation), a developed point-of-care blood-derived EV-enrichment system.

Methods: A structured narrative review was conducted using PubMed/MEDLINE as the principal biomedical index, with cross-checking against publisher and version-of-record pages. Peer-reviewed literature available through August 2026 was considered. Evidence was weighted toward human studies, then large-animal and rodent in vivo studies, primary-cell or organoid experiments, and mechanistic cell studies. Preprints, promotional webpages, unverified testimonials, and unsupported commercial claims were excluded from the evidentiary core. Vesicle terminology was interpreted in accordance with MISEV2023.

Results: Direct mechanistic evidence shows that hepatocyte-derived exosomes can promote hepatocyte proliferation through neutral ceramidase/sphingosine kinase 2/sphingosine-1-phosphate signalling. Hepatic-progenitor- and mesenchymal-stromal-cell-derived EVs have demonstrated regenerative, antioxidant, anti-apoptotic, immunomodulatory, and antifibrotic effects in experimental liver models through cargo including miR-183-5p and GPX1 and through pathways involving Akt/GSK3beta/beta-catenin, TXNIP/NLRP3, SLC7A11/GPX4, TGF-beta/SMAD, Wnt/beta-catenin, and Hedgehog/SMO. Circulating EV profiles also change during human liver regeneration, but human evidence remains predominantly observational. Autologous serum EVs show source- and disease-state-dependent biological activity, and no controlled human study was identified in which intravenously administered autologous blood-derived EVs regenerated damaged liver tissue.

Conclusion: Exosome- and EV-mediated liver repair is supported by substantial mechanistic and preclinical evidence, whereas therapeutic efficacy of autologous blood-derived EV preparations in human liver disease remains unestablished. Translation therefore depends on product-specific demonstration of EV identity, contaminant depletion, reproducible liver-relevant potency, haemocompatibility and safety, biodistribution, pharmacokinetics, and controlled clinical efficacy.

Keywords: exosomes; extracellular vesicles; autologous exosomes; hepatocyte regeneration; liver repair; partial hepatectomy; acute liver injury; hepatic progenitor cells; mesenchymal stromal cells; regenerative medicine.

Abbreviations

ACLF, acute-on-chronic liver failure; **ADSC**, adipose-derived stromal/stem cell; **ALF**, acute liver failure; **ALIX**, ALG-2-interacting protein X; **ApoA1**, apolipoprotein A1; **ApoB**, apolipoprotein B; **ATP**, adenosine triphosphate; **BECN1**, beclin 1; **CCl4**, carbon tetrachloride; **CDK1**, cyclin-dependent kinase 1; **CYTOR**, cytoskeleton regulator RNA; **EdU**, 5-ethynyl-2-deoxyuridine.

ERK, extracellular signal-regulated kinase; **EV**, extracellular vesicle; **GPX1**, glutathione peroxidase 1; **GPX4**, glutathione peroxidase 4; **HepLPC**, hepatocyte-derived liver progenitor-like cell; **HPC**, hepatic progenitor cell; **HSC**, hepatic stellate cell; **hBM-MSC**, human bone-marrow-derived mesenchymal stromal cell; **hUC-MSC**, human umbilical-cord-derived mesenchymal stromal cell; **hUCB-MSC**, human umbilical-cord-blood-derived mesenchymal stromal cell; **I/R**, ischemia-reperfusion; **IL**, interleukin.

LSEC, liver sinusoidal endothelial cell; **MIBlood-EV**, minimal information for blood extracellular vesicle research; **MISEV2023**, Minimal Information for Studies of Extracellular Vesicles 2023; **MSC**, mesenchymal stromal cell; **MVB**, multivesicular body; **NLRP3**, NLR family pyrin domain containing 3; **NTA**, nanoparticle tracking analysis; **PCNA**, proliferating cell nuclear antigen; **PHx**, partial hepatectomy; **PRP**, platelet-rich plasma; **ROS**, reactive oxygen species.

S1P, sphingosine-1-phosphate; **SK2**, sphingosine kinase 2; **SLC7A11**, solute carrier family 7 member 11; **SMO**, smoothened; **TEM**, transmission electron microscopy; **TGF-beta**, transforming growth factor beta; **TRPS**, tunable resistive pulse sensing; **TSG101**, tumor susceptibility gene 101 protein; **TXNIP**, thioredoxin-interacting protein; **VEGF**, vascular endothelial growth factor; **xCT**, cystine/glutamate antiporter light chain (SLC7A11).

1. Introduction

The liver is unusual among adult mammalian organs because it can restore functional mass after substantial tissue loss without requiring de novo organogenesis. Following partial hepatectomy or recovery from a reversible toxic insult, mature hepatocytes re-enter the cell cycle, non-parenchymal cells coordinate inflammatory and vascular responses, and extracellular-matrix remodelling allows reconstruction of tissue architecture. This regenerative response is clinically important after liver resection and transplantation and is equally relevant to acute toxic injury, ischemia-reperfusion injury, and chronic liver disease. Regeneration nevertheless has biological limits. Severe hepatocyte necrosis, sustained oxidative stress, mitochondrial dysfunction, dysregulated innate immunity, ferroptotic cell death, impaired sinusoidal perfusion, and persistent hepatic stellate-cell activation can convert an otherwise reparative response into progressive dysfunction or fibrosis [1,2].

Classical models of liver regeneration emphasise cytokines, growth factors, metabolic signals, portal hemodynamics, extracellular-matrix remodelling, and transcriptional control. Hepatocyte growth factor, epidermal growth-factor-family ligands, tumour necrosis factor, interleukin-6, Wnt/beta-catenin, Hippo-YAP/TAZ, PI3K-AKT, MAPK, and other pathways cooperate in temporally ordered phases of priming, cell-cycle progression, tissue expansion, angiogenic adaptation, and termination [1,2]. Increasing evidence now indicates that membrane-bound extracellular particles constitute an additional layer of intercellular communication within this network. These particles can transport proteins, lipids, metabolites, messenger RNAs, microRNAs, and other regulatory molecules between hepatocytes, macrophages, liver sinusoidal endothelial cells, hepatic stellate cells, cholangiocytes, and progenitor populations.

Among these particles, exosomes have attracted particular attention because they can protect and deliver biologically active cargo to recipient cells. A landmark study by Nojima and colleagues demonstrated that exosomes released by primary hepatocytes directly stimulated hepatocyte proliferation in vitro and promoted regeneration after ischemia-reperfusion injury and partial hepatectomy in mice. The proliferative effect depended on transfer of neutral ceramidase and sphingosine kinase 2 to recipient hepatocytes, resulting in increased intracellular sphingosine-1-phosphate; removal of the relevant sphingosine-kinase activity abrogated the effect [3]. This study provided unusually direct mechanistic evidence that hepatocytes can use exosome-mediated paracrine communication as part of a regenerative program rather than merely releasing vesicles as cellular waste.

Independent lines of evidence have extended this concept. Mesenchymal stromal-cell-derived exosomes increased hepatocyte proliferation and expression of regenerative proteins in carbon-tetrachloride injury and improved hepatocyte viability in acetaminophen and hydrogen-peroxide injury models [4]. Human umbilical-cord-derived mesenchymal stromal-cell exosomes were subsequently shown to deliver glutathione peroxidase 1 and reduce oxidative injury and apoptosis in experimental liver failure [5]. Exosomal microRNA-124 from human umbilical-cord-blood mesenchymal stromal cells promoted regeneration after partial hepatectomy in rats by suppressing Foxg1 [6]. More recently, human hepatocyte-derived liver progenitor-like-cell extracellular vesicles accelerated primary human hepatocyte cell-cycle progression and promoted regeneration in carbon-tetrachloride- and acetaminophen-induced acute liver failure, with miR-183-5p acting through suppression of FoxO1 and activation of Akt/GSK3beta/beta-catenin signalling [7].

The concept that extracellular vesicle signalling is intrinsic to normal liver regeneration has also gained support from tissue-level studies. Ying and colleagues reported that hepatocytes were the predominant source of liver tissue extracellular vesicles during regeneration after partial hepatectomy. Targeted inhibition of hepatocyte vesicle release through Rab27a interference impaired regeneration, whereas supplementation with hepatocyte-derived vesicles from regenerating liver improved insufficient regeneration; proteomic and functional analyses implicated cell-cycle regulation, including CDK1-associated activity [8]. These observations suggest that the regenerative vesicle response is not simply a pharmacological phenomenon produced by cultured stem cells, but may form part of the endogenous communication system used by the regenerating liver.

Human evidence remains more limited and is largely observational. In patients with chronic hepatitis B and acute-on-chronic liver failure, Jiao and colleagues reported that circulating vesicles with hepatocyte-associated albumin, CD63, and VEGF profiles differed according to disease state and survival, supporting their potential as markers of endogenous regenerative activity [9]. In 2026, Sun and colleagues analysed plasma small extracellular vesicles from 12 liver-transplant recipients at regenerating and non-regenerating phases and identified 25 differentially expressed microRNAs whose predicted targets were enriched in cell-cycle and Hippo-related regenerative pathways [10]. These studies demonstrate that circulating vesicle composition changes during human liver regeneration, but they do not establish that infusion of such vesicles is therapeutically effective.

The possibility of using a patient's own vesicles introduces the concept of autologous exosome therapy. Candidate sources include plasma or serum, platelet-rich preparations, adipose- or bone-marrow-derived mesenchymal stromal cells, and autologous hepatocyte or hepatic-progenitor-lineage cultures. Autologous origin could reduce certain donor-matching concerns and may allow repeated patient-specific production. However, the biological assumption that “self-derived” equals “regenerative” is unsafe. Vesicles released during steatohepatitis, alcohol-associated liver injury, fibrosis, viral infection, or hepatocellular carcinoma may carry inflammatory, profibrotic, immune-modulatory, or oncogenic cargo. Therefore, the scientifically meaningful unit is not simply the autologous vesicle but the autologous vesicle preparation with defined cellular origin, composition, purity, potency, and safety.

This review therefore focuses specifically on exosome-mediated hepatocyte repair and liver regeneration rather than on extracellular vesicles in liver disease broadly. It distinguishes direct hepatocyte proliferation from general hepatoprotection; separates endogenous regenerative signalling from experimentally administered products; evaluates hepatocyte-, progenitor-, mesenchymal-stromal-cell-, blood-, and platelet-derived preparations; and critically examines the translational rationale for autologous

strategies. Particular emphasis is placed on mechanistic evidence, source-dependent biological activity, disease-state-dependent risk, vesicle characterisation, potency testing, intravenous biodistribution, and the present absence of definitive clinical evidence for autologous exosome therapy in human liver regeneration.

2. Literature Search and Evidence Appraisal

This article was designed as a structured narrative review rather than a systematic review or meta-analysis. The aim was to identify and critically appraise peer-reviewed academic literature directly relevant to exosome biology, hepatocyte survival, hepatocyte proliferation, liver regeneration, acute liver injury, partial hepatectomy, hepatic fibrosis, and autologous or patient-derived exosome strategies. Particular priority was given to original mechanistic research rather than secondary narrative summaries.

Literature searches were conducted using PubMed/MEDLINE as the principal biomedical index and were cross-checked, where accessible, against publisher or version-of-record pages from Elsevier/ScienceDirect, Springer Nature, Wiley, Taylor & Francis, BioMed Central, and other peer-reviewed journal platforms. Search concepts combined terms including “exosome”, “small extracellular vesicle”, “hepatocyte-derived exosome”, “liver regeneration”, “hepatocyte proliferation”, “partial hepatectomy”, “acute liver failure”, “ischemia reperfusion”, “hepatic progenitor exosome”, “mesenchymal stromal cell exosome”, “serum exosome”, “plasma exosome”, “platelet exosome”, “autologous exosome”, “fibrosis”, “oxidative stress”, “ferroptosis”, “microRNA”, and “biodistribution”. Literature available through August 2026 was considered.

