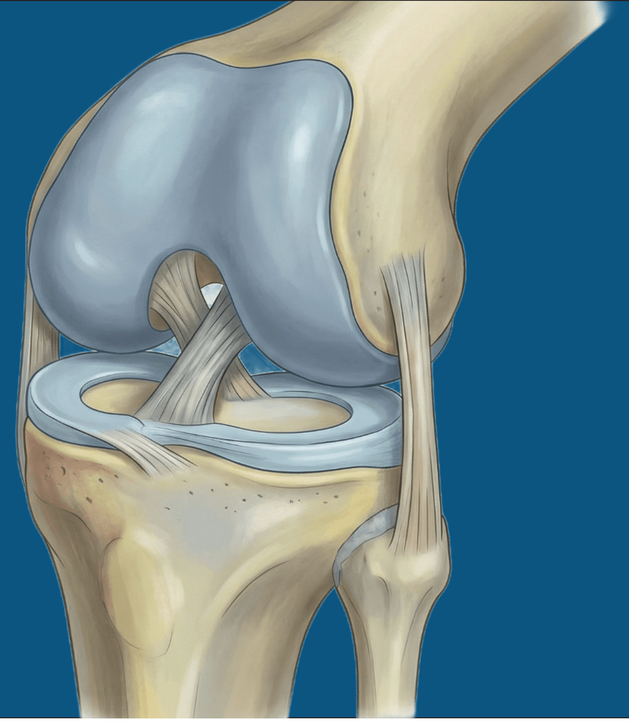
Extended ex vivo preservation of human osteochondral tissue at ambient conditions through enhanced culture medium formulation



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1 Introduction

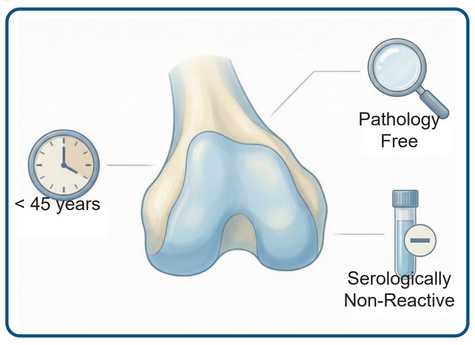
Preserving the structural integrity and celular viability of human osteochondral tissues is essential for succesful transplantation and functional cartilage repair.

Current strategies rely on refrigeration and cold-chain logistics, which increase operational complexity and limiting accesibility. Extending tissue viability at room temperature could significantly simplify tissue banking processes and enhance graft avaliability.

This study aims to develop a room-temperature preservation strategy using a specialized medium.



2 Methodology



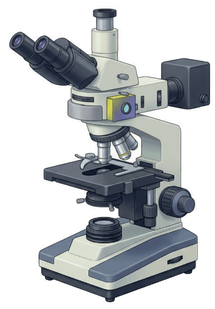
M1: Nutrient rich medium (aminoacids, vitamins, plasma proteins, human growth factors)

Room Temperature: 22 - 25 °C
14, 28 and 42 days

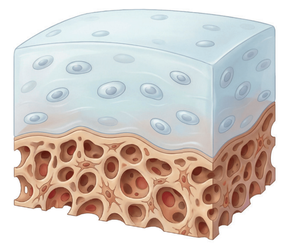
M2: High glucose DMEM supplemented with growth factors, sodium pyruvate and ascorbic acid



