High-density culture of bovine embryonic stem cell–derived mesenchymal cells on edible scaffolds for structured cultivated meat

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Abstract

Developing structured cultivated meat requires integrated solutions that combine scalable cell sources with edible, food-grade materials capable of supporting high-density growth and differentiation. Here, we evaluate bovine mesenchymal stem cells derived from embryonic stem cells (ESC-derived iMSCs) as a scalable adipogenic cell source and develop an integrated workflow combining these cells with edible plant-based scaffolds for structured biomass generation. Cell identity and functionality were assessed using transcriptomic, morphological, gene expression, flow cytometric, and adipogenic differentiation analyses, in both adherent and suspension culture systems. In parallel, lentil, pea and soybased scaffold formulations were screened for cell attachment, proliferation, and biomass accumulation. Soy-based scaffolds supported uniform cell distribution and robust growth and outperformed lentilbased scaffolds. Under dynamic culture conditions, bovine iMSCs cultured on soybased scaffolds achieved high-density growth, showing biomass accumulation (cell wet weight/scaffold wet weight) reached an average cell wet weight to scaffold wet weight ratio of 15% within three days. Cultures demonstrated active glucose metabolism and retained adipogenic differentiation capacity, confirmed by lipid accumulation and positive oil red O staining. These findings demonstrate an integrated cell–scaffold platform for rapid three-dimensional biomass generation. This approach supports the development of a cell culture strategy for structured cultivated meat by combining defined cell sources with food-grade scaffold technologies to improve scalability, structure, and nutritional relevance.

Keywords

cultivated meat; mesenchymal stem cells; adipogenesis; edible soybased scaffold; highdensity 3D culture; additive manufacturing

Highlights

• Bovine ESC-derived iMSCs enable scalable adipogenic cell production

• Edible soy-based scaffolds support 3D attachment and biomass accumulation

• Dynamic culture achieved ~15% cell wet weight fraction within 3 days

• iMSCs retained adipogenic differentiation capacity on edible scaffolds

• Integrated cell–scaffold culture supports structured cultivated meat prototypes

Introduction

Cultivated meat manufacturing requires cell sources that can be expanded reproducibly, directed toward relevant tissue phenotypes, and incorporated into edible three-dimensional structures. Cell sourcing is a core consideration across the cultivated meat production workflow, influencing achievable scale, differentiation outcomes, media requirements, and compatibility with downstream structuring approaches (Reiss et al., 2021). In parallel, the sensory and nutritional characteristics of cultivated products—particularly texture, color, flavor, and lipid content—are key determinants of product quality and consumer relevance (Fraeye et al., 2020; Lew et al., 2024).

Current approaches to cultivated meat commonly employ both primary animal cell sources and research model systems. For example, bovine muscle-derived fibro-adipogenic progenitor cells have been shown to support extensive proliferation and robust adipogenic differentiation, including within three-dimensional edible scaffold formats (Dohmen et al., 2022). In method development for structured tissues, murine cell lines such as C2C12 myoblasts and 3T3-L1 preadipocytes are frequently used to establish scaffold designs and fabrication strategies, including stacked muscle- and adipose-like constructs on edible protein-based materials (Li et al., 2022; Shahin-Shamsabadi & Selvaganapathy, 2022; Yao et al., 2024). These model systems are valuable for rapid prototyping of culture and structuring methods; however, translation to scalable livestock-derived cell platforms suitable for food production remains an active area of development (Reiss et al., 2021; Fish et al., 2020).

Mesenchymal stem or stromal cells (MSCs) represent a practical intermediate cell type for cultivated meat applications because they can be expanded *in vitro* and possess mesodermal lineage potential, including adipogenic differentiation. Bovine MSCs isolated from bone marrow have been shown to exhibit multilineage differentiation capacity, including adipogenic commitment under defined culture conditions, supporting their relevance as a livestock-derived cell source (Bosnakovski et al., 2005; Okamura et al., 2018; Hill et al., 2019). Adipose tissue is particularly relevant for cultivated meat products because fat contributes substantially to flavor, texture, and overall organoleptic perception, and recent studies demonstrate that cultivated fat can generate volatile and sensory profiles that overlap with those of conventional animal fat (Fraeye et al., 2020; Lew et al., 2024).

A second major challenge for cultivated meat is structurization; the formation of edible, tissue-like architectures that retain biomass while enabling nutrient transfer and handling during culture. In other biological contexts, self-assembly approaches have demonstrated that multicellular progenitor populations can form higher-order organoid-like structures under defined conditions (EbnerPeking et al., 2021; LewisIsraeli et al., 2021). However, producing macroscale structured biomass for food applications typically requires supporting biomaterials that provide mechanical integrity, facilitate cell attachment, and are compatible with manufacturing constraints.

Product-relevant scaffolds for cultivated meat must be edible, scalable, and permissive for cell adhesion, proliferation, and differentiation. Reviews of cultivated meat scaffolding emphasize the importance of food-grade materials and fabrication strategies that avoid scaffold removal

and reduce downstream processing complexity (Moslemy et al., 2023). Recent work using decellularized plant-derived scaffolds further demonstrates that edible architectures can support muscle and adipose cell attachment and differentiation and yield prototypes with measurable textural properties (Murugan et al., 2024). From a bioprocess perspective, porous edible scaffolds can intensify culture by increasing available surface area for cell adhesion and supporting high biomass accumulation, aligning scaffold design with scalable culture formats (Millbank et al., 2026).

In this study, we evaluate an integrated workflow combining bovine embryonic stem cell-derived mesenchymal stem cells (ESCderived iMSCs) with edible soy-based scaffolds for three-dimensional, high-density culture relevant to structured cultivated meat production (Figure 1). Cell identity, adipogenic competence, scaffold compatibility, and short-term high-density culture performance are assessed across a staged experimental framework encompassing cell characterization, scaffold screening, and dynamic culture evaluation.