Eligible evidence was restricted to peer-reviewed academic publications, with priority assigned in the following order: human interventional studies, human longitudinal or mechanistic studies, large-animal studies, rodent in vivo studies, primary-cell or organoid experiments, and established cell-line studies. Preprints were excluded from the evidentiary core unless explicitly discussed as emerging and non-peer-reviewed. Commercial webpages, promotional reports, non-academic product claims, and unverified clinical testimonials were excluded. Review articles were used for contextual background or reference discovery but not as substitutes for primary experimental evidence when an original study was available.

Each high-priority paper was assessed for four questions: (1) whether the isolated material was legitimately described as exosomes or more conservatively as extracellular vesicles/small extracellular vesicles; (2) whether the biological effect represented direct hepatocyte proliferation, cytoprotection, anti-inflammatory activity, antifibrotic activity, or only an association; (3) whether causality was tested by cargo depletion, pathway inhibition, dose-response analysis, loss-of-function manipulation, or equivalent controls; and (4) whether the model supported translation to autologous human liver therapy. Studies were not considered direct evidence of regeneration solely because they reduced aminotransferases or histological injury. Greater evidentiary weight was assigned to studies demonstrating cell-cycle entry, Ki67/PCNA/BrdU/EdU responses, liver-mass recovery, survival after major tissue loss, or pathway-dependent hepatocyte proliferation.

Vesicle nomenclature was interpreted in accordance with MISEV2023. Because commonly used isolation methods do not establish endosomal biogenesis, this review uses “extracellular vesicle” or “small extracellular vesicle” when the source paper does not adequately demonstrate an exosomal origin, while retaining “exosome” when describing studies that used and substantially supported that designation. MISEV2023 further emphasises rigorous separation from non-vesicular particles, orthogonal characterisation, transparent reporting, and functional controls, all of which are particularly important for blood-derived and proposed autologous preparations [11].

3. Exosomes: Nomenclature, Biogenesis, Cargo, and Functional Delivery

3.1 Exosomes within the extracellular-vesicle spectrum

Extracellular vesicles are lipid-bilayer-delimited particles released from cells that cannot replicate independently. They encompass multiple populations that overlap in size and composition. Exosomes are conventionally defined by biogenesis: they originate as intraluminal vesicles within multivesicular endosomes and are released when those endosomal compartments fuse with the plasma membrane. By contrast, other vesicles bud directly from the plasma membrane. In practice, particle diameter, morphology,

or expression of CD9, CD63, or CD81 alone cannot establish an endosomal origin. This is why MISEV2023 recommends descriptive terminology based on demonstrable physical or biochemical characteristics when biogenesis has not been proven [11].

In this context, the distinction between exosomes and broader EV populations is more than semantic. An autologous serum or plasma isolate may contain bona fide endosome-derived exosomes, plasma-membrane-derived vesicles, lipoproteins, protein aggregates, and other extracellular particles. Attribution of a therapeutic effect specifically to exosomes requires evidence that the biologically active fraction is vesicular and that the effect is not produced by co-isolated proteins, lipids, cytokines, or lipoproteins.

3.2 Biogenesis and release

Exosome formation begins with inward budding of the limiting membrane of endosomes to generate multivesicular bodies containing intraluminal vesicles. Cargo selection can involve ESCRT-associated machinery, tetraspanins, membrane lipids, RNA-binding proteins, and other sorting systems. Release depends on trafficking of multivesicular bodies to the plasma membrane and regulated fusion. Rab-family GTPases are important components of this process. Ostrowski and colleagues demonstrated experimentally that Rab27a and Rab27b control different steps of the exosome-secretion pathway, providing a molecular basis for later studies that manipulate vesicle release to test causality [12].

This principle has become particularly relevant in liver-regeneration research. Ying and colleagues used hepatocyte-targeted Rab27a interference to suppress hepatocyte-derived tissue-vesicle release after partial hepatectomy and observed impaired regeneration [8]. Although the preparation was reported as tissue extracellular vesicles rather than exclusively endosome-proven exosomes, the loss-of-function approach substantially strengthens the interpretation that vesicle release itself participates in the regenerative response.

3.3 Cargo selection and biological information transfer

Exosomes can carry membrane proteins, enzymes, signalling lipids, metabolites, messenger RNAs, microRNAs, long noncoding RNAs, and other molecular species. One of the seminal demonstrations of functional RNA transfer was provided by Valadi and colleagues, who showed that exosomes contained both mRNA and microRNA and that transferred mRNA could be translated in recipient cells [13]. Subsequent liver studies have extended the same general biological principle to regenerative cargo. In the Nojima model, the relevant transferred machinery was enzymatic rather than RNA-based: hepatocyte exosomes delivered neutral ceramidase and sphingosine kinase 2, allowing recipient hepatocytes to generate sphingosine-1-phosphate and enter a proliferative state [3].

Other liver-regeneration models emphasise regulatory microRNAs. Human umbilical-cord-blood MSC exosomal miR-124 promoted regeneration after partial hepatectomy through Foxg1 suppression [6], whereas human liver progenitor-like-cell vesicles were enriched in miR-183-5p, which suppressed FoxO1 and activated Akt/GSK3beta/beta-catenin signalling in primary human hepatocytes [7]. These studies illustrate a key feature of exosome biology: the regenerative phenotype is source- and state-dependent because the cargo reflects the biology of the producing cell.

3.4 Interaction with recipient liver cells

Extracellular vesicles can influence recipient cells through surface-receptor interactions, endocytosis, macropinocytosis, phagocytosis, or membrane fusion. The biologically important event is not merely particle uptake but functional delivery or receptor engagement that alters the phenotype of the target cell. In the liver, candidate recipient populations include hepatocytes, Kupffer cells and recruited macrophages, hepatic stellate cells, liver sinusoidal endothelial cells, cholangiocytes, and immune-cell subsets. A regenerative vesicle can therefore act directly by stimulating hepatocyte proliferation or indirectly by reducing inflammatory injury, modifying macrophage phenotype, restoring endothelial support, or suppressing stellate-cell activation.

Accordingly, liver accumulation and damaged-cell targeting are distinct concepts. Intravenous vesicles can accumulate in the liver because of sinusoidal anatomy and uptake by the mononuclear

phagocyte system, yet this does not prove selective delivery to injured hepatocytes. Product- and disease-specific experiments are needed to define cellular distribution, uptake mechanisms, and the functionally relevant recipient cells. The principal steps in exosome biogenesis, cargo incorporation, release, and recipient-cell interaction are summarised in Figure 1.

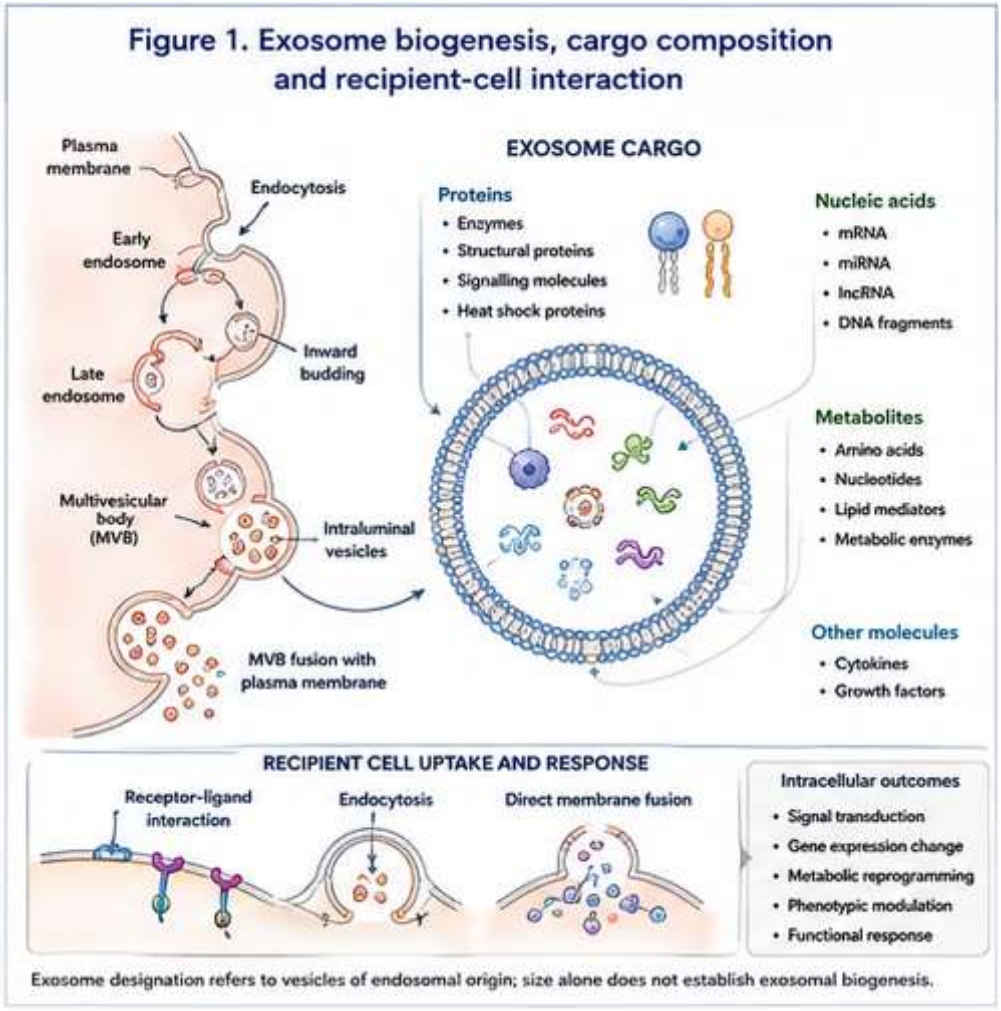

Figure 1. Exosome biogenesis, molecular cargo, and recipient-cell interaction. Exosomes arise through the endosomal pathway and may transfer proteins, lipids, nucleic acids, metabolites, and signalling molecules to recipient cells. Endosomal origin rather than size alone defines an exosome.

3.5 Methodological implications for autologous exosome research

Autologous approaches intensify the need for careful characterisation because body-fluid preparations are among the most compositionally complex extracellular-particle samples. Plasma contains abundant lipoproteins and coagulation-related components, whereas serum preparation induces platelet activation and release of platelet-derived vesicles. Consequently, the choice of serum versus plasma is not a trivial preanalytical variable; it changes the particle population being studied.

Relevant characterisation domains for an autologous product intended for liver-regeneration research include source, collection procedure, cellular origin where possible, isolation and purification method, particle concentration, size distribution, morphology, positive EV-associated markers, negative or contaminant markers, sterility, endotoxin, storage conditions, and a liver-relevant functional potency assay. Particle number alone cannot establish regenerative quality. These principles form the analytical basis for the later sections of this review on autologous serum/plasma vesicles, platelet-derived exosomes, autologous MSC exosomes, and hepatocyte-lineage exosome strategies.

3.6 Translational relevance of patient-specific point-of-care EV processing

Point-of-care processing is relevant to autologous extracellular-vesicle research because blood-derived preparations are highly sensitive to collection, handling, centrifugation, residual platelet content, and downstream separation conditions [11,16,17,21,22]. A standardised patient-specific platform may therefore improve procedural consistency, but process standardisation alone does not establish exosome identity, purity, liver-regenerative potency, or clinical efficacy.

4. Extracellular-Vesicle-Mediated Intercellular Communication During Liver Regeneration

4.1 Biological phases of liver regeneration

Liver regeneration is a coordinated restoration of functional mass rather than simple proliferation of one cell type. After partial hepatectomy, abrupt changes in portal flow, shear stress, metabolic demand, matrix organisation, cytokine signalling, and growth-factor availability initiate a priming phase in which quiescent hepatocytes become competent to enter the cell cycle. Hepatocyte DNA synthesis and mitosis are followed by proliferation and remodelling of non-parenchymal populations, including sinusoidal endothelial cells, stellate cells, macrophages, and biliary cells. Growth-control pathways subsequently terminate proliferation as appropriate liver mass is restored [1,2].

