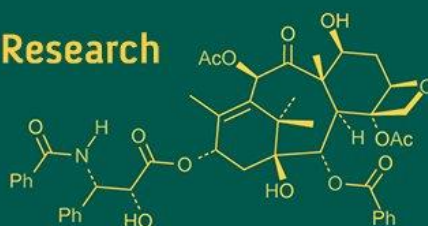
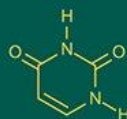
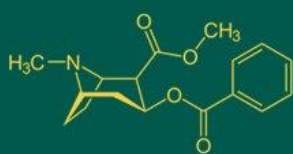


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Standardized isolation and culture of primary hepatic stellate cells from murine liver using enzymatic digestion and OptiPrep density gradient centrifugation

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Abstract

Hepatic stellate cells (HSCs) are the principal fibrogenic cells of the liver and play a critical role in extracellular matrix deposition and lipid droplet metabolism during hepatic injury. Isolation of viable and highly enriched primary HSC populations is essential for investigating cellular and molecular mechanisms underlying liver fibrosis. The present study describes a detailed and reproducible protocol for the isolation of primary HSCs from murine liver through controlled enzymatic digestion followed by iodixanol (OptiPrep) density gradient centrifugation. Murine livers were aseptically dissected, enzymatically dissociated using collagenase B and pronase, and subjected to discontinuous density gradient centrifugation under optimized conditions. HSCs were identified as a distinct interphase band and further purified by DNase treatment and red blood cell lysis. Isolated cells were cultured under standard conditions and stabilized through sequential washing steps. The protocol yielded viable, morphologically characteristic HSCs suitable for downstream biochemical, molecular, and lipid metabolism studies. This method provides a reliable platform for experimental investigations in liver fibrosis and stellate cell biology.

Keywords: Hepatic stellate cells, murine liver, enzymatic digestion, iodixanol gradient, liver fibrosis, primary cell isolation, lipid droplets

Introduction

Liver fibrosis is a progressive wound-healing response to chronic hepatic injury characterized by excessive extracellular matrix (ECM) deposition. Activation of hepatic stellate cells (HSCs) is widely recognized as the central event in fibrogenesis [1, 2]. In their quiescent state, HSCs store retinoids within cytoplasmic lipid droplets; however, upon activation, they lose lipid droplets and acquire a proliferative, contractile, myofibroblast-like phenotype, expressing α -smooth muscle actin and collagen type I [3, 4].

Recent advances in HSC biology highlight their dynamic involvement in metabolic reprogramming, inflammatory signaling, and autophagy regulation during fibrosis progression [5, 6]. Murine models remain indispensable for studying these mechanisms due to genetic manipulability and translational relevance [7].

Efficient isolation of primary HSCs is critical for mechanistic studies. Iodixanol-based density gradients exploit the lower buoyant density of lipid-rich HSCs compared to hepatocytes and other non-parenchymal cells, enabling high-purity separation [7, 8]. Updated methodologies emphasize controlled enzymatic digestion and optimized centrifugation conditions to improve yield and viability [8, 9].

The present study describes a standardized and reproducible method for isolation and culture of primary hepatic stellate cells from murine liver using enzymatic digestion followed by OptiPrep density gradient centrifugation.

Materials and Methods

Experimental animals

Adult mice (8-12 weeks old) were maintained under standard laboratory conditions. All procedures were performed according to the Institutional animal ethics guidelines.

Liver dissection

Mice were euthanized using chloroform followed by cervical dislocation. The abdominal cavity was opened under aseptic conditions, and the liver was excised carefully to avoid tissue damage. The excised liver was transferred into ice-cold HBSS to remove residual blood and preserve

cell integrity, as recommended in optimized murine HSC isolation protocols [8].

Enzymatic digestion

Efficient dissociation of hepatic tissue is essential for maximizing HSC recovery. The liver was minced into small fragments under sterile conditions.

Collagenase B and Pronase were pre-warmed at 37 °C for 15 minutes to ensure optimal enzymatic activity. The minced tissue was incubated with 10 ml Collagenase B and 10 ml Pronase. DNase I (2 ml) was added to prevent aggregation caused by released DNA from damaged cells, consistent with previously established protocols [7-9].