Materials and Methods

Cell proliferation assays

Cell number and cell wet weight (ww) were determined using standard curves correlating MTT absorbance with both cell number and biomass. For the 40 min MTT incubation (Figure 5), cell wet weight was linearly related to cell number (cell wet weight = $2.7369 \times 10^9 \times$ number of cells), while exponential relationships were established between MTT absorbance and both cell number (number of cells = $5.5192 \times 10^5 \times \exp(4.1167 \times$ MTT absorbance)) and cell wet weight (cell wet weight = $5.0319 \times 10^{-3} \times \exp(1.5471 \times$ MTT absorbance)). For the 60 min MTT incubation (Figure 4), similar relationships were obtained, with cell wet weight linearly correlated to cell number (cell wet weight = $2.6138 \times 10^9 \times$ number of cells), and exponential correlations observed for cell number (number of cells = $2.7918 \times 10^5 \times \exp(3.8473 \times$ MTT absorbance)) and cell wet weight (cell wet weight = $9.8314 \times 10^4 \times \exp(3.3955 \times$ MTT absorbance)).

Cell attachment efficiency (η_a) was calculated as the ratio of the number of cells measured after 1 h of attachment (C_{xn}) to the initial number of cells seeded (C_{xo}), providing a measure of the proportion of cells successfully adhering to the scaffold ($\eta_a = C_{xn} / C_{xo}$). The fold increase (F_i) in cell number was determined by dividing the cell concentration at a given time point (C_{xn}) by the initial cell concentration at day 0 (C_{xo}), reflecting overall cell expansion during culture ($F_i = C_{xn} / C_{xo}$). The population doubling number (PD) was calculated as $\log(C_{xn} / C_{xo})$ divided by log(2), in accordance with De La GarzaRodea et al. (2012).

Cell coverage of scaffolds was defined as the percentage of scaffold mass occupied by cells and was calculated as the ratio of cell wet weight to scaffold wet weight. Specifically, cell wet weight (as determined from the standard curve and corrected for the proportion of scaffold analysed) was divided by the total scaffold wet weight to yield the percentage cell coverage.

iMSC culture

Bovine ESCderived mesenchymal stem cells (iMSCs; RT Bovine iMSC_5A, Holstein/Female; RT Bovine iMSC8A, Angus x Simmental/Male) were maintained in adherent culture on tissue culture-treated plates or flasks (StarLab, CC76827506; Avantor, 7342313) coated with 0.2% (w/v) gelatin (Sigma-Aldrich, G1393). Cells were cultured in a proprietary, serum-free MSC proliferation medium (Roslin Technologies, UK), with medium exchange every 2–3 days. The exact media formulation is proprietary and supplied by the manufacturer.

For suspension culture, bovine iMSCs were maintained in MSC proliferation medium in an Ambr® 250 unbaffled stirred-tank vessel (Sartorius, 0015A33). Ambr® 250 run parameters were maintained at 38.5 °C with a deadband of 0.5, pH was maintained at 7.4 with a deadband of 0.05, dissolved oxygen was maintained at a minimum of 30% by controlled sparging of air, equating to a minimum of 6.3% pure oxygen, stirring speed was maintained in the range from 100 – 110 rpm, the initial working volume was 100 mL and cultures were maintained by fed-batch process up to a volume of 200 mL. Suspension culture methods were additionally validated in ultra-low attachment 6-well plates (Corning, 3471) and 125 mL shaker flasks (Corning, 431143) cultured on an orbital shaker platform (orbital diameter 19 mm) (Thermo

Fisher Scientific, 88881102) set to 90–110 rpm, using the same proliferation medium. Cell seeding density was maintained at 5×10^5 cells mL^{-1} across all suspension culture systems.

Cells were passaged every 4–7 days when confluency of approximately 80% was reached and dissociated to single cells using TrypLE™ Select Enzyme (Gibco, 12563029). For adherent culture, cells were reseeded at 1×10^4 cells cm^{-2}, while suspension cultures were reseeded at 5×10^5 cells mL^{-1}. Cells used for experiments were between passages 3 and 5.

For adipogenic differentiation, bovine iMSCs were expanded in adherent or scaffold-based culture until reaching confluency (approximately 90100%). Cells were then cultured in a proprietary, serum-free adipogenic induction medium (Roslin Technologies) for 4 days, followed by a proprietary adipogenic maintenance medium (Roslin Technologies). Adipogenic maintenance was continued for an additional 3 days for adherent and scaffold cultures, or 10 days for suspension cultures. The media formulations are proprietary and supplied by the manufacturer.

All iMSC cultures were maintained in a humidified incubator or bioreactor at 38.5 °C with 5% CO_2. Routine sterility testing was performed throughout the study, and all cultures were confirmed to be free of mycoplasma contamination using a VenorGEM qEP Mycoplasma Detection Kit assay (Cambio, 119250).

Bovine satellite cell and murine cell culture

Primary bovine satellite cells (OpoMooM18; Opo Bio, New Zealand) were cultured according to the manufacturer's protocol. Briefly, cells were seeded at 3×10^3 cells cm^{-2} in T300 tissue culture flasks (TPP, 90301) in proliferation medium containing 20% fetal bovine serum (SigmaAldrich, F7524) and 5 ng mL^{-1} basic fibroblast growth factor (bFGF; SigmaAldrich, GF446). Cells were maintained until reaching 70–80% confluency before harvesting by enzymatic dissociation using TrypLE™ Select Enzyme (Gibco, 12563029).

Murine muscle precursor cells (C2C12, ATCC) were seeded at 1,500 cells cm^{-2} in T300 tissue culture flasks (TPP, 90301) and cultured in proliferation medium containing 10% fetal bovine serum (SigmaAldrich, F7524). Cells were harvested at 80–90% confluency using TrypLE™ Select Enzyme.

Primary bovine and murine cell cultures were maintained at 37 °C with 5% CO_2 in a humidified incubator.

Scaffold generation

Porous scaffolds were fabricated using a negative salt templating approach. Salt templates were produced using stereolithography (Prusa SL1S) with proprietary ink based on NaCl particles. Following printing, the salt templates were washed and underwent a proprietary thermal treatment to remove the binder. These templates were then used as sacrificial molds for scaffold generation.

Highprotein dispersions were prepared by mixing 25% (w/v) plant protein powders (pea (Bulk, 000273994), lentil (HSN, 220201C3), or soy (myvegan, P410615528); in a proprietary liquid

medium (sallea AG, Switzerland). Protein dispersions were prepared in a foodsafe environment and mechanically infiltrated into the printed salt templates using a 10mL syringe.

