Hyaluronic acid microparticles as a versatile platform for spheroid-based 3D cell culture

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ABSTRACT

Three-dimensional (3D) cell culture technologies have emerged as essential tools in regenerative medicine, drug screening, and disease modeling. Among these, spheroid culture provides a physiologically relevant environment that enhances cell-cell interactions and mimics native tissue microenvironments. However, traditional spheroid culture methods often face challenges such as poor structural integrity, limited nutrient diffusion, and difficulty in maintaining long-term viability.

Hyaluronic acid (HA) microparticles offer a promising solution by serving as a biomimetic scaffold that supports spheroid formation across various cell types. HA microparticles provide a tunable microenvironment that enhances cell aggregation, extracellular matrix formation, and long-term cell viability, while simultaneously enabling efficient nutrient exchange. In this study, we have demonstrated that HA microparticle-based spheroid culture can be applied to hepatic [1], cartilage [2], and other tissue engineering models [3], showing improved functional outcomes compared to conventional methods. Additionally, HA microparticles facilitate spheroid transplantation, supporting cell survival and integration within host tissues, making them a valuable tool in regenerative therapies.

INTRODUCTION

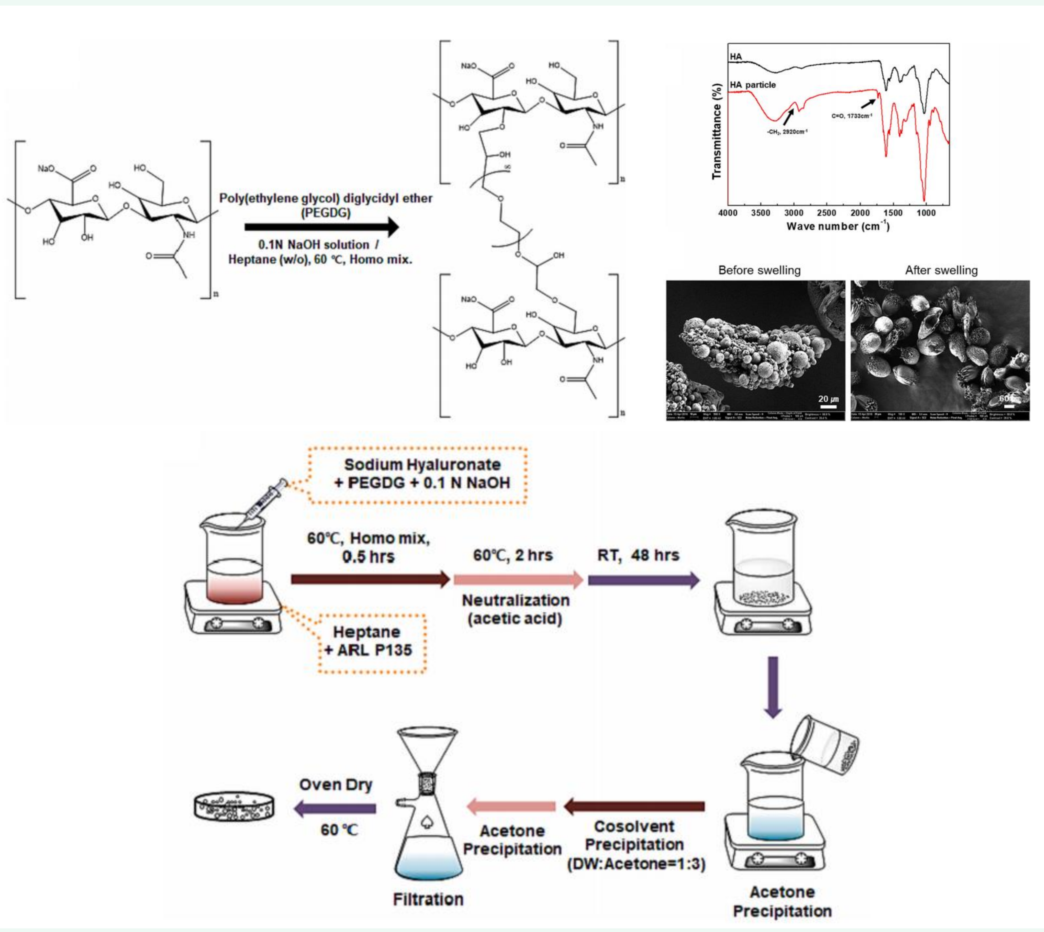
• Limitations of conventional systems:

- **Structural instability leads to inconsistent morphology and poor reproducibility.**
- **Limited nutrient diffusion causes necrotic core formation, reducing cell viability.**
- **Difficulty in maintaining stable and heterogeneous spheroids hinders complex applications.**

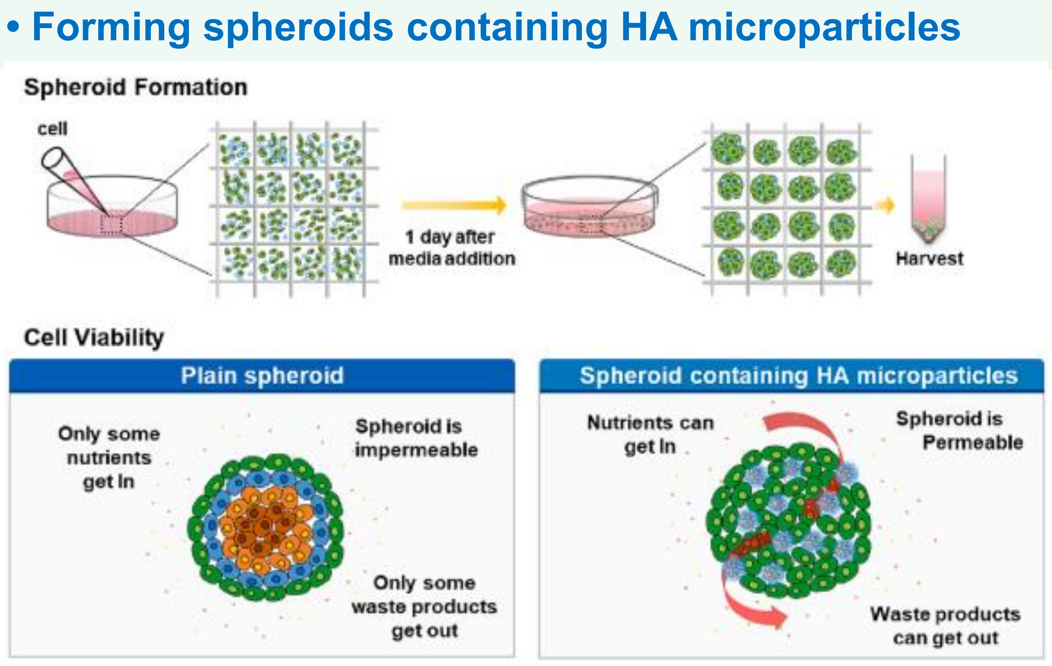
• Our solution:

- **HA microparticles provide a biomimetic scaffold for controlled spheroid formation.**
- **Their porous structure enhances nutrient exchange, preventing necrotic cores.**
- **HA microparticles support extracellular matrix development and improve transplantation outcomes.**