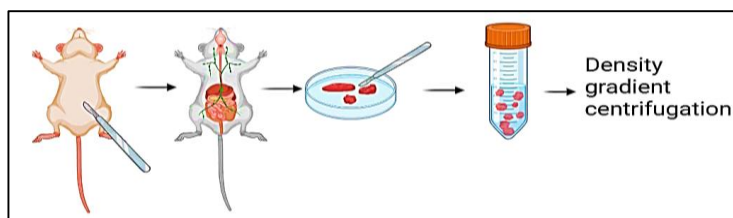


Fig 1: Workflow for isolation of hepatic stellate cells (HSCs) from mouse liver.

The mixture was incubated at 37 °C in a shaking incubator for 20 minutes. Following digestion, the suspension was filtered through a 70 µm cell strainer and centrifuged at 600 × g for 7 minutes.

Livers were surgically excised from mice following dissection, minced into small fragments, and subjected to enzymatic digestion. The resulting cell suspension was processed by density gradient centrifugation to isolate hepatic stellate cells for subsequent culture and analysis.

OptiPrep Density Gradient Centrifugation

Iodixanol (OptiPrep) density gradient centrifugation was performed based on established high-yield murine HSC isolation methods [8, 9].

A discontinuous gradient was prepared by mixing OptiPrep with HBSS to create bottom and intermediate phases. The cell suspension was layered carefully to avoid mixing of interfaces. Centrifugation was performed at 1, 400 × g for 20 minutes at 4 °C with slow acceleration and no brake to preserve gradient integrity.

Following centrifugation, hepatic stellate cells appeared as a distinct opaque white interphase band, consistent with prior reports [7, 8].

After enzymatic digestion, the cell pellet was resuspended in OptiPrep-HBSS and overlaid with HBSS to form a density gradient. Following centrifugation, hepatic stellate cells (interphase pericytes) were collected from the interface layer for further analysis.

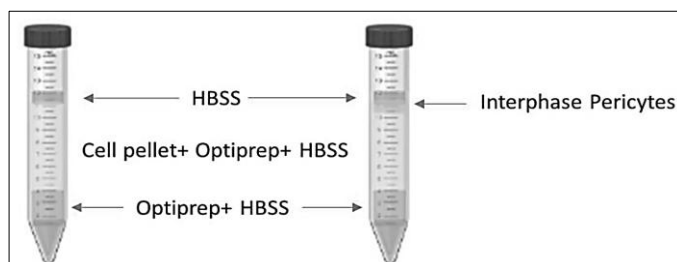


Fig 2: Isolation of hepatic stellate cells (HSCs) by OptiPrep density gradient centrifugation.

Purification and Culture

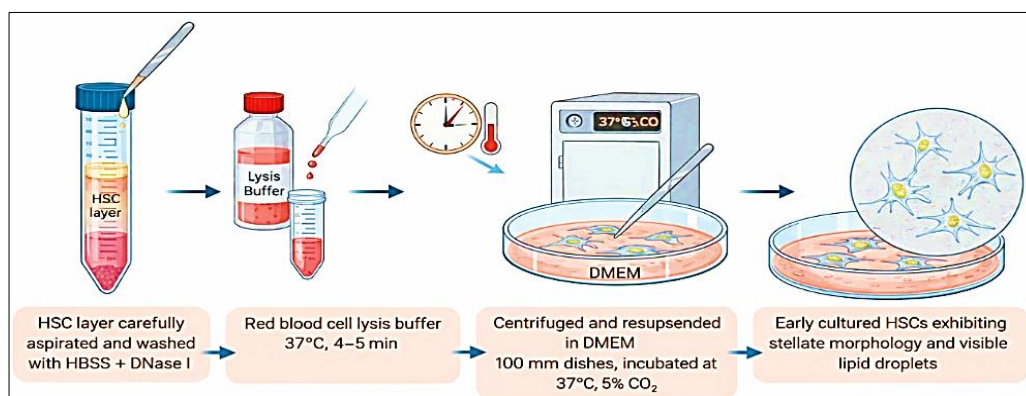


Fig 3: Purification and culture of primary hepatic stellate cells (HSCs).

The HSC layer was aspirated carefully and washed with HBSS containing DNase I. Red blood cell lysis buffer was applied for 4-5 minutes at 37 °C to eliminate erythrocyte contamination. Cells were centrifuged again at 600 × g and resuspended in complete DMEM. Cells were plated in 100 mm dishes and incubated at 37 °C with 5% CO₂. After 24 hours, non-adherent debris was removed by sequential PBS washing. Early cultured cells displayed typical stellate morphology with visible lipid droplets, as previously described in murine HSC isolation studies ^[1, 3].