Following infiltration, the protein dispersions were cured within the salt templates for 60 min. The sacrificial salt templates were subsequently removed by dissolution in tap water at room temperature, yielding freestanding porous protein scaffolds. Scaffolds were then freezedried for up to 24h with a minimum of 8h using Home Freeze Dryer (Harvest Right). Initial freeze was performed at 17°C and the final drying was performed at 51°C. Scaffolds were stored dry at room temperature until use.

The resulting scaffolds were cylindrical in geometry, with an approximate height of 10 mm and diameter of 7 mm.

Preculture scaffold preparation and cell seeding

Prior to cell seeding, dried scaffolds were placed in a heat-resistant glass beaker and covered with aluminum foil before sterilization by autoclaving. Scaffolds were sterilized using a custom solids dry autoclave cycle at 121 °C for 15 min, followed by a 16-min drying phase. After sterilization, scaffolds were allowed to cool under sterile conditions before further handling.

Autoclaved scaffolds were transferred with sterile tweezers to 24well plates (TPP, 92124) preconditioned by incubation with 100 µL of undiluted FBS for 1 h at 37 °C (C2C12 and OpoMooM18) or a 1% (w/v) bovine serum albumin (BSA) solution (SigmaAldrich, A1470) for 1 h at 38.5 °C (iMSCs). Excess solution was removed immediately prior to cell seeding.

For seeding, bovine iMSCs were prepared as a concentrated singlecell suspension at a density of 5×10^6 cells per 80 µL. C2C12 and OpoMooM18 cells were prepared at a concentrated singlecell suspension at a density between 1 and 5×10^6 cells per 80 µL. A volume of 80 µL cell suspension was carefully applied directly onto each scaffold, corresponding to 5×10^6 cells per scaffold. Seeded scaffolds were incubated statically for 1 h at 37 °C (C2C12 and OpoMooM18) or 38.5 °C (iMSCs) to allow initial cell attachment.

Cells were seeded onto scaffolds either immediately following thawing of cryopreserved cell stocks (Figure 9) or following expansion in adherent culture prior to seeding (Figure 10). Subsequent scaffold culture was performed as described in the relevant experimental sections.

All scaffold handling and cell seeding steps were carried out using aseptic technique within a class 2 biological safety cabinet.

Scaffold culture

Screening

Following cell seeding, soybased (SP25) or lentilbased (LP25) scaffolds were transferred to ultralow attachment 6well plates (Corning, 3471), with one scaffold per well, and cultured in 3 mL of proprietary, serumfree MSC proliferation medium (RBDM1; Roslin Technologies, Edinburgh, UK). Plates were placed on a Benchmark OrbiShaker BT3001 rocking platform

operating at 30 rpm and maintained for 4 days in a humidified incubator at 38.5 °C with 5% CO_2. Culture medium was replaced daily.

Site validation

Following cell seeding, soy-based scaffolds (SP25) were cultured either in ultra-low attachment 6-well plates (Corning, 3471) or 125 mL shaker flasks (Corning, 431143), with three scaffolds per flask. Scaffolds cultured in 6-well plates were maintained in 3 mL of MSC proliferation medium (Roslin Technologies) per well, with daily complete medium changes.

For shaker flask cultures, scaffolds were maintained in 35 mL of MSC proliferation medium on an orbital shaker (orbital diameter 19 mm) (Thermo Fisher Scientific, 88881102) operating at 70 rpm. On day 2 of culture, 50% of the culture medium was exchanged for an equal volume of fresh medium. All cultures were maintained for a total of 3 days in a humidified incubator at 38.5 °C with 5% CO_2.

Scaffold processing and viability analysis

At the end of the culture period, scaffolds were removed from culture and bisected longitudinally using a sterile scalpel. One half of each scaffold was used for metabolic activity assessment by MTT staining and quantification, while the remaining half was reserved for nuclear staining and imaging as described below. Scaffold halves were briefly placed on absorbent paper to remove surface liquid before weighing to determine wet weight (ww) using an analytical balance (Veritas, LW203I).

MTT assay and biomass quantification

Scaffold halves allocated for metabolic analysis were incubated in 0.5 mL of 0.5 mg mL^{-1} MTT solution (Sigma Aldrich, M5655) prepared in phosphatebuffered saline. Incubations were performed for 40 or 60 min at 38.5 °C under static conditions. Following incubation, scaffolds were imaged using a Keyence VHXS660E stereomicroscope to document spatial distribution of formazan formation.

To solubilize the reduced MTT product, scaffold halves were submerged in 0.5 mL acidic ethanol 0.01 M HCl (Fisher Scientific, 12943544) in absolute ethanol (Fisher Scientific, 16685992) and incubated overnight at 4 °C. The resulting eluates were transferred to a transparent 96-well plate, and absorbance was measured at 600 nm using a plate reader (PerkinElmer, VICTOR® Nivo™).

Total biomass and the ratio of cell wet weight to scaffold wet weight (cell%, expressed as ww/ww) were calculated using standard curves generated from defined bovine iMSC numbers. Cell pellets of known cell numbers were prepared, centrifuged at 400 x *g* to remove excess liquid and weighed to determine pellet wet weight. Pellets were incubated under identical conditions and for the same duration as scaffold samples during the MTT assay and dye solubilization steps. Standard curves were generated for the following relationships: number of cells versus pellet wet weight, number of cells versus MTT absorbance, and pellet wet weight versus MTT absorbance.

Metabolic analysis

Culture supernatant samples were collected at the end of the indicated culture periods and clarified by brief centrifugation 400 × *g* for 2 min) prior to analysis. Supernatant pH, dissolved oxygen, dissolved carbon dioxide, and metabolite concentrations were analyzed using a MetaFLEX analyser (Beckman Coulter/Vi-Cell, Brea, CA, USA).

Metabolite analysis included quantification of glucose and lactate according to the manufacturer's protocols. The analyzer was calibrated prior to sample measurement using manufacturer-supplied standards and quality controls.

RTqPCR

Cell pellets were collected by centrifuging samples for 4 min at 400 × *g*, washing with DPBS (Gibco, 14190) and snap freezing at 80ºC. RNA was extracted using the RNeasy Mini kit (Qiagen, 74136), quantified by Nanodrop, and reverse transcribed using AffinityScript cDNA synthesis kit (Agilent, 200436) following manufacturer's instructions. qPCR reactions were prepared with 1x PowerSYBR green Mastermix (Applied Biosystems, 4367659), custom primers for the relevant target (IDT) at optimized concentrations of 50300nM, and 10ng sample cDNA per reaction and analyzed in a qPCR machine (Applied Biosystems, Quantstudio3). Sample results were normalized to a housekeeping gene HMBS and to a calibrator sample according to the delta Ct method (Schmittgen & Livak, 2008). Relative gene expression data was compared between test samples and undifferentiated controls as log2 fold change and analyzed by oneway ANOVA (GraphPad Prism).