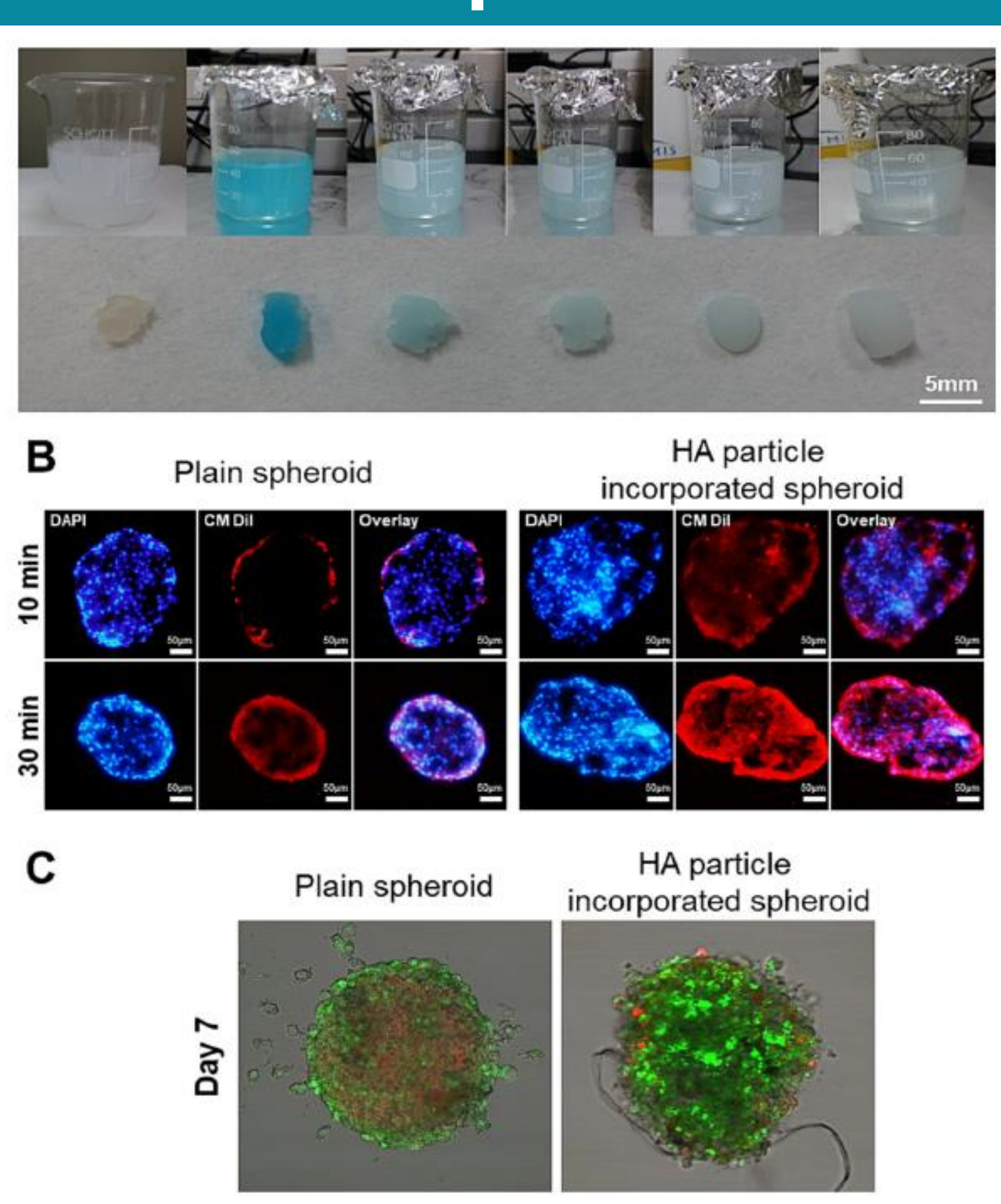
• Synthesis & fabrication of HA Microparticle



• Forming spheroids containing HA microparticles



Role of HA particles



The role: delivers nutrients and oxygen to the inside of the spheroid and increase survival rate

RESULTS

• BAN-Assisted Formation and Characterization of High-Density, Uniform HepG2 Spheroids