Kupffer cells and recruited macrophages influence this process by producing cytokines and growth-regulating signals; depletion studies have demonstrated delayed hepatocyte proliferation and impaired regeneration when Kupffer-cell function is reduced [14]. Hepatocytes themselves contribute to vascular adaptation by producing factors such as VEGF, which can stimulate sinusoidal endothelial-cell proliferation during regeneration [15]. These bidirectional interactions make the regenerating liver an ideal biological system in which short-range and blood-borne vesicle communication could have functional importance.

4.2 Endogenous hepatocyte exosomes as direct regenerative mediators

The strongest direct evidence for an exosome-dependent hepatocyte-to-hepatocyte regenerative pathway remains the study by Nojima and colleagues. Exosomes isolated from primary murine hepatocytes induced dose-dependent proliferation of primary hepatocytes, whereas exosomes from Kupffer cells or sinusoidal endothelial cells did not reproduce the same proliferative effect. The hepatocyte exosomes fused with target hepatocytes and transferred neutral ceramidase and sphingosine kinase 2, increasing intracellular sphingosine-1-phosphate. Genetic loss or functional removal of the relevant sphingosine-kinase activity prevented the proliferative response [3].

The study also showed in vivo relevance. Hepatocyte exosome administration increased hepatocyte proliferation after ischemia-reperfusion injury and supported regeneration after partial hepatectomy. Circulating exosomes with proliferative activity increased after injury, consistent with the possibility that the damaged or regenerating liver releases an endogenous pro-regenerative signal. This mechanism is especially important for the present review because it demonstrates direct functional transfer between hepatocytes rather than a nonspecific anti-inflammatory effect.

4.3 Regenerating-liver vesicles and cell-cycle control

The endogenous model was strengthened by the 2024 work of Ying and colleagues. Using transcriptomic, single-cell, ultrastructural, proteomic, and functional approaches, the investigators reported that hepatocytes were the major source of tissue extracellular vesicles during regeneration after partial hepatectomy. Hepatocyte-derived vesicles from the regenerating liver carried enhanced proliferative information, and targeted inhibition of hepatocyte vesicle release impaired liver regeneration. Mechanistic analysis implicated acceleration of cell-cycle progression through CDK1-associated activity [8].

Importantly, supplementation with hepatocyte-derived vesicles from regenerating liver improved insufficient regeneration in the experimental system. These findings do not yet establish a clinically usable hepatocyte-exosome product, and the study appropriately used the broader EV terminology. Nevertheless, they provide strong evidence that vesicle-mediated signalling is not only associated with regeneration but functionally contributes to it.

4.4 Human evidence for regeneration-associated circulating vesicles

Human evidence currently supports the existence of regeneration-associated circulating vesicle signatures rather than therapeutic efficacy. Jiao and colleagues analysed plasma exosomes in chronic hepatitis B and acute-on-chronic liver failure. Vesicle profiles carrying albumin, CD63, and VEGF differed between disease groups, and ALB+/CD63+/VEGF+ profiles were higher among surviving patients with acute-on-chronic liver failure than among those who died. The authors proposed these hepatocyte-associated vesicle profiles as potential non-invasive indicators of liver regeneration and prognosis [9]. Because the study was observational, it cannot establish that the circulating vesicles caused recovery.

Sun and colleagues provided a complementary human dataset in 2026. Plasma small extracellular vesicles were collected from 12 liver-transplant recipients at time points corresponding to regenerating and non-regenerating phases. Twenty-five microRNAs differed between phases, and bioinformatic analyses linked their predicted targets to cell-cycle and Hippo signalling. The study supports dynamic changes in circulating vesicle cargo during human liver regeneration but did not test reinfusion or regenerative potency of the isolated vesicles [10].

Together, these human studies provide an important bridge between experimental exosome biology and clinical liver regeneration. They establish that circulating vesicle composition changes in association with regeneration, but they also define the current evidence boundary. The next translational question is not simply whether patients possess circulating exosomes, but whether a specific autologous vesicle fraction can be isolated reproducibly, shown to possess hepatocyte-regenerative potency ex vivo, demonstrated to be free of dominant pathological cargo, and administered safely with measurable biological benefit. Those questions are addressed in the subsequent sections of this review. The major EV-mediated intercellular interactions involved in hepatic injury and regeneration are illustrated in Figure 2.

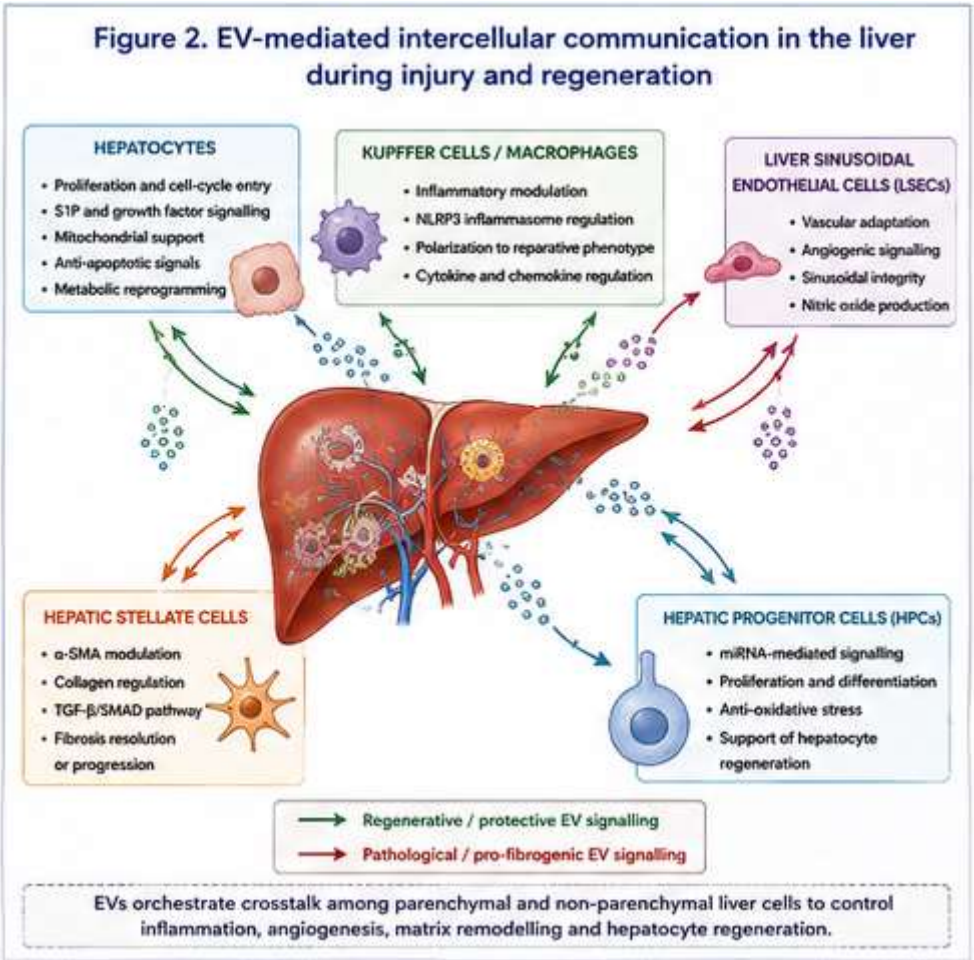

Figure 2. Extracellular-vesicle-mediated intercellular communication during liver injury and regeneration. Hepatocytes, macrophages, hepatic stellate cells, liver sinusoidal endothelial cells, and hepatic progenitor populations exchange EV-associated signals that may support repair or, depending on source and disease

state, propagate inflammatory and profibrogenic activity. Green and red arrows highlight representative protective and pathological signalling, respectively; other arrow colours identify cell-specific communication routes and do not assign a fixed biological valence.

Representative mechanistic and human observational studies linking EV signalling with hepatocyte repair and liver regeneration are summarised in Table 1.

Table 1: Representative evidence linking extracellular vesicles with hepatocyte repair and liver regeneration

Study	Model	Vesicle/source	Key finding used here	Evidence interpretation
Nojima et al [3].	Primary hepatocytes; I/R; PHx	Primary hepatocyte exosomes	Neutral ceramidase/SK2 -> S1P -> hepatocyte proliferation	Direct mechanistic regeneration
Tan et al [4].	CCl4 mouse; APAP/H2O2 in vitro	MSC exosomes	PCNA/cyclin D1 and hepatocyte survival increased	Regenerative + cytoprotective
Song et al [6].	Rat PHx	hUCB-MSC exosomes	miR-124 suppresses Foxg1; regeneration enhanced	Direct post-hepatectomy evidence
Chen et al [7].	Human primary hepatocytes; mouse ALF	HepLPC-EVs	miR-183-5p/FoxO1/Akt/GSK3beta/beta-catenin	Strong mechanistic regeneration
Ying et al [8].	Mouse PHx	Hepatocyte tissue EVs	Rab27a inhibition impairs regeneration; CDK1-linked effect	Endogenous vesicle requirement
Jiao et al [9].	Human ACLF/CHB	Plasma hepatocyte-associated exosomes	ALB/CD63/VEGF profiles associated with regeneration/prognosis	Human biomarker, not therapy
Sun et al [10].	12 human transplant recipients + mice	Circulating small EVs	Regeneration-phase miRNA signatures linked to cell cycle/Hippo	Human temporal association

5. Hepatocyte-Derived Exosomes and Direct Hepatocyte Regeneration

5.1 Evidence for hepatocyte-to-hepatocyte regenerative signalling

Among the available experimental studies, hepatocyte-derived exosomes provide the most direct biological argument that an exosome population can participate in liver regeneration rather than merely reduce tissue injury. Nojima and colleagues isolated exosomes from primary murine hepatocytes and compared their proliferative activity with exosomes released by other liver-cell populations. Hepatocyte exosomes produced a dose-dependent increase in primary-hepatocyte proliferation, whereas exosomes from Kupffer cells or sinusoidal endothelial cells did not reproduce the same response [3]. This lineage-dependent difference is important for autologous therapeutic concepts because it suggests that the regenerative phenotype of a vesicle preparation depends on its cellular origin and molecular cargo rather than on particle number alone.

The mechanistic component of the Nojima study was unusually strong. Hepatocyte exosomes directly interacted with recipient hepatocytes and transferred neutral ceramidase together with sphingosine kinase 2 (SK2), increasing intracellular generation of sphingosine-1-phosphate (S1P). Loss or inhibition of the relevant sphingosine-kinase activity markedly reduced the proliferative response [3]. S1P is a bioactive sphingolipid involved in survival, proliferation, migration, vascular biology, and tissue repair; in this

model, exosomal transfer therefore supplied an enzymatic module capable of changing the recipient hepatocyte's own lipid-signalling environment. This differs conceptually from a simple growth-factor exposure because the vesicle transfers machinery that enables signal generation within the target cell.

5.2 In vivo regeneration after injury and hepatectomy

The same study demonstrated in vivo relevance in two distinct contexts. Administration of hepatocyte exosomes increased hepatocyte proliferation following hepatic ischemia-reperfusion injury and supported regeneration after partial hepatectomy [3]. The partial-hepatectomy model is especially informative because recovery requires controlled expansion of the surviving hepatocyte population rather than only suppression of an acute toxin. These observations support the concept that hepatocyte-derived exosomes may participate in restoration of functional liver mass.

The 2024 work of Ying and colleagues strengthened the endogenous-regeneration model using tissue-derived extracellular vesicles from regenerating liver [8]. Hepatocytes represented the major vesicle source in the regenerating tissue; suppression of hepatocyte vesicle release impaired regeneration, and vesicles obtained from regenerating hepatocytes promoted cell-cycle progression and improved insufficient regeneration experimentally. Because this work used the broader EV terminology rather than proving endosomal origin for every particle, it does not establish that every observed effect was mediated exclusively by classical exosomes. Nevertheless, when interpreted together with Nojima, it supports a biologically coherent model in which vesicle release is an active component of the regenerative hepatocyte program.