RNAsequencing

Total RNA was extracted from samples and quantified as described above. Library preparation and sequencing were performed by an external service provider (GENEWIZ, Azenta Life Sciences). RNA-sequencing libraries were prepared from RNA isolated from ESCs, MSCs, and MP samples using the TruSeq Stranded mRNA Library Preparation Kit (Illumina). Libraries were sequenced on an Illumina NovaSeq 6000 platform to generate 150 bp paired-end reads, with an average depth of approximately 30 million reads per sample.

Raw sequencing reads were quality-checked using FastQC (v0.12.1). Adapter sequences and low-quality reads were removed using Cutadapt (v3.2) (Martin, 2011). Filtering criteria included removal of reads containing adapter sequences, reads with a Phred quality score <30, reads shorter than 50 bp after trimming, and reads with undetermined base calls (N) at the 5′ or 3′ ends.

Filtered reads were aligned to the *Bos taurus* reference genome (NCBI ARS-UCD2.0; assembly GCF_002263795.3) using STAR (v2.7.11a) (Dobin et al., 2013) with default parameters. Gene-level read counts were generated using featureCounts (v2.0.6) (Liao et al., 2014).

Differential gene expression analysis was performed using edgeR (v4.2.2) (Chen et al., 2025). Genes with an adjusted *P*-value < 0.05 and an absolute $\log_2$ fold change ≥ 2 were considered significantly differentially expressed.

Flow cytometry

Single-cell suspensions were prepared as described above and incubated in cell staining buffer consisting of Dulbecco's phosphate-buffered saline (DPBS; Gibco, 14190144) supplemented with 2% (v/v) chicken serum (Gibco, 16110). Cells were stained with a fixable viability dye (Invitrogen, L34963) and fluorophore-conjugated primary antibodies against CD44 (BioLegend), CD73 (Life Technologies), and SSEA-4 (BioLegend). Antibody incubations were performed for 40 min at ambient room temperature (2023 °C) protected from light.

Following primary antibody staining, cells were washed with staining buffer and incubated with an AlexaFluor™ 647-conjugated donkey anti-IgG secondary antibody (Invitrogen, A21448) at a dilution of 1:1000 for 30 min at ambient room temperature (2023 °C) protected from light. Cells were then washed again with staining buffer and resuspended in 100 µL for analysis.

Flow cytometric analysis was performed using a CytoFLEX™ 5 flow cytometer (Beckman Coulter). Compensation was carried out using fluorescence minus one (FMO) and unstained controls, and data were acquired and analyzed using CytExpert™ software (Beckman Coulter). Gating strategies were based on forward and side scatter to exclude debris, followed by viability gating and marker-specific fluorescence thresholds.

Staining and imaging

Adipogenic differentiation was assessed by lipid staining. For Oil Red O (ORO) staining, cultures were fixed with 4% (w/v) paraformaldehyde (Thermo Fisher Scientific, J61889) and rinsed with 60% isopropanol (Sigma-Aldrich, I9516). Fixed samples were then incubated with a 60% ORO solution (Sigma-Aldrich, O0625) for 10 min at room temperature, followed by washing with sterile distilled water to remove excess stain prior to imaging.

Alternatively, lipid accumulation in live cells was visualized by incubation with a fluorescent lipid dye. Live cultures were incubated with BioTracker™ 488 lipid dye (Merck, 3CT144) for 30 minutes at 38.5 °C according to the manufacturer's instructions before imaging.

For nuclear staining, live cell samples were incubated with NucBlue™ nuclear stain (Invitrogen, R37605) for 30 min at 38.5 °C prior to imaging.

Imaging was performed using an inverted fluorescence microscope equipped for brightfield and fluorescence imaging with 488 nm and 360 nm filter sets (Nikon Eclipse TS2). Where indicated, higher-resolution imaging was carried out using confocal laser scanning microscopy (Zeiss LSM 880). For all imaging modalities, acquisition and exposure settings were kept constant between experimental samples and corresponding controls.

Fatty acid analysis

Cell samples were collected by centrifugation at $400 \times g$ for 4 min, washed once with Dulbecco's phosphate-buffered saline (DPBS; Gibco, 14190-144), and snap-frozen at −80 °C. Control tissue samples representing conventional beef fat and muscle were obtained from a commercially available beef sirloin steak (Sainsbury's, UK), portioned into sterile tubes, and snap-frozen at −80 °C.

Frozen samples were lyophilized using a bench-top freeze dryer (Frozen in Time, Lablyo Mini) prior to weighing. Lyophilized samples were subsequently analyzed for fatty acid composition and quantification by gas chromatography at an external analytical service provider (Vitas Analytical Services, Oslo, Norway).

Results

Generation and characterization of bovine iMSCs in 2D and 3D culture

To establish a scalable bovine MSC source with adipogenic potential, pluripotent RT bovine embryonic stem cells (ESCs) were differentiated through an intermediate mesodermal progenitor (MP) stage to an induced mesenchymal stem cell (iMSC) state (Figure 2). This staged differentiation strategy generated a cryopreservable mesodermal progenitor intermediate population and a downstream iMSC population suitable for expansion and functional evaluation.

To assess transcriptional changes throughout the differentiation pathway, RNA-sequencing was performed on ESCs, MPs, and iMSCs. Unsupervised clustering revealed that each differentiation stage formed a transcriptionally distinct population, indicating clear transitions in gene expression programs during lineage progression (Figure 3). ESCs derived from three individual animals clustered tightly by cell type, while MP and iMSC populations derived from two ESC lines (BEIH5 and BEIH8) formed separate, stage-specific clusters with high similarity between biological replicates (Figure 3, Supplementary Figure 1). These data demonstrate that differentiation from ESCs to iMSCs is associated with reproducible and well-defined transcriptional remodeling rather than gradual or heterogeneous drift.