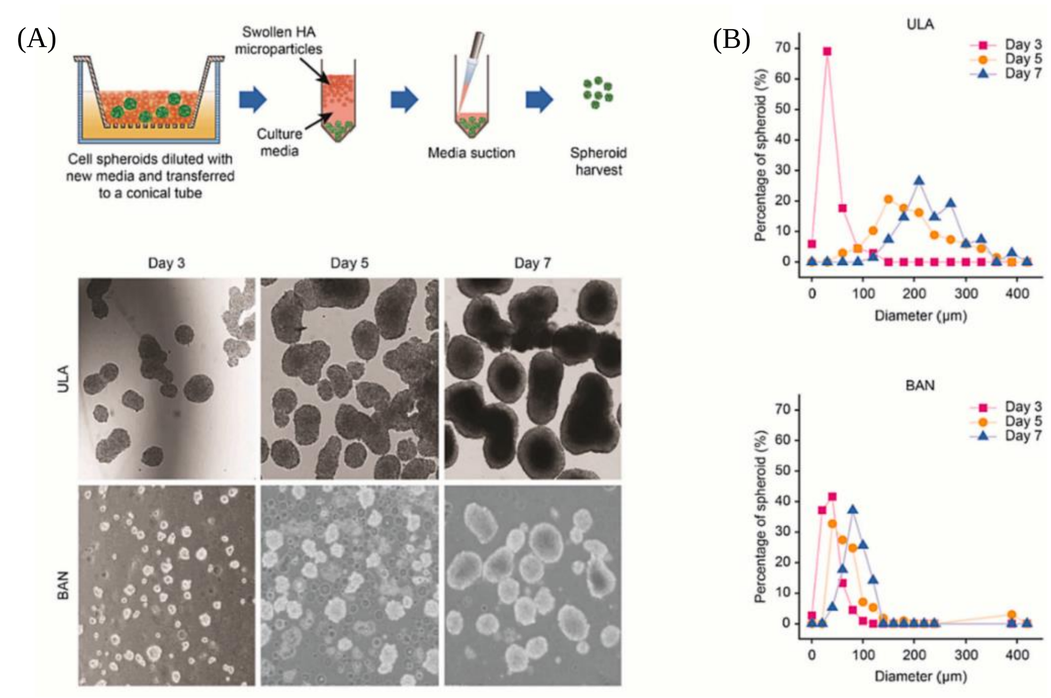


Figure 1. Generation of uniform HepG2 spheroids with high cell density. (A) Schematic representation of the harvested HepG2 spheroids initiated with 5 × 10^4 cells generated from BAN (upper). Phase contrast micrographs of harvested HepG2 spheroids (lower). (B) Size analysis of the HepG2 spheroids generated from BAN and the ULA plates for 7 days using Leica LAS X Core software.

• Viability and Apoptotic Profiling of HepG2 Spheroids Cultured via BAN and ULA Platforms