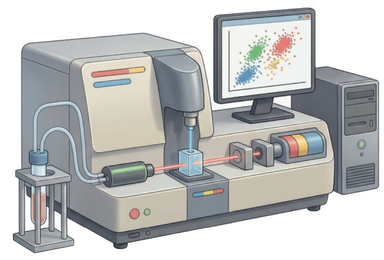
Viability
Live/Dead



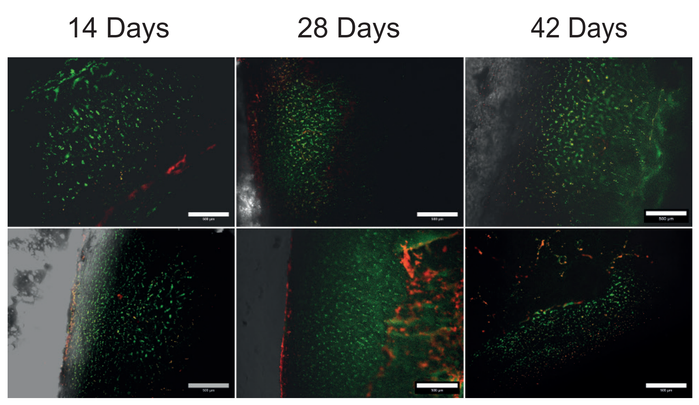
Histology
H&E, Alcian Blue
Masson's Trichrome