Following differentiation, bovine iMSCs were evaluated for their capacity to expand under both two-dimensional (2D) and three-dimensional (3D) culture conditions. In adherent 2D culture, iMSCs formed a monolayer and exhibited an elongated spindle-shaped morphology characteristic of mesenchymal stem cells (Figure 4A). In parallel, iMSCs were successfully expanded in 3D suspension culture within stirred-tank bioreactors, where they self-assembled into multicellular aggregates with diameters below 400 µm (Figure 4B). These observations indicate that bovine iMSCs can be propagated in both planar and suspension formats without loss of viability or gross morphological abnormalities.

To confirm acquisition of a mesenchymal phenotype, expression of canonical MSC markers was assessed following expansion in both culture formats. After seven population doublings in suspension culture and ten population doublings in adherent culture, iMSC populations showed significantly increased expression of the MSC markers CD90 and CD105 compared to undifferentiated ESCs, as measured by RT-qPCR ($p < 0.0001$, $n = 3$) (Figure 4C). These results confirm successful transition from a pluripotent to mesenchymal transcriptional state and demonstrate that marker expression is maintained during early expansion.

Cell type purity within expanded iMSC banks was further assessed by flow cytometry. After 12 population doublings in adherent culture, more than 85% of cells exhibited co-expression of the MSC-associated surface markers CD73 and CD44 (Figure 4D), indicating a highly enriched mesenchymal population. Due to the lack of species-compatible antibodies, hematopoietic exclusion markers (CD45, CD34, CD14, and HLA-DR) could not be evaluated; however, residual expression of the ESC-associated marker SSEA-4 was detected in fewer than 3% of cells (Figure 4D), suggesting minimal persistence of undifferentiated cells. Consistent

with their mesenchymal identity, iMSCs showed strong adherence to tissue-culture plastic and could be readily expanded under standard adherent conditions (data not shown).

Collectively, these results demonstrate that bovine iMSCs can be reproducibly derived from pluripotent ESCs, exhibit stable mesenchymal marker expression following expansion, and are compatible with both adherent and suspension culture formats suitable for downstream bioprocessing applications.

Adipogenesis of bovine iMSCs is possible in 2D and 3D culture

To assess whether bovine iMSCs retained functional lineage competence following expansion, cells cultured in either three-dimensional (3D) suspension culture (7 population doublings) or two-dimensional (2D) adherent culture (12 population doublings) were induced toward adipogenic differentiation. This enabled evaluation of adipogenic potential following expansion under both planar and suspension formats relevant to bioprocess development.

Following adipogenic induction, cultures in both expansion formats accumulated visible intracellular lipid droplets (Figure 5Ai, Bi). Lipid deposition was evident by brightfield imaging and was further confirmed by positive Oil Red O staining and fluorescent lipid labeling (Figure 5Aii, Bii). In contrast, undifferentiated control cultures showed no detectable lipid accumulation (Supplementary Figure 2), indicating that lipid formation was dependent on adipogenic induction rather than prolonged culture.

At the molecular level, differentiated adipogenic cultures exhibited significantly increased expression of the adipogenic latestage marker genes *FABP4* and *PPARγ* relative to undifferentiated controls ($p < 0.005$, $n = 3$) (Supplementary Figure 3), consistent with activation of an adipogenic gene regulatory program.

To quantify lipid accumulation, fatty acid (FA) composition was measured in differentiated cultures and compared with undifferentiated cell populations. Adipogenic differentiation under 3D conditions resulted in a four-fold increase in total FA abundance relative to undifferentiated ESCs and a 2.4-fold increase compared to undifferentiated iMSCs (Figure 6A), consistent with the establishment of a lipid-rich phenotype.

In addition to increased lipid abundance, differentiated adipogenic cultures displayed a fatty acid composition broadly comparable to that of conventional bovine adipose tissue. Palmitic acid, oleic acid, and stearic acid were the most abundant fatty acids detected in both differentiated cultures and supermarket beef steak fat samples (Figure 6B). This similarity indicates that iMSC-derived adipogenic cultures generate lipid profiles aligned with native bovine fat.
Together, these results demonstrate that bovine iMSCs retain robust adipogenic differentiation capacity following both 2D and 3D expansion, resulting in increased lipid accumulation and fatty acid compositions characteristic of bovine adipose tissue, despite current limitations in species-specific reagent availability for some adipogenic markers.

Scaffold design for optimal cell culture conditions

To evaluate scaffold architectures suitable for scalable cell culture, structured protein-based scaffolds were fabricated with a highly ordered gyroid geometry and compared with conventional sponge-like scaffolds. Key physical properties relevant to cell culture performance (including pressure loss, internal surface area, and mechanical stability) were assessed.

Computational and experimental characterization showed that gyroid structured scaffolds had up to 2 x 10^1 Pa and exhibited a 22fold reduction in pressure loss compared with spongelike constructs (Figure 7Ai). This resulted in a uniform flow pattern in the interior of the scaffold with a flow rate lower than 4.3 x 10^2 m/s, in a range suitable for cell proliferation (Figure 7Aii). In parallel, the internal surface area of the gyroid scaffolds increased approximately 3.5fold relative to sponge controls (Figure 7A). These combined changes indicate substantially improved internal mass transfer characteristics while increasing available surface area for cell attachment.
Mechanical performance of the scaffolds was evaluated during extended exposure to culture conditions. Scaffolds retained structural integrity for up to 11 days in culture medium at 37°C, both in the presence and absence of cells, indicating that the material and architecture were stable under biologically relevant conditions.

Quantitative mechanical testing further showed that the elastic modulus (Emodulus) of soy-based scaffolds fell within a range of 3560 kPA, previously reported to support growth and differentiation of mesodermderived cells (Figure 7Bi) (Pavan et al., 2020; Ward et al., 2009). These measurements indicate that scaffold stiffness is compatible with MSC culture without imposing excessive mechanical constraint.

To benchmark scaffold textural properties against conventional meat tissues, texture profile analysis was performed. No statistically significant differences were observed between hydrated soy-based scaffolds without cells and beef samples across most measured parameters, with the exception of increased cohesiveness in the scaffold material (Figure 7Bii). This result indicates partial overlap between scaffold mechanical properties and those of native muscle tissue, while highlighting a distinct difference in cohesiveness.

Together, these results demonstrate that gyroid-structured protein scaffolds provide favorable mass-transfer properties, mechanical stability, and tissue-relevant textural characteristics, supporting their suitability for subsequent high-density cell culture applications.