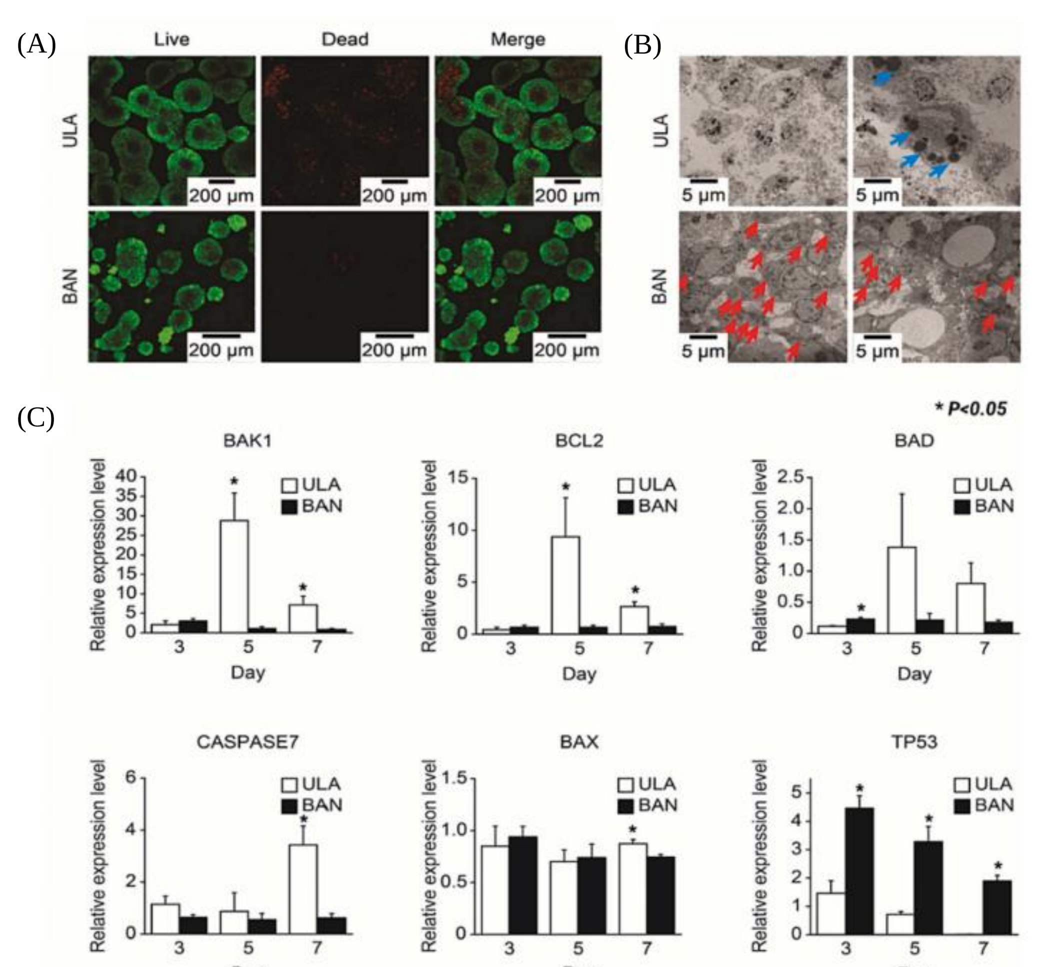


Figure 2. Viability of HepG2 spheroids generated from BAN and ULA plates. (A) Cell viability assay of HepG2 spheroids on day 7 using a live/dead assay kit. (B) Microscopic necrosis analysis of the HepG2 spheroids at 7 days: BAN (red arrow; uniform mitochondria) and ULA plates (blue arrow; distorted irregular nuclei with fragmented chromatin). (C) Expression of apoptosis markers (BAK1, BCL2, BAD, CASPASE7, CASPASE3, BAX, and TP53) in HepG2 spheroids generated from BAN and ULA plates using real-time PCR analysis.

• Structural, Viability, and Functional Characterization of HepaRG Spheroids with HA Microparticles