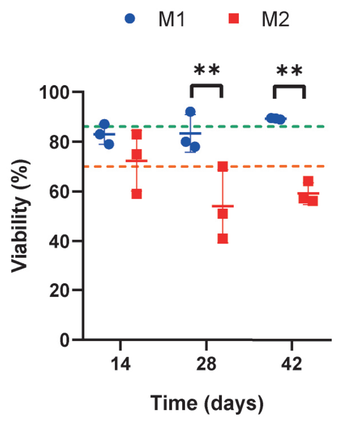


Cell characterization
CD44, CD54, CD90
CD34, CD45

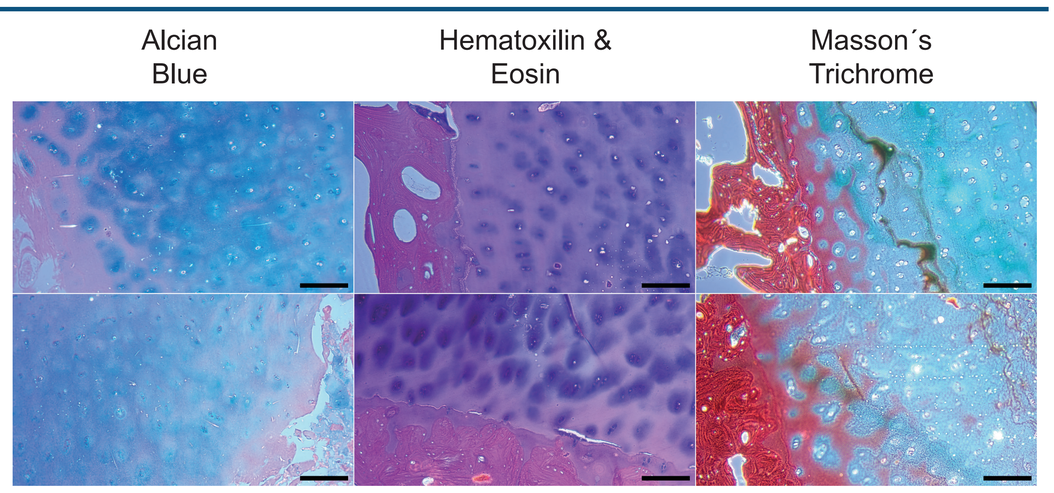


3 Results

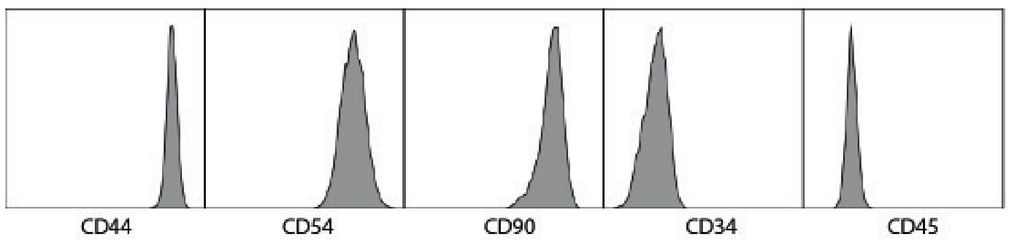


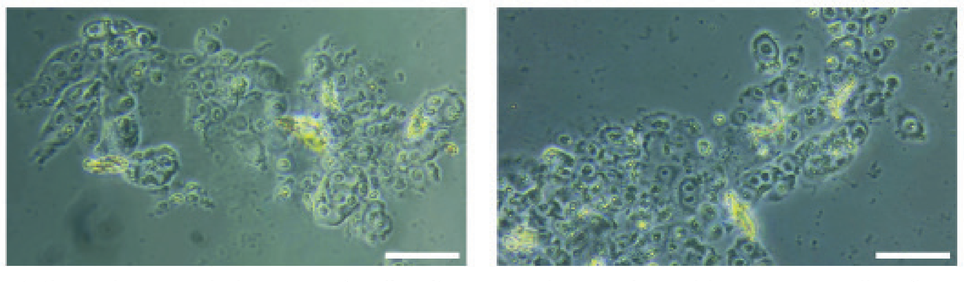


M1 vastly outperformed M2. In M1, chondrocyte viability remained >85% at day 14, 84% at day 28, and >85% at day 42. In contrast, M2 showed a marked decline over the same time points, dropping to 75% at day 14, 55% at day 28, and ~60% at day 42. Scalebar: 500µm



Both media preserved overall osteochondral architecture; however, M1 showed superior tissue quality, characterized by higher lacunar occupancy, viable chondrocyte clusters, enhance collagen expression and stronger proteoglycan retention compared to M2. Scalebar: 1 mm





Representative microscopic images of cell culture are shown alongside corresponding flow cytometry histograms. The immunophenotypic profile demonstrates positive expression of CD44, CD54 and CD90, and negative expression of CD34 and CD45. Scalebar: 100µm

4 Discusion

M1 sustains chondrocyte viability (~83–89%) over 42 days, while M2 shows a significant time-dependent decline.

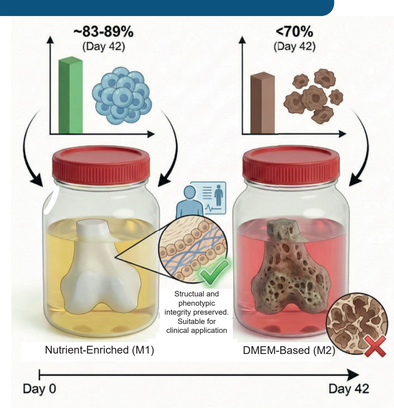
Medium composition is critical, with nutrient-rich conditions enabling prolonged cellular survival at room temperature.

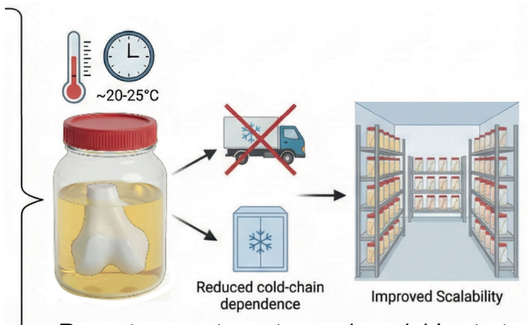
M1 exceeds the recommended clinical threshold of 70% chondrocyte viability for OCA implants [1].

Matrix integrity correlates with viability, as M1 better preserves lacunar occupancy and proteoglycan content.

Room-temperature preservation is feasible, offering a scalable alternative to cold-chain–dependent osteochondral graft storage [2]

5 Conclusion





Room temperature storage is a viable strategy, reducing coldchain dependance and improving tissue banking scalability

6 References

[1] J. L. Cook et al., "A Novel System Improves Preservation of Osteochondral Allografts," Clinical Orthopaedics & Related Research, vol. 472, no. 11, pp. 3404–3414, Nov. 2014, doi: 10.1007/s11999-014-3773-9.

[2] A. L. Pallante, W. C. Bae, A. C. Chen, S. Görtz, W. D. Bugbee, and R. L. Sah, "Chondrocyte Viability is Higher after Prolonged Storage at 37°C than at 4 C for Osteochondral Grafts," Am J Sports Med, vol. 37, no. 1_suppl, pp. 24–32, Nov. 2009, doi: 10.1177/0363546509351496.

Illustrations in Sections 1, 2, 4, and 5 were generated using Illustrae AI under the direction of Leonardo Prieto.