Experimental conditions for seeding research cell lines on plant scaffolds

To determine suitable experimental seeding and culture conditions on plant-derived scaffolds, multiple culture configurations were evaluated with research cell lines, including variations in scaffold material, medium grade, seeding density, and culture format (Figure 8). Murine and primary bovine progenitor cells were initially used as a screening model to compare scaffold materials and media formulations under controlled conditions.

During a four-day culture period, murine progenitor cells were seeded onto pea-based (PP25), soybased (SP25) and lentilbased (LP25) scaffolds under rocking conditions in low attachment 6-well plates and accumulated measurable biomass in both food-grade (FG) and pharmaceutical-grade (PG) basal media (Figure 8B). Comparative analysis of scaffold materials showed that murine progenitor cells preferentially accumulated biomass on pea-based scaffolds relative to lentil- or soy-based scaffolds (Figure 8B), indicating scaffold-specific differences in support of cell attachment and growth. Cultures maintained in FG medium achieved a mean biomass content of 12.5%, compared with 8% in PG medium (Figure 8B). These results demonstrate that medium formulation influences biomass accumulation under otherwise identical culture conditions.
Based on these outcomes, both pea and soybased scaffold types were screened in subsequent experiments with bovine iMSCs.

To assess scalability and transferability between culture formats, scaffold cultures were compared between 6-well rocking conditions and 125 mL shaker flask cultures. Cultures maintained in dynamic rocking conditions showed increasing cell growth over 7 days (Figure 8C). No statistically significant differences in final cell numbers were observed between the two systems (Figure 8C), indicating that scaffold-based cultures could be transferred from static or semi-static formats to more dynamic, scalable culture conditions without loss of cell yield.

Biomass production efficiency was further evaluated by analyzing the ratio of final cell density to initial seeding density using a machine learning–based analytical approach (LABMaiTE GmbH). This analysis revealed that, in FG medium, both murine progenitor cells and bovine satellite cells achieved higher biomass production efficiency when seeded at lower initial densities (Figure 8D). In contrast, bovine cells cultured in PG medium showed higher efficiency at increased seeding densities. These results indicate that optimal seeding density is both cell type and medium dependent on soybased scaffolds.

Collectively, these data demonstrate that scaffold material, medium grade, and seeding density significantly influence biomass accumulation and production efficiency, and that optimized scaffold cultures are transferable between laboratory-scale and dynamically mixed culture systems.

Comparison of bovine iMSC growth on soy and lentilbased scaffolds

To assess scaffold compatibility for bovine iMSC culture, cell attachment and short-term expansion were compared across plant-derived scaffold materials. Initial screening indicated that bovine iMSCs attached more efficiently to pea- and soy-based scaffolds than to lentil-based scaffolds (Figure 9A), suggesting similar material-dependent differences in early cell–scaffold interactions (Figure 8B).

To further compare scaffold performance, bovine iMSCs were cultured on lentil- and soy-based scaffolds for four days under identical conditions. Cells cultured on soy-based scaffolds

exhibited a more homogeneous spatial distribution throughout the scaffold compared with those on lentil-based scaffolds (Figure 9B). Quantitative analysis showed significantly higher total cell number on soy-based scaffolds after four days of culture (Figure 9C), together with an increased ratio of cell wet weight to scaffold wet weight (Figure 9D), indicating greater biomass accumulation than that on lentilbased scaffolds.

In addition to culture in low volume static and rocked 6well plate culture formats, the shortterm compatibility of bovine iMSC–scaffold constructs was evaluated in dynamic systems. iMSC-seeded scaffolds were successfully maintained during shortterm culture in perfusion and TideMotion bioreactor systems with cell attachment and without observable loss of scaffold integrity (data not shown), supporting the transferability of the soybased scaffold–cell combination to more dynamic culture environments.

Taken together, these results demonstrate that soy-based scaffolds provide superior support for bovine iMSC attachment, distribution, and early biomass accumulation compared with lentil-based scaffolds and are compatible with both static and dynamic culture formats relevant to scalable cultivation.

Bovine iMSC culture optimized for dynamic culture on soybased scaffolds

To evaluate short-term, high-density bovine iMSC culture under conditions relevant to dynamic processing, soy-based scaffolds were used in a site-validation experiment designed to assess reproducibility in a different laboratory environment and to compare static versus shaker-flask culture over a three-day period (Figure 10A). The experiment focused on high-density seeding and short culture durations to enable direct comparison of early attachment, distribution, metabolic performance, and biomass accumulation under dynamic mixing conditions.

After three days of culture, MTT staining indicated that iMSC coverage on soy-based scaffolds under dynamic shaker-flask conditions was comparable to the coverage observed previously at four days in the earlier scaffold comparison study and was increased relative to day 0 controls and statically cultured scaffolds (Figure 10). Consistent with this, nuclear staining showed regions in which cells began bridging scaffold pores under dynamic conditions, indicating progression from surface attachment toward three-dimensional coverage within the scaffold architecture (Figure 10B).

Culture supernatant analysis demonstrated distinct metabolic profiles between dynamic and static conditions (Figure 10C). Shaker-flask cultures showed higher glucose consumption and lactate production rates relative to static controls, alongside lower oxygen consumption per scaffold (Figure 10C, 10D, 10E). Together, these measurements are consistent with increased overall metabolic activity and greater biomass accumulation under dynamic culture conditions (Figure 10).

Quantitative MTT-based analysis further supported enhanced biomass accumulation in shaker-flask cultures. Biomass accumulation (cell wet weight/scaffold wet weight) was

significantly higher under dynamic conditions than under static culture, with an average 3.5-fold increase in cell wet weight fraction to approximately 15% after three days (Figure 10F). This corresponded to an approximately 8.7-fold increase in biomass, with mean yields of approximately 25×10^6 cells per scaffold (Figure 10F–G). These quantitative outcomes align with the higher metabolite turnover observed in dynamic cultures (Figure 10C).

Collectively, these results show that bovine iMSCs can be cultured reproducibly at high density on soy-based scaffolds under dynamic conditions, achieving rapid increases in scaffold coverage and biomass accumulation over short culture durations, with accompanying increases in measurable metabolic activity.