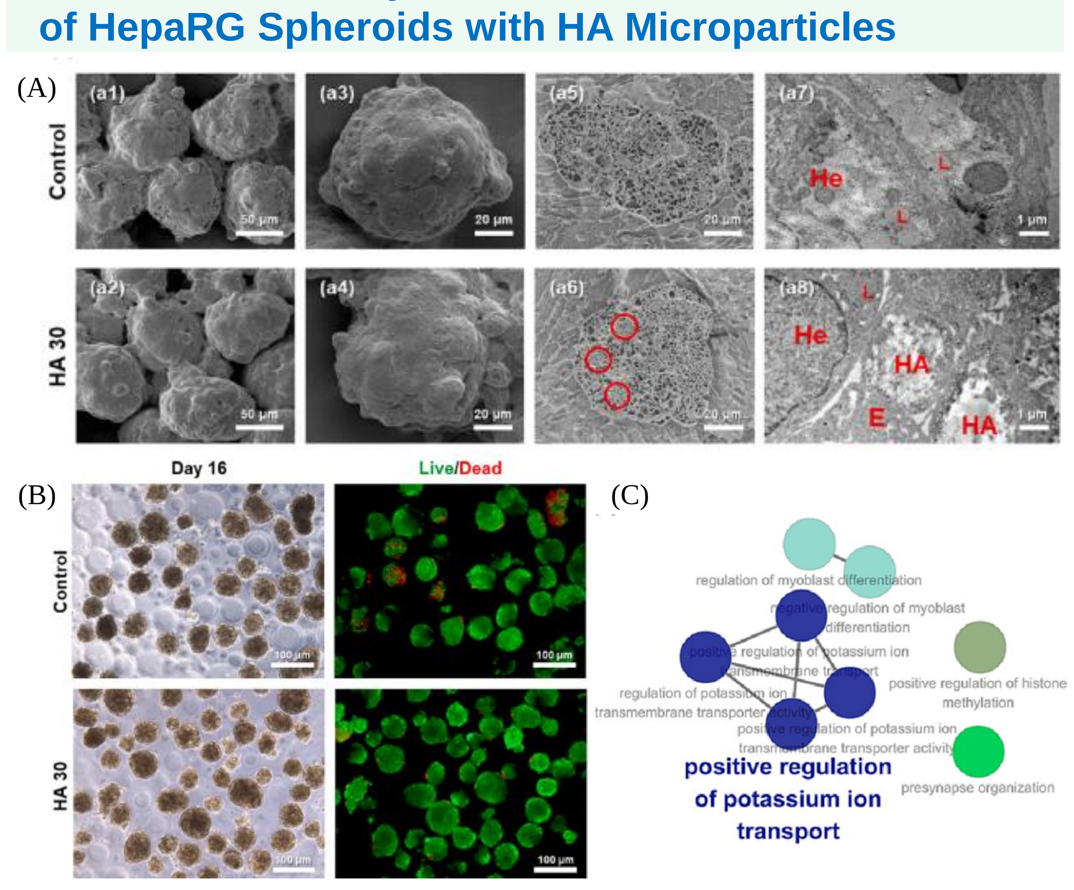


Figure 3. (A) Cryo-SEM and TEM images of HepaRG spheroids without (upper) or with HA microparticles (lower). a1-a4; cross-sectional Cryo-SEM images of the surfaces. a7-a8; TEM microstructures of the spheroids without or with HA microparticles. (B) LIVE/DEAD cell staining of the spheroids without (upper) or with HA microparticles (lower). The spheroids were cultured for 16 days and stained using calcein AM (green for live cells) and EthD-1 (red for dead cells). (C) ClueGO analysis of upregulated DEGs in HepaRG spheroids containing HA microparticles compared to spheroids without HA microparticles.

RESULTS

• Histological Analysis of Chondrocyte Spheroids with and without HA Microparticles Over Time

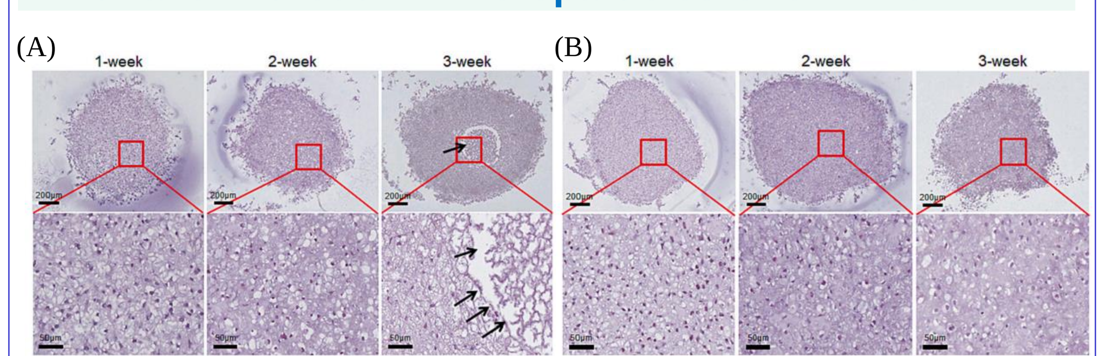


Figure 4. Histological analysis using H&E staining of (A) chondrocyte spheroids without HA microparticles and (B) chondrocyte spheroids containing HA microparticles at 1, 2, and 3 weeks of culture. The arrows point to the necrosis part.

• In Vivo Imaging and Histological Assessment of Chondrocyte Spheroids for Cartilage Regeneration