Results

The described protocol enabled efficient separation of

hepatic stellate cells using iodixanol density gradient centrifugation. A clearly distinguishable interphase band corresponding to HSCs was observed after centrifugation. The isolated cells adhered within 24 hours and demonstrated characteristic morphology. At 24 hours post-seeding, HSCs exhibited typical stellate morphology with cytoplasmic extensions and visible lipid droplets. By 48 hours, cells showed increased spreading and more pronounced stellate projections, indicating progressive morphological adaptation in culture. Phase-contrast microscopy at 10× and 20× magnification confirmed time-dependent changes in cell morphology.

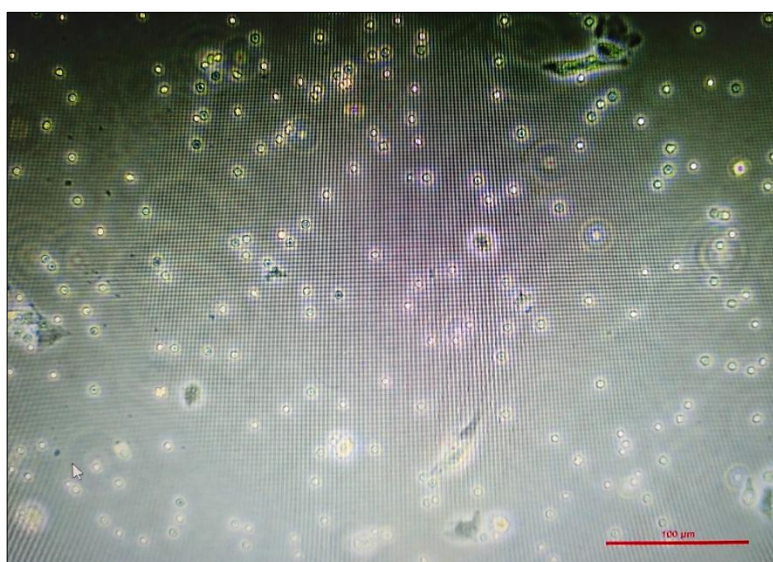


Fig 4: Phase-contrast micrograph of freshly isolated hepatic stellate cells (HSCs).

Primary murine HSCs displaying characteristic round morphology with prominent cytoplasmic lipid droplets

(refractile ring-like structures). The presence of abundant lipid droplets indicates quiescent stellate cells.

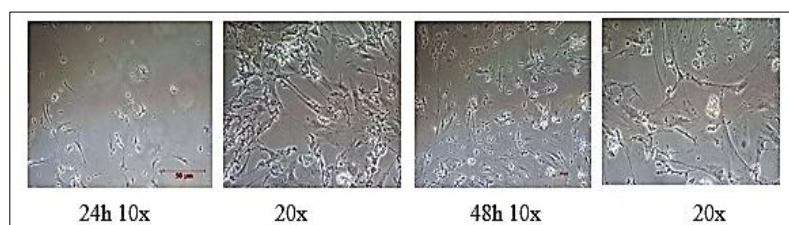


Fig 5: Time-dependent morphological changes of cultured hepatic stellate cells (HSCs).

Phase-contrast images of primary HSCs at 24 h and 48 h post-seeding, captured at 10× and 20× magnification. Cells progressively exhibit spreading and pronounced stellate morphology with cytoplasmic extensions over time. The combination of controlled enzymatic digestion and density-based separation improved cell yield and purity, consistent with recent optimized murine HSC isolation methods ^[8, 9].

Discussion

Isolation of primary HSCs requires precise enzymatic digestion and density gradient separation. Collagenase disrupts extracellular matrix components, whereas pronase enhances dissociation of intercellular connections. DNase prevents cell aggregation, thereby improving recovery ^[7]. Iodixanol gradients offer improved reproducibility and purity compared to traditional Percoll-based methods ^[8]. Recent studies emphasize that proper centrifugation

parameters—particularly slow acceleration and avoidance of braking—are critical for preserving gradient layers and maximizing HSC yield ^[8, 9].

The isolated HSCs obtained through this method are suitable for studying fibrogenic signaling pathways, lipid droplet remodeling, metabolic reprogramming, and autophagy-related mechanisms involved in liver fibrosis ^[5, 6].

Conclusion

The present study provides a comprehensive and reproducible protocol for isolation and culture of primary hepatic stellate cells from murine liver using enzymatic digestion and iodixanol density gradient centrifugation. The method yields viable and enriched HSC populations suitable for advanced biochemical and molecular investigations in liver fibrosis research.

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