Functional analysis of bovine iMSCscaffold conditions by adipogenic differentiation

To determine whether bovine iMSCs cultured on soy-based scaffolds under dynamic conditions retained functional differentiation capacity, scaffold-cultured cells were subjected to a seven-day adipogenic differentiation protocol following high-density shaker-flask culture for 7 days (Figure 11A). This experiment was designed to assess adipogenic competence after exposure to scaffold materials and dynamic culture conditions relevant to scale-up.

Following adipogenic differentiation, ORO staining revealed accumulation of intracellular lipid droplets within iMSCs cultured and differentiated on soy-based scaffolds in shaker-flask conditions (Figure 11B). In contrast, undifferentiated iMSC–scaffold cultures maintained under identical conditions showed no detectable ORO–positive staining (Figure 11C), indicating that lipid accumulation was differentiation-dependent rather than an artefact of scaffold association or dynamic culture.

Taken together, the observed biomass accumulation and preserved adipogenic differentiation highlight the suitability of this scaffold-based dynamic culture strategy for structured cultured tissue applications.

Discussion

Cultivated meat manufacturing remains constrained by three linked challenges: (i) establishing renewable, well-characterized livestock cell sources that can be banked, expanded and differentiated to key target cell types reproducibly; (ii) achieving high-density culture at scale while maintaining acceptable nutrient delivery and waste removal; and (iii) enabling edible structurization so that biomass can be formed into food-relevant architectures efficiently and without costly scaffold removal steps. Recent techno-economic syntheses emphasize that feasibility at industrial scale depends strongly on productivity and time-in-culture, and that many published laboratory workflows still do not align with the assumptions used in scale-up models (Goodwin et al., 2024). In parallel, recent reviews of cultivated meat scaffolds stress that edible, non-animal materials must satisfy both biological requirements (attachment, differentiation, mass transfer) and food constraints (safety, manufacturability, and ingredient compatibility) (Seibert et al., 2025; Qin et al., 2026). Finally, generating fat is increasingly recognized as essential for food relevance, because adipose tissue strongly affects organoleptic perception, and scaffold engineering can modulate differentiation quality and downstream food-relevant attributes (Lee et al., 2024).

The present study demonstrates an integrated workflow that addresses each of these constraints by combining a renewable bovine ESCderived iMSC platform with edible soybased scaffolds and dynamic culture to achieve rapid biomass accumulation while retaining adipogenic differentiation capacity. The iMSC platform offers a reproducible starting point aligned with the need for stable, banked cell sources highlighted by technoeconomic analysis (TEA) literature (Goodwin et al., 2024). At the materials level, soybased scaffolds were selected because plantprotein scaffold systems are increasingly supported as foodcompatible structuring substrates, and soy in particular has been reported to support strong adhesion and proliferation in stemcellrelevant contexts (Kim et al., 2024). Consistent with that direction, our results show preferential bovine iMSC attachment, distribution, and early biomass accumulation on soy-based scaffolds, with peabased scaffolds as an alternative regarding seeding, compared with lentilbased scaffolds, supporting soy as a practical edible scaffold material for shortterm, high-density culture.

A key operational finding was that dynamic culture improved short-term productivity relative to static controls. Under shaker-flask conditions, cellscaffold cultures exhibited increased glucose consumption and lactate production alongside increased biomass accumulation, consistent with the general observation that proliferative mammalian cells can display high glycolytic flux with lactate formation under common culture conditions (Hefzi et al., 2025). While mechanistic interpretation should remain conservative without pathway-level flux analysis, the observed increase in net biomass formation under dynamic culture conditions is consistent with improved effective mass transfer during the rapid expansion phase, which is precisely the type of process-intensification lever highlighted as important for bridging bench workflows to manufacturing-relevant processes (Goodwin et al., 2024).

Beyond expansion, the study also demonstrates functional adipogenesis after scaffoldbased culture, addressing a major gap in many structurization workflows where expansion and

differentiation are not integrated in a foodcompatible manner. Lipid accumulation was confirmed by staining following adipogenic induction. This aligns with recent work showing that scaffold engineering can be used to regulate differentiation quality and improve food-relevant attributes in cultured meat constructs (Lee et al., 2024). Our findings also sit alongside emerging evidence that dynamic 3D culture strategies can produce stable adipogenic building blocks suitable for downstream structuring approaches (Klatt et al., 2024). While composition of FA on scaffolds was not analyzed, evidence showing the nutritional similarity of differentiated adipogenic iMSCs to bovine tissue shows the potential of this cell source to provide lipids to improve the nutritional composition of a cellscaffold food prototype. Importantly, our workflow links highdensity scaffold culture directly to onscaffold adipogenic differentiation, supporting a more integrated "culturetostructured prototype" approach consistent with the direction suggested by recent scaffold and cultivated meat structurization reviews (Seibert et al., 2025; Qin et al., 2026).

In terms of edible scaffold choice and broader comparability, recent studies have shown soy-based edible scaffolds can support 3D muscle tissue formation and that scaffold microstructure can influence tissue yield and matrix deposition, reinforcing the broader potential of soy as a structuring substrate for cultivated meat rather than merely a carrier (Mariano et al., 2025). Complementary findings in cultivated meat bioprocess intensification (e.g., scalable operational strategies in serum-free contexts) further support the general principle that unit-operation optimization materially improves manufacturing-relevant outcomes (Bodiou et al., 2025). Taken together, these comparisons suggest that the key value of the present work is not simply demonstrating scaffold compatibility, but demonstrating a short-cycle, dynamic, edible scaffold workflow that enables cell expansion and differentiation for a production relevant output.

Several limitations should be considered when interpreting and extending these findings. Firstly, while adipogenesis and FA composition support functional lipid formation, broader nutritional equivalence would benefit from additional characterization beyond bulk FA profiles (e.g., lipid class distribution and stability under relevant processing conditions), as emphasized by recent organolepticfocused cultivated meat studies (Lee et al., 2024). Secondly, because high lactate production can become inhibitory at larger scales, further work should define operating windows (agitation and feeding strategies, oxygen transfer) that sustain productivity while limiting accumulation of growthlimiting byproducts, consistent with broader mammalian cell bioprocess considerations (Hefzi et al., 2025). Similar shifts toward elevated glycolytic byproduct formation under intensified or stressassociated culture conditions have been widely reported in mammalian cell systems, reinforcing that increased lactate production is a common feature of highdensity culture rather than a systemspecific metabolic mechanism (da Veiga Moreira et al., 2021). Thirdly, as scale increases, gradients and heterogeneity become more influential; this is repeatedly flagged as an open challenge in TEA literature and motivates future experiments in more controlled and scalable bioreactor formats (Goodwin et al., 2024).