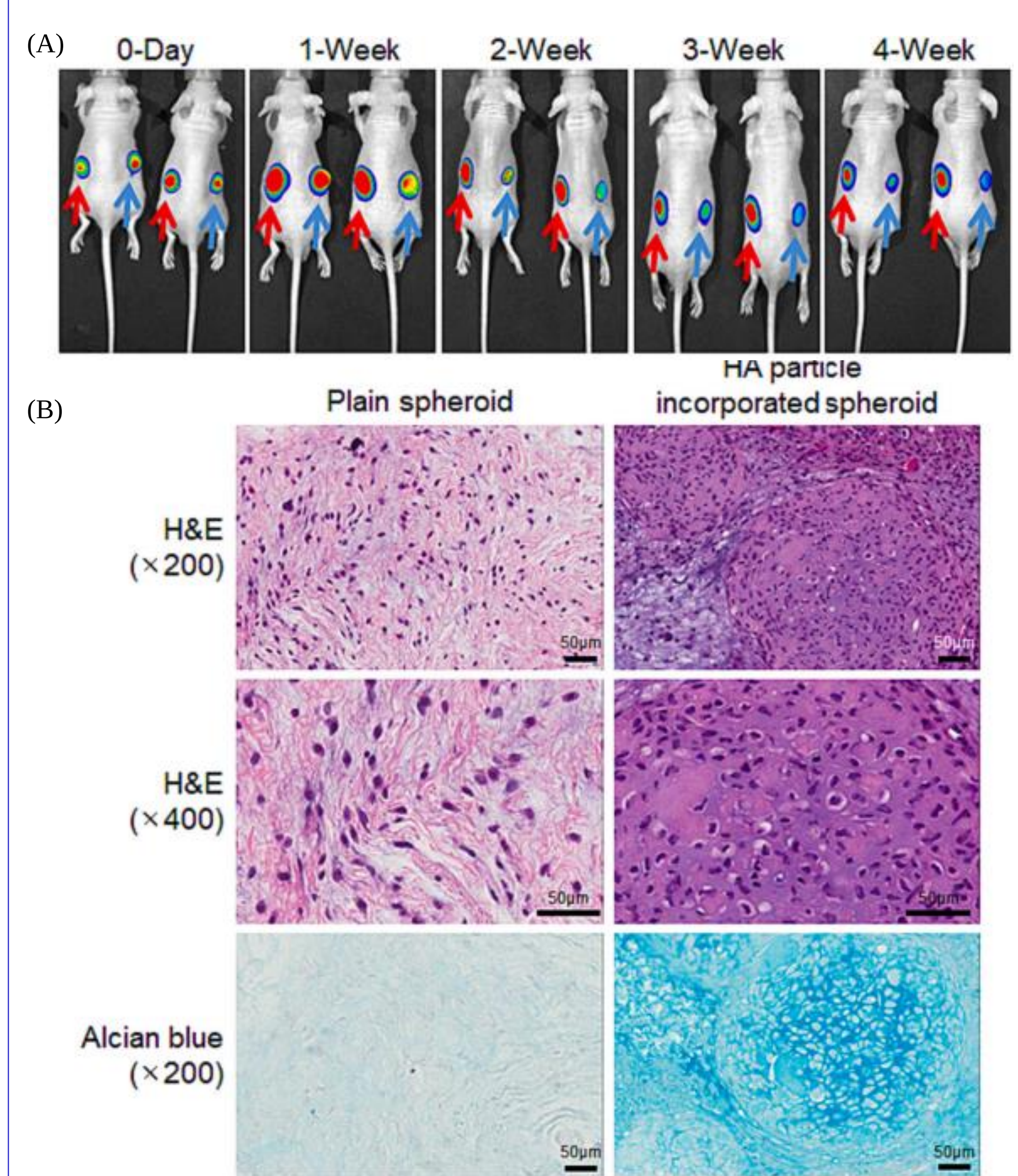


Figure 5. In vivo tracking and cartilage regeneration analysis. (A) Whole-body images using the IVIS Spectrum system at 1, 2, 3, and 4 weeks after injection of chondrocyte spheroids. Injected cells were labeled with CM DiI. Red arrows indicate chondrocyte spheroids containing HA microparticles, and blue arrows indicate chondrocyte spheroids without HA microparticles. (B) Histological analysis using H&E staining and alcian blue staining for in vivo cartilage regeneration after injection of chondrocyte spheroids.

CONCLUSIONS

- This study presents a scalable method for generating homogeneous chondrocyte spheroids using HA microparticles, with potential applications in regenerative medicine and drug screening.
- The potential of HA hydrogel microparticles in 3D spheroid culture to enhance cell survival and therapeutic efficiency for regenerative medicine.
- Spheroid culture with HA microparticles enhances cartilage regeneration by preserving chondrocyte function and supporting tissue formation, offering therapeutic potential.

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