5.3 Implications for autologous therapy

For an autologous strategy, hepatocyte-lineage exosomes are conceptually attractive because the source is biologically matched to the target tissue. However, obtaining and expanding patient hepatocytes is considerably more complex than processing peripheral blood. The current evidence therefore supports hepatocyte-derived exosomes primarily as a mechanistic benchmark: a candidate blood-derived autologous preparation is most appropriately benchmarked against measurable hepatocyte outcomes such as EdU incorporation, Ki67 or PCNA expression, cell-cycle entry, survival after standardised injury, and restoration of synthetic function. A patient-specific blood product cannot be assumed to reproduce hepatocyte-exosome biology unless such activity is demonstrated directly.

6. Hepatic-Progenitor-Derived Exosomes

6.1 Chemically induced human hepatic progenitors

Human hepatic progenitor platforms provide a second liver-lineage source with greater expansion potential than mature primary hepatocytes. Hyung and colleagues studied exosomes released by chemically induced human hepatic progenitor cells and found that suppression of exosome secretion increased reactive oxygen species and reduced cell viability, whereas progenitor-derived exosomes supported antioxidant responses and protected cells exposed to hypoxic or oxidative stress [25]. The study therefore links liver-lineage exosomes with cytoprotection as well as regeneration. Importantly, protection of stressed but viable hepatocytes is biologically complementary to proliferation: preservation of the surviving hepatocyte pool increases the substrate from which regeneration can proceed.

A 2024 preclinical comparison by Kim and colleagues evaluated exosomes derived from human chemically derived hepatic progenitors against bone-marrow-MSC exosomes [26]. In vitro, the hepatic-progenitor exosomes reduced TGF-beta1-induced stellate-cell activation markers; in a carbon-tetrachloride liver-injury model, they alleviated histological and biochemical injury, with stronger effects than the BM-MSC exosome preparation in that experimental system. This does not establish universal superiority of hepatic-progenitor exosomes, because manufacturing methods, dose, culture conditions, and model selection can change relative potency. It does, however, justify direct source-comparison studies rather than treating all exosome products as therapeutically interchangeable.

6.2 Hepatocyte-derived liver progenitor-like cells and miR-183-5p

Chen and colleagues provided a more defined regenerative mechanism using extracellular vesicles from hepatocyte-derived liver progenitor-like cells (HepLPCs) [7]. HepLPC-derived vesicles promoted cell-cycle progression and proliferation in primary human hepatocytes and accelerated regeneration in carbon-tetrachloride- and acetaminophen-associated acute-liver-failure models. MicroRNA sequencing identified enrichment of miR-183-5p; functional experiments linked this cargo to suppression of FoxO1 and activation of the Akt/GSK3beta/beta-catenin pathway. The mechanistic sequence can therefore be summarised as HepLPC vesicle -> miR-183-5p transfer -> FoxO1 downregulation -> Akt/GSK3beta/beta-catenin activation -> hepatocyte cell-cycle progression [7].

6.3 Relevance to future autologous liver-lineage platforms

These hepatic-progenitor studies suggest a future autologous model in which patient-derived cells are converted or expanded into a liver-lineage progenitor population, used to manufacture a standardised vesicle product, and then tested for patient-matched hepatoregenerative potency. Such an approach is substantially more complex than point-of-care blood purification, but it offers better control over the producing cell, culture environment, cargo profile, and release testing. In the present review, this strategy serves as a high-specificity comparator for the Biotex Autologous Exosome Isolation Kit: the Biotex kit has logistical advantages, while the liver-lineage approach provides stronger control over biological source identity.

7. Mesenchymal Stromal-Cell-Derived Exosomes in Liver Repair

7.1 Regeneration in drug-induced liver injury

Mesenchymal stromal-cell-derived exosomes form the largest preclinical therapeutic literature in liver injury, although they are generally allogeneic or experimentally produced rather than autologous blood-derived preparations. Tan and colleagues showed that MSC-derived exosomes protected hepatocytes against acetaminophen and hydrogen peroxide injury in vitro and improved recovery in a carbon-tetrachloride liver-injury model [4]. Treated livers showed increased proliferating-cell nuclear antigen and cyclin D1, supporting a regenerative effect rather than biochemical hepatoprotection alone. The study helped establish the concept that a cell-free MSC secretome component could reproduce part of the regenerative activity attributed to MSC conditioned medium.

7.2 Antioxidant cargo and GPX1-dependent rescue

Yan and colleagues subsequently identified glutathione peroxidase 1 (GPX1) as a functionally important component of human umbilical-cord-MSC exosomes [5]. The exosome preparation reduced oxidative stress and hepatic injury, while depletion of GPX1 from the producing cells weakened the protective effect. This provides a useful example of a potency-defining cargo: instead of judging a preparation only by particle abundance or canonical surface markers, functional activity can be linked to a defined molecular mechanism. The same principle applies to autologous-kit research: candidate release tests are most informative when they demonstrate a liver-relevant effect such as ROS suppression, hepatocyte survival, or proliferation and, where possible, associate that effect with a measurable cargo signature.

7.3 MSC exosomes versus autologous circulating exosomes

MSC exosomes and autologous circulating exosomes are biologically distinct products. MSC exosomes are generated from a selected and controlled cell population, whereas a blood-derived autologous fraction contains vesicles originating from platelets, erythrocytes, leukocytes, endothelium, hepatocytes, and other tissues. Thermal conditioning before separation may further change the relative contribution of these cellular sources. This distinction is central to interpretation of the Biotex kit: the kit offers a practical route to recover a same-patient vesicle-enriched fraction, but its liver-regenerative phenotype remains to be established empirically and cannot be inferred from MSC-exosome studies. The MSC literature provides mechanistic hypotheses and candidate potency endpoints, not proof that a blood-derived autologous preparation has the same composition or effect. The principal regenerative EV sources, their proposed mechanisms, and their relevance to autologous strategies are compared in Table 2.

Table 2: Principal extracellular-vesicle sources investigated for liver repair and regeneration

Source	Representative evidence	Principal regenerative mechanism	Relevance to autologous strategy
Primary hepatocyte exosomes	Nojima et al [3].	Neutral ceramidase/SK2 transfer -> S1P -> hepatocyte proliferation	Strong mechanistic benchmark; difficult direct autologous sourcing
Regenerating hepatocyte EVs	Ying et al [8].	Endogenous vesicle release supports cell-cycle progression	Supports natural vesicle-dependent regeneration; broader EV terminology
Human hepatic-progenitor exosomes	Hyung et al [25].; Kim et al [26].	Oxidative-stress protection; reduced HSC activation	Liver-lineage manufacturing platform with source control
HepLPC-derived EVs	Chen et al [7].	miR-183-5p -> FoxO1 down -> Akt/GSK3beta/beta-catenin up	Strong human-hepatocyte mechanistic evidence; preclinical therapy
MSC-derived exosomes	Tan et al [4].; Yan et al [5].	Regeneration, antioxidant activity, GPX1-dependent protection	Mechanistic comparator; not equivalent to circulating autologous blood EVs
Biotex kit-derived autologous blood EV-enriched fraction	Manufacturer-described protocol; validation pending	Mixed-source autologous vesicle population; potency to be established	High logistical feasibility; requires identity, contaminant and liver-potency testing

8. Oxidative Stress, Mitochondrial Dysfunction, and Exosome-Mediated Cellular Rescue

Oxidative stress is a major determinant of whether an injured hepatocyte remains viable, enters a reversible stress state, or progresses to regulated or necrotic cell death. Reactive oxygen species (ROS) generated during toxic injury, ischaemia-reperfusion, metabolic overload, and inflammatory activation can oxidise membrane lipids, damage proteins and nucleic acids, impair respiratory-chain function, and reduce mitochondrial ATP production. Because the liver has a high metabolic demand, restoration of mitochondrial quality control is particularly important for regeneration: surviving hepatocytes must not only avoid death but also regain sufficient bioenergetic capacity to re-enter the cell cycle and support protein synthesis, membrane biogenesis, and tissue growth.

8.1 Antioxidant cargo and restoration of redox balance

The clearest cargo-specific evidence comes from Yan et al., who demonstrated that human umbilical-cord MSC exosomes delivered glutathione peroxidase-1 (GPX1) and reduced hepatic oxidative injury [5]. The protective effect was weakened when GPX1 expression was suppressed in the parent cells, providing stronger mechanistic evidence than a simple association between exosome exposure and lower ROS. This finding supports the broader concept that exosomal protein cargo can transiently augment antioxidant capacity in damaged hepatocytes. For a regenerative interpretation, however, reduced oxidative injury is best interpreted primarily as preservation of the viable hepatocyte pool; it does not by itself prove restoration of functional liver mass.

8.2 Mitochondrial dynamics after hepatectomy and ischaemia-reperfusion

Zhang et al. examined adipose-derived MSC exosomes in rats subjected to hepatic ischaemia-reperfusion injury following hepatectomy [27]. Exosome treatment improved liver function, reduced oxidative stress and hepatocyte apoptosis, increased hepatic ATP, and improved mitochondrial ultrastructure. Mechanistically, the study reported increased expression of the mitochondrial-fusion proteins OPA1, MFN1, and MFN2; decreased expression of the fission-associated proteins DRP1 and FIS1; and increased PGC-1alpha, NRF1, and TFAM, consistent with enhanced mitochondrial biogenesis. These findings are

particularly relevant to regeneration because the post-hepatectomy liver must rapidly restore metabolic capacity while simultaneously supporting cellular proliferation.

Another experimental study of adipose-stromal-cell exosomes in hepatic ischaemia-reperfusion reported lower ROS, higher superoxide dismutase activity, preservation of mitochondrial integrity, reduced inflammatory mediators, and modulation of ERK1/2 and GSK-3beta signalling [28]. Taken together, these studies suggest that exosome-mediated hepatoprotection can involve several levels of mitochondrial control: antioxidant buffering, preservation of membrane integrity, suppression of excessive mitochondrial permeability, normalisation of fusion-fission balance, and induction of mitochondrial biogenesis. None of these mechanisms has yet established a validated autologous blood-exosome potency marker for clinical liver therapy.

8.3 Implications for an autologous blood-derived preparation

For an autologous blood-derived exosome-enriched preparation, mitochondrial rescue cannot be assumed from particle recovery alone. A clinically meaningful product-development program would need to demonstrate that the recovered fraction reproducibly decreases hepatocyte ROS, preserves mitochondrial membrane potential, maintains ATP production, and improves survival in a standardised liver-injury assay. These functional measurements are more informative than particle concentration alone and could eventually form part of a liver-specific potency panel for the Biotex kit. The manufacturer-defined isolation process described in Section 13 therefore represents the collection and enrichment step; whether the recovered autologous fraction contains functionally regenerative cargo remains an empirical question requiring direct testing.

9. Exosomes, Apoptosis, Ferroptosis, and Hepatocyte Survival

Liver injury involves multiple forms of cell death. Apoptosis is characterised by mitochondrial outer-membrane permeabilisation, caspase activation, and orderly cellular dismantling, whereas ferroptosis is driven by iron-dependent lipid peroxidation and failure of the cystine-glutathione-GPX4 antioxidant axis. Exosome-based interventions have been reported to modify both processes, but the direction of effect depends on the recipient cell. This distinction is important: inhibition of ferroptosis may protect hepatocytes during acute injury, whereas induction of ferroptosis in activated HSCs may reduce fibrosis.

9.1 Anti-apoptotic signalling in injured hepatocytes

In toxic and ischaemic liver-injury models, MSC-derived exosome preparations have repeatedly been associated with lower hepatocyte apoptosis, higher BCL-2 relative to BAX, and reduced caspase activation. These changes are commonly accompanied by improvement in aminotransferases and tissue morphology. Such findings support a cytoprotective role but do not establish direct replacement of dead hepatocytes. Exosomes can influence cells that remain biologically recoverable; they cannot revive irreversibly necrotic tissue.

9.2 Hepatocyte ferroptosis and stabilisation of SLC7A11

Lin et al. identified ferroptosis as a major component of carbon-tetrachloride-induced acute liver injury and showed that both MSCs and MSC-derived exosomes reduced lipid-peroxidation-associated injury [29]. In primary hepatocytes and mouse liver, exosome treatment restored the protein level of SLC7A11, the light-chain component of system xc-, without a parallel increase in SLC7A11 messenger RNA. The investigators further implicated CD44-mediated stabilisation of SLC7A11. By preserving cystine uptake and glutathione-dependent antioxidant defence, this mechanism can reduce hepatocyte ferroptosis and conserve viable parenchymal cells during acute injury.

9.3 Cell-specific ferroptosis: protecting hepatocytes while eliminating activated stellate cells

The ferroptosis literature also demonstrates why recipient-cell identity matters. Tan et al. reported that hUC-MSC-derived exosomes delivered BECN1 to activated HSCs and promoted their ferroptosis through suppression of the xCT/GPX4 axis [33]. In experimental fibrotic liver, this was associated with reduced alpha-SMA expression and collagen deposition. Thus, the desirable direction of ferroptosis regulation is cell-specific: hepatocyte ferroptosis is generally detrimental during acute injury, whereas selective

ferroptotic removal of persistently activated HSCs may be antifibrotic. Evaluation of an autologous product intended for liver repair therefore benefits from more than one recipient-cell assay rather than reliance on a single generic antioxidant endpoint.

10. Macrophage Modulation and the Regenerative Microenvironment

Macrophages are central regulators of the transition from hepatic injury to repair. Kupffer cells and recruited monocyte-derived macrophages remove cellular debris, produce inflammatory mediators, coordinate neutrophil and lymphocyte responses, and later contribute to resolution and tissue remodelling. Exosomes can alter this macrophage response without necessarily entering hepatocytes directly, creating an indirect route by which a vesicle preparation may support regeneration.

10.1 miR-17-TXNIP-NLRP3 signalling in acute liver failure

In a lipopolysaccharide/D-galactosamine model of acute liver failure, Liu et al. showed that adipose-MSC-derived exosomes colocalised with hepatic macrophages and suppressed inflammasome activation [30]. The exosomes were enriched in miR-17, which targeted thioredoxin-interacting protein (TXNIP) and reduced downstream NLRP3 inflammasome activation. Importantly, knockdown of miR-17 in the parent MSCs weakened the anti-inflammatory effect of the resulting exosomes, supporting a cargo-dependent mechanism. This study illustrates how exosomes may preserve hepatocytes indirectly by reducing macrophage-derived IL-1beta and IL-18 rather than by acting only on the damaged parenchymal cell.

10.2 miR-455-3p and inflammatory macrophage regulation

Shao et al. demonstrated that hUC-MSC-derived exosomes ameliorated IL-6-associated acute liver injury through miR-455-3p [31]. The study linked exosomal miR-455-3p with attenuation of inflammatory signalling and improvement of experimental liver injury. Although the model does not represent all causes of human acute liver injury, it reinforces the concept that defined exosomal microRNAs can modify the hepatic immune microenvironment.

10.3 Consequences for autologous exosome potency testing

For autologous preparations, macrophage effects deserve dedicated assessment because circulating vesicles from an inflammatory or fibrotic patient may not reproduce the immunoregulatory activity of cultured MSC exosomes. A useful translational assay could expose primary human macrophages or a validated macrophage model to the isolated fraction and measure inflammasome activation, IL-1beta/IL-18 release, pro-inflammatory versus reparative markers, and the ability of conditioned media to support hepatocyte survival. Such an approach would directly test whether a patient-specific preparation creates a regenerative rather than inflammatory microenvironment.

11. The Fibrotic Barrier to Liver Regeneration and Exosome-Mediated HSC Modulation

In chronic liver disease, hepatocyte regeneration occurs within an extracellular matrix that can become progressively rigid, vascularly distorted, and rich in profibrotic cytokines. Activated HSCs are major sources of collagen and other extracellular-matrix components. Consequently, a therapy that protects hepatocytes but fails to address persistent stellate-cell activation may be insufficient in advanced fibrosis. Exosome studies have identified several pathways through which selected preparations can attenuate HSC activation, but they have also shown that vesicles released from damaged hepatocytes can intensify fibrosis.

11.1 TGF-beta/Smad and collagen deposition

Li et al. provided early evidence that hUC-MSC-derived exosomes could reduce carbon-tetrachloride-induced liver fibrosis [32]. Treatment was associated with lower collagen I and III, reduced TGF-beta1 and phosphorylated Smad2, and improvement in liver injury markers. Although some mechanistic interpretations in this early exosome literature predate current MISEV standards, the study remains an important proof-of-concept that the MSC secretome can be transferred through a vesicle-enriched fraction and influence fibrogenic signalling.

11.2 Wnt/beta-catenin modulation

Rong et al. investigated human bone-marrow-MSC exosomes in an eight-week carbon-tetrachloride rat fibrosis model and reported improvement in histopathology, liver function, inflammatory mediators, and HSC activation [34]. Their mechanistic analysis implicated inhibition of Wnt/beta-catenin signalling. This pathway is relevant because Wnt activity participates in stellate-cell activation and tissue remodelling, but its role is context-dependent and also intersects with hepatocyte proliferation. Therapeutic interpretation therefore depends on cell-specific and disease-stage-specific effects rather than an assumption that global Wnt inhibition is intrinsically regenerative.

11.3 BECN1-xCT-GPX4 and selective HSC ferroptosis

As discussed in Section 9, Tan et al. identified BECN1 delivery as a mechanism by which hUC-MSC exosomes suppress the xCT/GPX4 defence system in activated HSCs, induce ferroptosis, and reduce experimental fibrosis [33]. This finding is conceptually attractive because it couples selective removal of a pathological mesenchymal cell population with preservation of hepatocytes by other exosome mechanisms. Experimental demonstration of cell selectivity remains essential before such a mechanism can be considered therapeutically safe.

11.4 Hedgehog/SMO signalling

Zong et al. reported in 2024 that hUC-MSC-derived exosomes alleviated thioacetamide-induced liver fibrosis and downregulated smoothened (SMO), a central component of Hedgehog signalling [35]. Pharmacological activation of SMO weakened the anti-inflammatory and antifibrotic effects, supporting involvement of the Hedgehog/SMO axis. This study adds another candidate mechanism through which MSC exosomes may restrain activated HSCs and matrix accumulation.

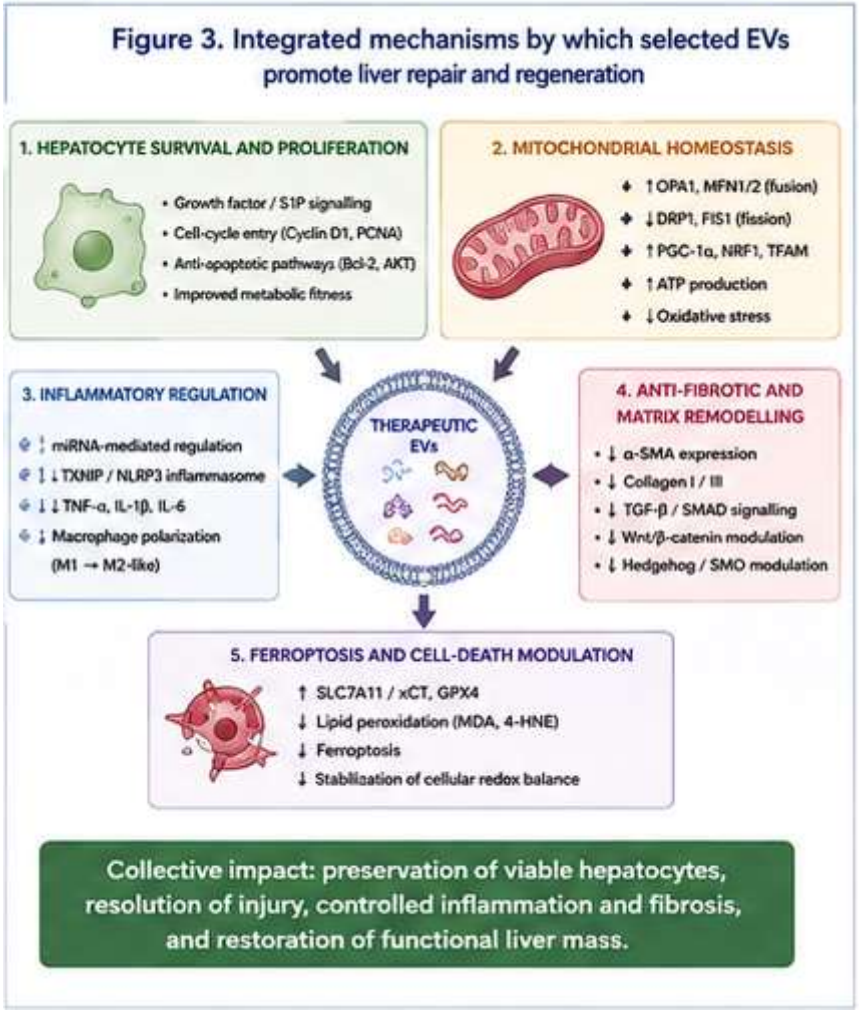

Figure 3. Integrated preclinical mechanisms of selected EV preparations in liver repair. Proposed effects include hepatocyte survival, mitochondrial homeostasis, inflammatory regulation, matrix remodelling, and ferroptosis; they are source- and model-dependent and do not establish clinical efficacy.

Key experimentally supported molecular pathways relevant to hepatocyte survival, inflammatory regulation, and hepatic fibrosis are summarised in Table 3.

11.5 Pathological exosomes from damaged hepatocytes: a critical counterpoint

A central limitation of the autologous concept is that the patient's own circulating vesicle pool can contain disease-promoting cargo. Xu et al. showed that exosomes from damaged hepatocyte-like cells were enriched in the long noncoding RNA CYTOR and transferred this signal to HSCs, where a CYTOR/miR-125/GDNF regulatory circuit promoted stellate-cell activation and fibrosis [36]. Downregulation of CYTOR in the vesicle source reduced fibrogenic effects. This counter-evidence is directly relevant to the Biotex Autologous Exosome Isolation Kit: autologous origin may reduce donor incompatibility, but it does not establish that the recovered cargo is beneficial. A future therapeutic release criterion would therefore require evidence that the preparation is not strongly profibrotic in a validated HSC assay.

11.6 Integrated interpretation

The fibrosis literature suggests that successful exosome-mediated liver repair requires coordinated effects across at least two cellular compartments. In hepatocytes, a useful preparation would preserve redox balance, mitochondrial function, survival signalling, and regenerative capacity. In HSCs, it would ideally reduce persistent activation, collagen synthesis, and profibrotic signalling. These functions need not arise from the same molecular cargo, and a mixed autologous circulating fraction may contain both beneficial and detrimental vesicle subpopulations. This is why enrichment technology requires pairing with characterisation and functional potency testing rather than evaluation by particle yield or nominal purity alone. The interconnected pathways through which selected EV preparations may support hepatocyte survival, mitochondrial recovery, inflammatory regulation, ferroptosis control, and matrix remodelling are integrated in Figure 3.

Table 3: Experimentally supported EV-associated pathways involved in hepatocyte survival, inflammatory regulation, and hepatic fibrosis

Context	Exosome source	Pathway/cargo	Recipient	Interpretation
Oxidative injury	hUC-MSC exosomes	GPX1 delivery; ROS reduction	Hepatocytes	Preserves viable hepatocytes; cargo-specific evidence [5]
I/R after hepatectomy	ADSC exosomes	OPA1/MFN1/MFN2 up; DRP1/FIS1 down; PGC-1alpha/NRF1/TFAM up	Hepatocytes	Improved mitochondrial homeostasis and ATP [27]
Acute liver injury	MSC exosomes	SLC7A11 stabilisation; reduced lipid peroxidation	Hepatocytes	Inhibits hepatocyte ferroptosis [29]
Acute liver failure	Adipose-MSC exosomes	miR-17 -> TXNIP down -> NLRP3 down	Hepatic macrophages	Reduces inflammasome signalling [30]
IL-6-associated liver injury	hUC-MSC exosomes	miR-455-3p-mediated inflammatory modulation	Macrophage/inflammatory compartment	Reduces experimental inflammatory injury [31]
CCl4 fibrosis	hUC-MSC exosomes	TGF-beta1/p-Smad2 down; collagen I/III	HSC/fibrotic liver	Early antifibrotic proof-of-concept [32]

		down		
CCl4 fibrosis	hBM-MSC exosomes	Wnt/beta-catenin modulation	HSCs	Reduced activation and fibrosis [34]
Fibrosis	hUC-MSC exosomes	BECN1 -> xCT/GPX4 down	Activated HSCs	Induces HSC ferroptosis and reduces fibrosis [33]
TAA fibrosis	hUC-MSC exosomes	Hedgehog/SMO inhibition	HSCs	Anti-inflammatory and antifibrotic effect [35]
Damaged hepatocyte signalling	Hepatocyte-derived exosomes	CYTOR/miR-125/GDNF	HSCs	Profibrotic counter-evidence; important for autologous safety [36]

12. Autologous Blood-Derived and Platelet-Derived Exosome Strategies

The concept of autologous exosome therapy is attractive because the biological material originates from the same individual who may later receive the processed preparation. In principle, this reduces donor mismatch and may simplify repeat collection. However, the term autologous describes origin rather than function. A patient-derived preparation can contain regenerative, neutral, inflammatory, thrombogenic, fibrogenic, or tumour-associated vesicles simultaneously. The central translational problem is therefore not merely how to collect more particles, but how to define, enrich, characterise, and functionally qualify the fraction that is intended for therapeutic investigation [16,18,19,21].

12.1 Serum- and plasma-derived autologous vesicles

Blood is an accessible source of circulating vesicles, but plasma and serum are biologically different starting materials. Plasma retains soluble coagulation proteins and is strongly influenced by anticoagulant selection, whereas serum is generated after clotting and therefore contains additional vesicles released during platelet activation and coagulation. MIBlood-EV emphasises that collection tube, anticoagulant, processing delay, temperature, centrifugation, residual platelet content, haemolysis, and storage conditions can materially alter measured EV abundance and composition [16,21,22]. Consequently, interpretation of a blood-derived autologous preparation depends as strongly on its pre-analytical process as on its downstream purification method.

Chen and colleagues provided particularly relevant liver-specific evidence by comparing serum EVs from healthy and fibrotic sources. EVs from healthy animals suppressed experimental fibrosis, reduced hepatocyte injury and inflammatory indices, and increased proliferation of injured hepatocytes, whereas EVs from fibrotic animals did not show the same therapeutic profile [18]. The study also identified differentially represented regulatory microRNAs between healthy and fibrotic serum EVs. These findings are central to the present review because they demonstrate that the biological state of the donor can change the functional quality of a circulating-vesicle preparation. For autologous liver applications, the patient's disease state is therefore a potential determinant of potency and risk rather than a neutral background variable.

12.2 Platelet-derived exosomes as a potentially accessible autologous source

Platelets are among the most abundant sources of circulating vesicles and can be collected through established blood-processing procedures. Saumell-Esnaola and colleagues isolated platelet-derived exosomes from human platelet-rich plasma and demonstrated characteristic morphology and exosome-associated markers, while showing that platelet activation altered vesicle yield and protein cargo [37]. Platelet-derived exosomes are therefore an attractive autologous regenerative source because they can carry growth factors, cytokine-related proteins, lipids, and regulatory RNAs. Nevertheless, their direct efficacy in liver regeneration remains insufficiently established. Accordingly, platelet exosomes represent a plausible and experimentally testable autologous source rather than a clinically validated hepatic therapy.

The platelet contribution is especially relevant for point-of-care blood processing because warming, clotting, agitation, delayed processing, or residual platelet contamination can increase the apparent vesicle concentration in the final sample [21,22]. Such an increase may represent biologically active platelet release, but it may also complicate interpretation of whether the recovered particles originated from hepatocytes, leukocytes, endothelial cells, or platelets. Accordingly, platelet-associated markers such as CD41/CD61 are relevant to analytical characterisation when the starting material is whole blood or platelet-containing plasma.

12.3 Disease-state dependence and the need for patient-specific qualification

The strongest argument for autologous exosome research is also its principal limitation: each preparation is biologically individualised. Cavallari and colleagues showed that serum EVs from different individuals had markedly different angiogenic activity and that biological performance was related to EV cargo, including miR-130a and TGF-beta [19]. Although the study was cardiovascular rather than hepatic, the principle is directly transferable to regenerative-product development: release decisions for autologous vesicle preparations are therefore better linked to measurable biological function than to particle number alone. A patient-specific exosome product is therefore better conceptualised as a personalised biologic whose activity requires demonstration for each defined manufacturing process. The potential advantages and disease-state-dependent risks of autologous blood-derived EV strategies are summarised conceptually in Figure 4.

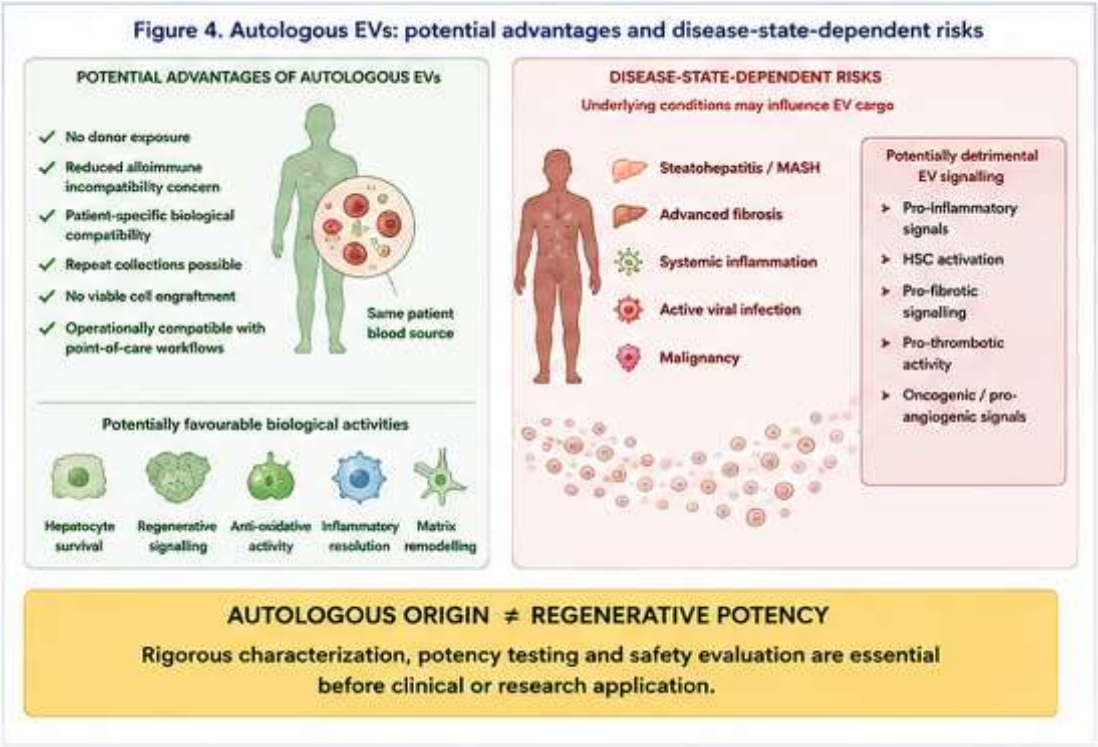

Figure 4. Dual biological potential of autologous blood-derived extracellular vesicles in liver-directed regenerative strategies. Autologous sourcing may reduce donor-related concerns and permit repeated patient-specific collection; however, liver disease, systemic inflammation, metabolic dysfunction, platelet activation, viral disease, and malignancy may alter EV cargo and biological activity. Autologous origin therefore does not establish regenerative potency.

Potential autologous EV sources and their principal translational advantages and limitations are summarised in Table 4.

Table 4: Potential autologous extracellular-vesicle sources for liver-regeneration research

Autologous source	Principal advantage	Major uncertainty	Liver evidence	Current interpretation

Plasma/serum vesicles	Simple repeat blood collection	Mixed cell origin; disease-state cargo; lipoproteins/platelets	Healthy-source serum EVs show antifibrotic and hepatocyte-proliferative effects in experimental liver injury [18]	Most directly compatible with point-of-care autologous collection, but requires potency testing
Platelet/PRP exosomes	Accessible, abundant, established blood collection infrastructure	Procoagulant biology; activation-dependent cargo; limited direct liver data	Well-characterised human platelet exosome preparations exist [37]	Promising source class; liver-specific efficacy not established
Autologous MSC exosomes	Potentially controllable cell source and secretome	Requires cell harvest/expansion, GMP culture and longer manufacturing	Strong liver preclinical MSC-exosome literature [4,5,27-35]	Scientifically strong but not a rapid point-of-care process
Autologous hepatocyte/progenitor exosomes	Highest liver-lineage biological specificity	Difficult cell procurement/expansion; complex manufacturing	Direct hepatocyte and hepatic-progenitor regenerative mechanisms [3,7,8,25,26]	High biological interest; currently experimental

13. Translational Case Framework: The Biotex Autologous Exosome Isolation Kit (manufacturer designation)

The Biotex Autologous Exosome Isolation Kit (manufacturer designation) provides a practical case example of how a same-patient blood-derived extracellular-vesicle strategy could be incorporated into a liver-regeneration research pathway. Within this review, the final material is described scientifically as an autologous blood-derived EV-enriched fraction unless product-specific studies establish a more specific exosomal identity. According to the manufacturer-defined working protocol, the kit contains eight proprietary blood-collection tubes, each with a nominal capacity of approximately 8 mL and designed to receive approximately 5 mL of peripheral blood, providing a total starting blood volume of approximately 40 mL. Each collection tube contains a proprietary gel formulation, and the collected blood remains non-clotted during the defined processing interval. These features are manufacturer-defined protocol parameters and do not represent independently validated therapeutic requirements. The physical Biotex Autologous Exosome Isolation Kit is shown in Figure 5.

Fig 5: Biotex Autologous Exosome Isolation Kit. The product image identifies the physical kit discussed as the translational case framework in Section 13.

13.1 Manufacturer-defined workflow and scientific terminology

After collection, the manufacturer-defined protocol incorporates thermal conditioning at approximately 38 °C for approximately 30 minutes before centrifugation. This step is not established as a method of de novo exosome production. Published studies provide only indirect evidence that thermal stress can influence extracellular-vesicle abundance or release in vivo and in cultured cells [23,24]; these studies do not validate the Biotex-specific ex vivo blood-conditioning procedure. Product-specific studies are needed to determine whether this step alters total EV recovery, cellular origin, cargo composition, purity, or biological potency. Centrifugation then separates the cellular components from the upper liquid fraction. Because the manufacturer-defined collection tubes maintain the blood in a non-clotted state during processing, the recovered upper liquid fraction is operationally described as plasma in this review. The detailed chemistry of the proprietary tube gel is manufacturer-confidential; therefore, technical characterisation of the Biotex kit still requires documentation of collection-tube composition, centrifugation conditions, processing delay, and residual platelet content [16,21,22]. The recovered plasma fraction is subsequently processed through the separate proprietary membrane-filtration unit of the Biotex kit using the defined connector-and-syringe workflow to obtain an autologous EV-enriched filtrate. The proprietary gel contained within the blood-collection tubes and the downstream proprietary membrane filter represent distinct components of the processing system. The filtration step therefore represents the principal enrichment/purification stage of the Biotex kit. Manufacturer-reported product-development studies indicate an approximately 90% purification efficiency under the tested conditions, operationally defined as removal of non-vesicular and oversized contaminants from clarified plasma. This reported value provides a favourable indicator of filtration performance, but it is not equivalent to demonstrating that 90% of all particles in the final fraction are definitively exosomes.

Operationally, centrifugation of the approximately 40 mL starting blood volume yields about 12–15 mL of clarified plasma across the eight tubes. This plasma is passed through the separate proprietary membrane assembly using the closed connector-and-syringe configuration, and approximately 3 mL of the processed autologous EV-enriched fraction is recovered. The combination of proprietary gel-containing collection tubes, controlled processing, centrifugation, and closed membrane filtration reduces open handling and unnecessary transfers while maintaining a same-patient workflow. The complete manufacturer-defined, same-patient processing sequence is illustrated in Figure 6.

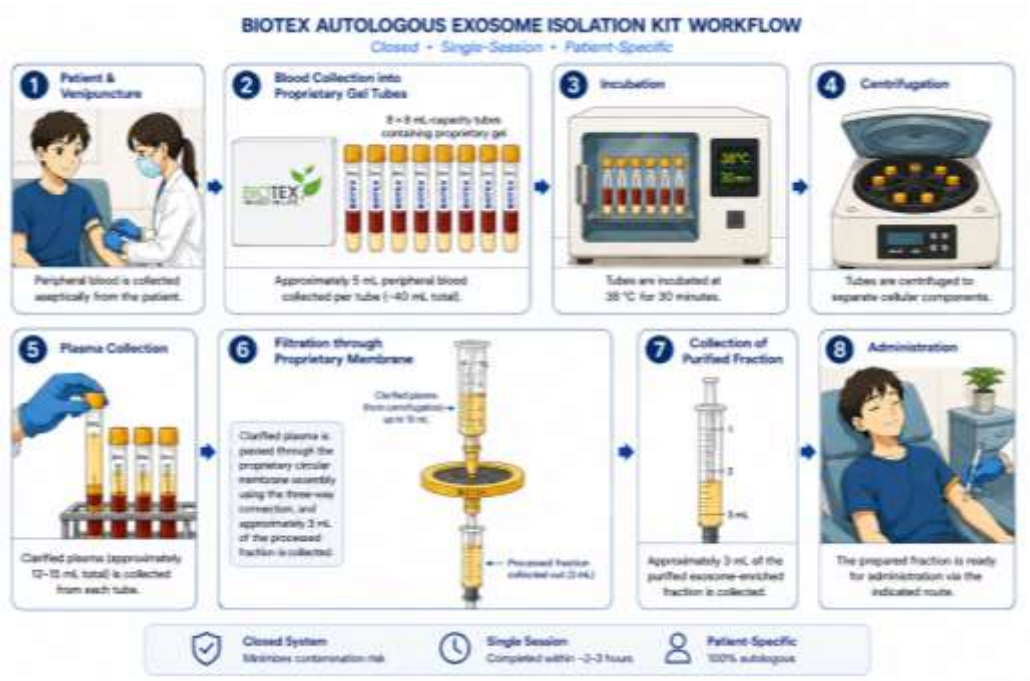

Fig 6: Manufacturer-defined Biotex Autologous Exosome Isolation Kit workflow. The schematic summarises peripheral blood collection into eight proprietary gel-containing tubes, controlled conditioning at approximately 38 °C for 30 minutes, centrifugation, plasma recovery, proprietary membrane filtration, and collection of an approximately 3 mL autologous EV-enriched fraction. The figure illustrates the processing workflow and does not itself establish product-specific clinical efficacy or route-specific safety.

13.2 Reported Purification Performance and Validation Boundaries

The kit can standardise the physical sequence of collection, conditioning, separation, filtration, and recovery. Such standardisation is valuable because blood-EV composition is highly sensitive to pre-analytical handling [16,21,22]. The process cannot, by description alone, establish that every recovered nanoparticle is an exosome, that the preparation is free from lipoproteins or soluble proteins, or that the recovered fraction is regenerative. Publication-grade validation requires quantitative assessment of both vesicle recovery and contaminant depletion using explicitly defined metrics. Depending on the analytical objective, purification performance may include depletion of soluble protein, lipoproteins, residual platelets, cell debris, or non-vesicular nanoparticles, together with recovery of EV-associated particles and markers. Comparative blood-EV studies show that separation methods produce different purity-yield trade-offs [17].

Taken together, the standardised collection format and the manufacturer-reported approximately 90% contaminant-removal performance provide a positive analytical basis for the Biotex Autologous Exosome Isolation Kit as a rapid, patient-specific EV-enrichment system. The reported filtration performance indicates substantial cleaning of the clarified plasma input and supports the practical value of the proprietary membrane stage for downstream research and translational development. At the same time, purification efficiency is analytically distinct from exosome identity, sterility, haemocompatibility, biodistribution, and clinical safety; these attributes require separate product- and route-specific testing.

13.3 Positioning in Liver-Regeneration Research

For liver-regeneration research, the Biotex Autologous Exosome Isolation Kit offers a practical, reproducible, and patient-specific point-of-care sample-preparation approach. Its closed workflow, standardised starting volume, distinct proprietary gel and membrane stages, and manufacturer-reported approximately 90% purification efficiency provide a favourable operational foundation for downstream biological evaluation. The resulting EV-enriched fraction can be assessed for hepatocyte uptake, survival, proliferation, oxidative-stress suppression, macrophage modulation, and HSC activity using the potency

framework described below. These characteristics support the Biotex kit as a promising translational research platform; however, use of the term hepatoregenerative, or claims of established clinical safety and efficacy, require direct product-specific evidence. Any same-patient administration remains investigational and requires defined product-release criteria, safety monitoring, appropriate ethics review, and regulatory oversight.

13.4 Comparative Operational Positioning Relative to Conventional EV-Isolation Approaches

Relative to conventional laboratory extracellular-vesicle isolation workflows, the Biotex Autologous Exosome Isolation Kit has several practical design features that may be advantageous for same-patient point-of-care processing. These include a fixed-volume collection format, proprietary gel-containing tubes, a closed single-session sequence, limited sample transfers, integrated centrifugation and membrane filtration, and recovery of the processed fraction directly into a syringe-compatible format. Conventional ultracentrifugation, polymer-precipitation, and size-exclusion approaches each involve characteristic purity-yield, equipment, processing-time, and handling trade-offs; comparative plasma studies have not identified a single universally optimal separation method [17]. Within that context, the Biotex kit is positively distinguished by workflow integration, same-session autologous processing, reduced open handling, and the manufacturer-reported approximately 90% contaminant-removal performance of its proprietary filtration stage. These characteristics make the system particularly attractive for rapid translational and point-of-care research where reproducibility, procedural simplicity, and patient-specific processing are important. However, no peer-reviewed head-to-head study currently establishes the Biotex kit as analytically or clinically superior to every commercial EV-isolation kit. Its comparative advantage is therefore most defensibly expressed as operational efficiency, closed-workflow practicality, and favourable reported filtration performance rather than as universal superiority or proven therapeutic safety.

14. Potency Testing and Release Criteria for an Autologous Liver-Regenerative Preparation

Potency is the measurable biological activity that links a manufactured preparation to its intended mechanism. For an autologous blood-derived exosome product, particle count is an inadequate release criterion because high particle abundance may reflect platelet activation, cellular stress, contamination, or disease-associated vesicles. Cavallari et al. demonstrated patient-to-patient functional variation in serum EVs [19], while Chen et al. showed that serum EVs from healthy and fibrotic sources have different effects on liver injury and fibrogenesis [18]. These observations support a multi-domain release strategy rather than a single particle-number threshold.

14.1 Identity and composition

- Particle size and concentration by a validated orthogonal method such as NTA or TRPS.
- Morphology by transmission electron microscopy or cryo-electron microscopy.
- Positive EV/exosome-associated markers such as CD9, CD63, CD81, ALIX, and/or TSG101, interpreted according to MISEV2023 [11].
- Assessment of blood-source contribution, including platelet-associated markers when relevant.
- Negative/contaminant markers and quantitative assessment of ApoA1/ApoB or other major lipoprotein contamination.
- Particle-to-protein or another validated enrichment metric, reported together with recovery yield.

14.2 Liver-relevant functional potency

A liver-regeneration potency panel is strengthened by inclusion of at least one direct hepatocyte assay and one non-parenchymal-cell assay. Direct hepatocyte endpoints could include EdU/BrdU incorporation, Ki67, PCNA, cyclin D1, viability after oxidative or toxic injury, ROS suppression, mitochondrial membrane potential, caspase activity, or albumin/urea function. Non-parenchymal assays could measure macrophage inflammatory signalling or HSC alpha-SMA, collagen-I, TGF-beta/Smad activity, and cell viability. A preparation intended for chronic fibrotic liver disease cannot be qualified solely based on hepatocyte proliferation if it simultaneously increases HSC activation.

14.3 Safety-related release attributes

At minimum, a clinical-development preparation would require a validated aseptic process, sterility testing appropriate to the intended use, endotoxin control, haemolysis assessment, visible and subvisible particulate assessment where applicable, and haemocompatibility or coagulation testing for systemic administration. In-vitro toxicology studies by Maji and colleagues demonstrated that EV toxicological profiles can differ substantially by source and preparation, including effects on haemolysis, platelet aggregation, complement, leukocyte responses, and endotoxin burden [43]. These findings support product-specific safety testing but do not provide estimates of clinical adverse-event incidence. A proposed analytical, functional, and safety qualification framework for an autologous blood-derived EV preparation intended for liver research is presented in Table 5.

Table 5: Proposed analytical, functional, and safety qualification framework for an autologous blood-derived EV preparation intended for liver research

Domain	Representative assessment	Why it matters	Status for Biotex kit
Identity	NTA/TRPS, TEM/cryo-EM, CD9/CD63/CD81, ALIX/TSG101	Confirms a vesicle-enriched population rather than particles alone	Requires formal product-specific validation
Purity	ApoA1/ApoB, soluble protein, residual cells/platelets, non-EV markers	Defines the degree of contaminant removal relative to EV recovery	Critical before making any quantitative purity claim in a journal paper
Recovery	Input-versus-output particle and marker recovery	Prevents high purity from masking poor yield	Interpreted together with purification
Hepatocyte potency	Viability, ROS, EdU/Ki67/PCNA, mitochondrial function	Links preparation to liver-repair mechanism	Primary functional release domain
Antifibrotic potency	HSC alpha-SMA, COL1A1, TGF-beta/Smad	Detects profibrotic versus antifibrotic activity	Especially important in chronic liver disease
Safety	Sterility, endotoxin, haemolysis, coagulation/complement where relevant	Required before systemic clinical investigation	Route-specific validation required

15. Intravenous Administration, Biodistribution, and the Question of Liver Targeting

Systemic administration is attractive for liver-directed exosome research because intravenously delivered vesicles are frequently detected in the liver. However, liver accumulation does not establish selective homing to damaged hepatocytes. In mouse studies, EV biodistribution depends on cellular source, route of administration, particle characteristics, and targeting modifications [39]. A systematic review of animal biodistribution studies concluded that administered EVs are commonly detected in liver, lung, kidney, and spleen, with substantial methodological heterogeneity between studies [40].

Haga and colleagues demonstrated that intravenously administered bone-marrow-derived MSC extracellular vesicles protected mice against hepatic ischaemia-reperfusion injury, reducing hepatic necrosis, apoptosis, aminotransferase release, and inflammatory signalling [38]. Separately, Wiklander and colleagues demonstrated that EV biodistribution is strongly influenced by the route of administration and cellular source, with intravenous administration producing substantial hepatic accumulation in their experimental models [39]. Together, these findings provide a liver-relevant proof of principle that systemic EV administration can reach the liver and modify injury, but they do not demonstrate selective targeting of

damaged hepatocytes or establish that a blood-derived autologous preparation will reproduce the same biodistribution.

Large-animal data reinforce the need for pharmacokinetic measurement. Driedonks and colleagues tracked intravenously administered EVs in macaques and detected them in liver and spleen within one hour; repeated administration altered circulation behaviour, illustrating that pharmacokinetics can differ between single and repeated dosing [41]. For a future autologous preparation derived using the Biotex kit, a preclinical programme would therefore need to define circulation time, organ distribution, cellular uptake, and repeat-dose behaviour rather than assume that autologous origin ensures favourable biodistribution.

16. Human Clinical Evidence: What Is Established and What Remains Unproven

16.1 Autologous EV therapy outside the liver

The strongest direct human precedent for autologous circulating-vesicle treatment identified in the academic literature is the pilot case-control study by Gibello and colleagues. Patient-derived serum EVs were applied to chronic venous ulcers that had not responded to conventional treatment, and EV-treated lesions showed improved granulation and greater surface reduction compared with sham-treated lesions [20]. This is important because it demonstrates the feasibility of collecting, processing, and therapeutically applying a same-patient circulating EV preparation. However, the treatment was local, the target tissue was skin, and the study does not establish safety or efficacy for intravenous liver therapy.

16.2 Human therapeutic EV literature overall

Published human EV studies have thus far shown a generally encouraging safety profile, although substantial heterogeneity in EV source, manufacturing, characterisation, dose reporting, route, and clinical indication prevents extrapolation to a specific autologous liver-directed preparation [42]. The 2024 systematic review and meta-analysis included both autologous and allogeneic products across multiple diseases and therefore cannot establish liver-specific safety or efficacy. Its principal value for the present review is to show that clinical translation is feasible while remaining insufficiently standardised.

16.3 The liver-specific autologous evidence gap

Within the peer-reviewed academic literature evaluated for this review, no controlled human study was identified in which an autologous blood-derived exosome preparation was intravenously administered specifically to regenerate damaged liver tissue. Human evidence is instead strongest for endogenous circulating vesicle signatures associated with regeneration [9,10], while therapeutic liver evidence remains predominantly preclinical [3-8,18,25-35,38]. This gap is central to interpretation of the current evidence. The absence of direct clinical evidence does not invalidate the biological hypothesis; it defines the next experimental question. The current hierarchy of evidence and the principal translational gap between preclinical EV studies and autologous human liver therapy are summarised in Figure 7.

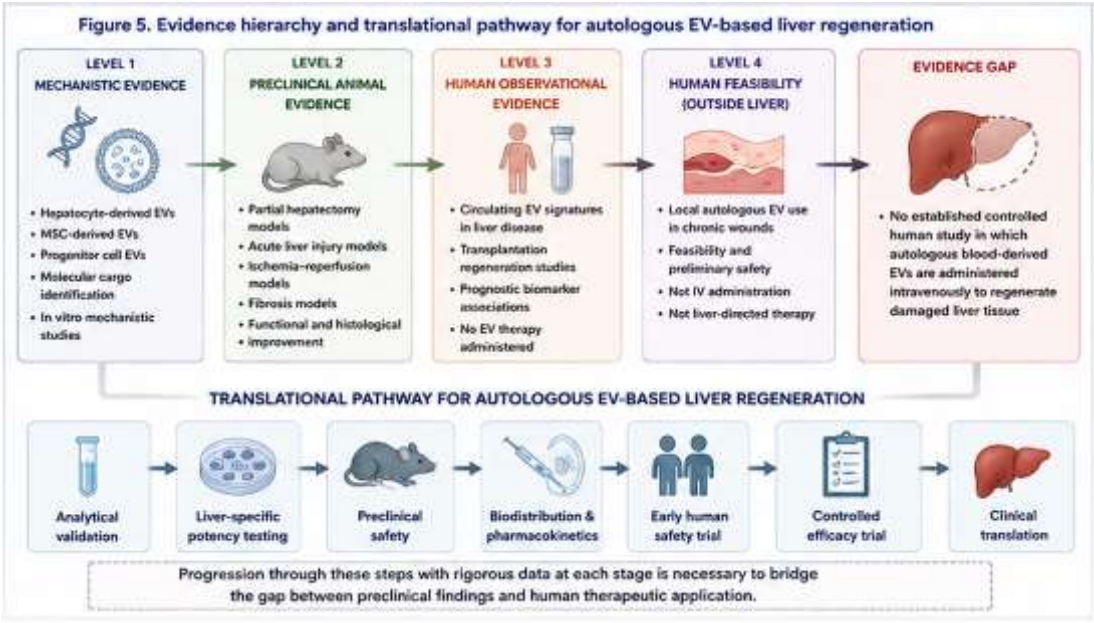

Figure 7. Current evidence hierarchy and translational pathway for autologous EV-based liver regeneration. Mechanistic and preclinical evidence is substantial, whereas human evidence remains predominantly observational or derives from autologous EV feasibility outside the liver. Controlled human evidence for intravenous autologous blood-derived EV therapy in liver regeneration remains insufficient; progressive analytical, functional, preclinical, and clinical validation is required.

17. Safety, Clinical Governance, and Claim Boundaries

Autologous origin is not synonymous with safety. Same-patient origin reduces some alloimmune concerns but does not remove the possibility of microbial contamination, endotoxin exposure, activation of coagulation or complement, inflammatory cargo, pro-fibrotic signalling, tumour-associated vesicles, or an ineffective preparation. Blood-derived EV composition is particularly sensitive to the patient's disease state and to processing variables [16,18,21,22]. In patients with hepatocellular carcinoma or another active malignancy, the possibility of tumour-associated circulating vesicles creates an additional theoretical and experimental concern and warrants explicit consideration as an exclusion or risk-analysis domain in early liver-regeneration studies.

Accordingly, the evidence supports a conservative interpretation: exosome-mediated liver regeneration is strongly supported by mechanistic and animal evidence, and autologous blood-derived vesicle therapy is biologically plausible and technically feasible as a development strategy, but therapeutic efficacy for human liver disease is not yet established. The Biotex Autologous Exosome Isolation Kit described in this review enables standardised collection and enrichment but does not constitute evidence that the final preparation treats liver disease. Jurisdiction-specific ethics and regulatory requirements apply to any interventional use.

18. Key Evidence Gaps and Research Priorities

- Cellular origin and composition of the Biotex kit-derived EV-enriched fraction before and after the controlled incubation step.
- Mechanism and magnitude of any change in recoverable vesicles associated with thermal conditioning, including the contribution of platelets and residual blood cells.
- Definition and validation of purification performance using prespecified contaminant-depletion and vesicle-recovery metrics.
- Reproducibility of hepatoprotective, proliferative, immunomodulatory, and/or antifibrotic activity in the final blood-derived fraction.
- Influence of liver-disease severity on the potency and risk profile of autologous preparations.

- Comparative performance of the Biotex kit workflow and at least one reference isolation method, such as size-exclusion chromatography plus concentration or another validated blood-EV method.
- Development of a release assay linked to hepatocyte-regenerative activity rather than particle number or surface markers alone.
- Characterisation of coagulation, complement, haemolysis, immunological, and tumour-related risks before systemic administration studies.
- Pharmacokinetics, hepatic cellular uptake, off-target organ exposure, and repeat-dose behaviour.
- Prospective human investigation following adequate analytical validation, preclinical potency assessment, and route-specific safety evaluation.

19. Proposed Translational Validation Roadmap for an Autologous Exosome Liver Programme

A staged translational programme provides a more rigorous bridge between device development and therapeutic claims. Each stage addresses a distinct analytical, functional, safety, or clinical question and provides evidence for progression to the next stage.

Stage 1 - Analytical identity: Characterisation of pre- and post-filter fractions by particle size/count, morphology, EV-associated markers, platelet contribution, soluble-protein/lipoprotein contamination, and recovery yield.

Stage 2 - Process validation: Quantification of the effects of incubation time/temperature, collection-tube chemistry, centrifugation, filtration, and operator variability on composition and yield.

Stage 3 - In-vitro liver potency: Assessment of human hepatocyte or liver-organoid survival, proliferation, ROS, mitochondrial function, apoptosis/ferroptosis, and secretory function, together with parallel HSC and macrophage assays.

Stage 4 - Mechanistic cargo analysis: Small-RNA sequencing, proteomics, and targeted validation to identify cargo associated with regenerative versus adverse activity.

Stage 5 - Preclinical biodistribution and safety: Evaluation of systemic and/or locally relevant routes, pharmacokinetics, liver-cell uptake, coagulation/complement, dose range, repeat dosing, and toxicology.

Stage 6 - Early human translational study: Prospective observational or ex-vivo autologous potency assessment during natural liver regeneration before consideration of therapeutic reinfusion.

Stage 7 - Interventional clinical development: If supported by preceding evidence, a regulated dose-escalation safety study with predefined product-release criteria and mechanistic pharmacodynamic endpoints.

20. Conclusion

Exosomes and related small extracellular vesicles form an important component of the liver's endogenous communication and repair network. The strongest mechanistic evidence demonstrates that hepatocyte-derived exosomes can directly promote hepatocyte proliferation through neutral ceramidase/sphingosine-kinase-2/sphingosine-1-phosphate signalling, while hepatic-progenitor- and MSC-derived preparations can support regeneration through microRNA transfer, antioxidant defence, mitochondrial preservation, suppression of apoptosis or hepatocyte ferroptosis, modulation of macrophage inflammation, and attenuation of HSC-driven fibrosis. These effects collectively support the concept that a regenerative vesicle preparation could influence several biological barriers to liver recovery simultaneously.

Autologous blood-derived exosomes represent a distinct translational strategy because collection is repeatable and patient-specific. The available evidence nevertheless shows that autologous origin is not equivalent to regenerative potency. Healthy- and fibrotic-source serum vesicles can differ functionally, platelet activation and blood handling can alter the recovered vesicle population, and patient-to-patient biological activity can vary. Accordingly, the biological interpretation of an autologous product depends on process, composition, potency, and safety rather than provenance alone.

The Biotex Autologous Exosome Isolation Kit (manufacturer designation) is presented in this review as a developed proprietary collection-and-enrichment system within this translational framework. The Biotex kit workflow provides a standardised route from peripheral blood to a same-patient EV-

enriched fraction. The next scientific requirement is product-specific validation: objective characterisation of identity and purity, definition of purification performance using explicit recovery and contaminant-depletion metrics, demonstration of liver-relevant regenerative potency, and route-specific safety and biodistribution. Until those steps and controlled human studies are completed, the evidence supports classification of the kit-derived preparation as an investigational autologous blood-derived EV-enriched preparation with a biologically plausible liver-regeneration rationale rather than an established treatment for liver disease.

Declarations

Author Contributions: All authors contributed to the conception, literature appraisal, drafting, critical revision, and final approval of the manuscript and accept accountability for the work.

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Conflict of Interest: All authors are employees of Biotex Life Solutions Pvt. Ltd., the company that developed the Biotex Autologous Exosome Isolation Kit discussed in this review. This employment and institutional relationship is disclosed as a potential conflict of interest. The manuscript is a scientific review of the published literature and references. The authors declare no other conflicts of interest.

Ethics Statement: Not applicable to the literature-review component. Any future study involving collection of human samples, product testing on identifiable human material, or administration to participants requires the applicable institutional ethics and regulatory approvals.

Data Availability: No new clinical dataset was generated for this review. The evidence synthesised is available in the cited peer-reviewed literature. Product-specific analytical data are not presented as evidence in this review.

Use of AI-Assisted Tools: Generative AI tools were used for language refinement and schematic figure development. Scientific interpretation, reference verification, claim assessment, and final manuscript content remain the responsibility of the authors.

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