Future experiments should therefore focus on: (i) systematic optimization of food-grade media composition and feeding strategies to improve yield and reduce waste metabolite accumulation;

(ii) finetuning of scaffold architecture (e.g., pore characteristics and surface properties) to further improve uniform cell distribution and maturation, consistent with scaffold-focused research trends (Seibert et al., 2025; Qin et al., 2026); and (iii) expanding food-relevant characterization toward texture evolution and sensory-linked chemistry, building on prior evidence that differentiation quality and matrix cues can influence organoleptic outcomes (Lee et al., 2024). Collectively, the results support an integrated strategy in which renewable bovine iMSC banks, edible soy-based scaffolds, and dynamic culture conditions combine to accelerate structured biomass generation in a manner aligned with current scale-up and structurization priorities in the cultivated meat field.

Data Availability Statement

The data supporting the findings of this study are available within the article and its supplementary materials. Sequence data that can be downloaded from Roslin Technologies servers on request from the corresponding author. Proprietary media and scaffolds are available from Roslin Technologies and sallea AG.

Ethics Statement

The bovine embryonic stem cell lines used in this study were provided by Genus plc and was generated, banked, and supplied under the appropriate regulatory approvals, licenses, and ethical oversight in accordance with relevant national and international guidelines. All work described in this manuscript used previously established cell lines; no new animalderived material was generated, and no live animal procedures were performed as part of this study.

Author Contributions

Madeleine Carter, Tim Spitters, and Deepika Rajesh contributed to the conceptualization of the study. Methodology was developed by Madeleine Carter and Tim Spitters. Investigation was carried out by Sarah Ho on adipogenesis, Sophia Webb on RNAseq analysis, and Niamh Hyland for the provision of cells and tissue culture expertise. Formal oversight was performed by Patrick Joseph Mee and Deepika Rajesh. Resources and data relating to scaffolds were provided by Simon Fehlmann. All authors contributed to construction, reviewing and editing the manuscript. All authors have read and approved the final manuscript.

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Conflict of Interest

All authors are either employees of Roslin Technologies Ltd or sallea AG. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest beyond employment.

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Figures

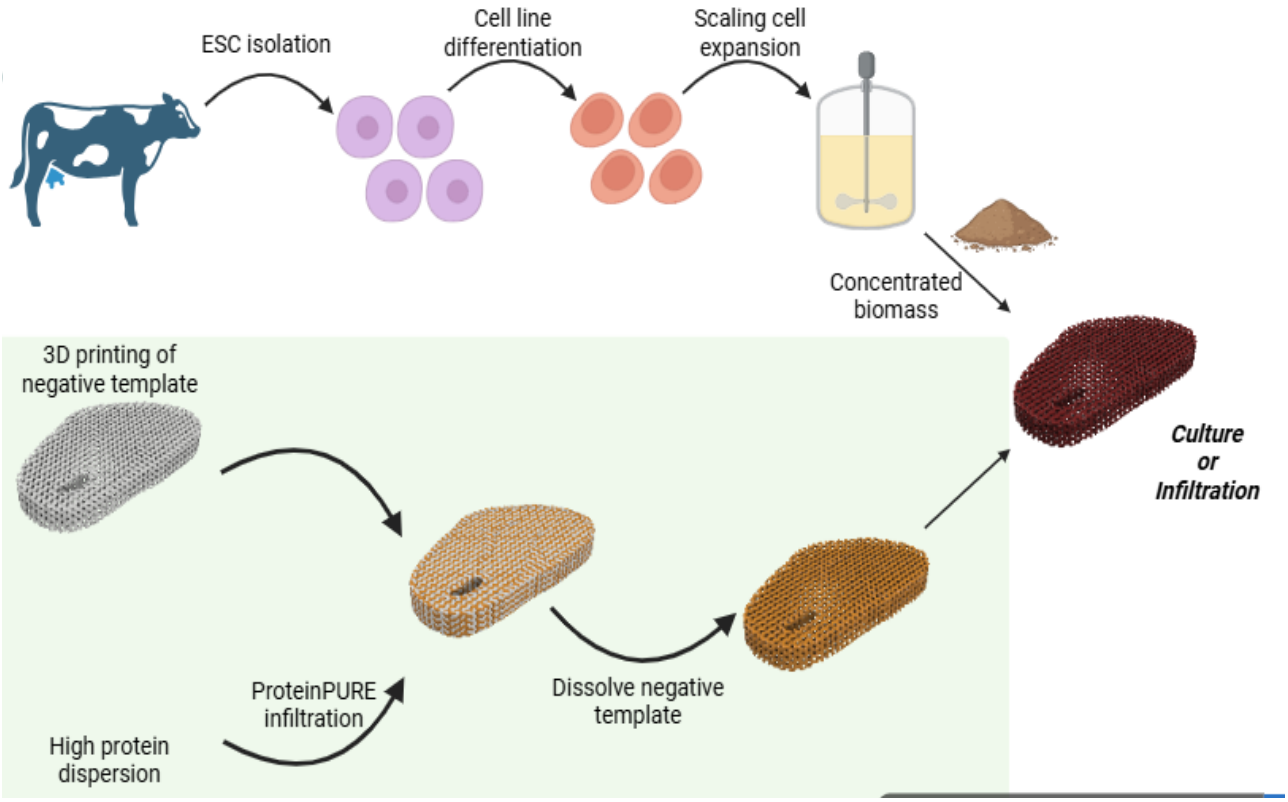


Figure 1. Integrated cell derivation and scaffold-based workflow for three-dimensional biomass generation. Schematic overview of the experimental strategy used to generate structured three-dimensional biomass for cultivated meat applications. Pluripotent bovine embryonic stem cells (ESCs) are isolated, expanded, and differentiated through defined lineage stages to generate mesenchymal stem cells, which are subsequently expanded for biomass production. In parallel, edible plant-protein scaffolds are fabricated using additive manufacturing of a sacrificial template followed by protein infiltration to create defined porous structures suitable for cell culture. The two components are combined by culturing or seeding cells onto the edible scaffolds, enabling three-dimensional cell attachment, expansion, and downstream differentiation within a food-grade matrix.

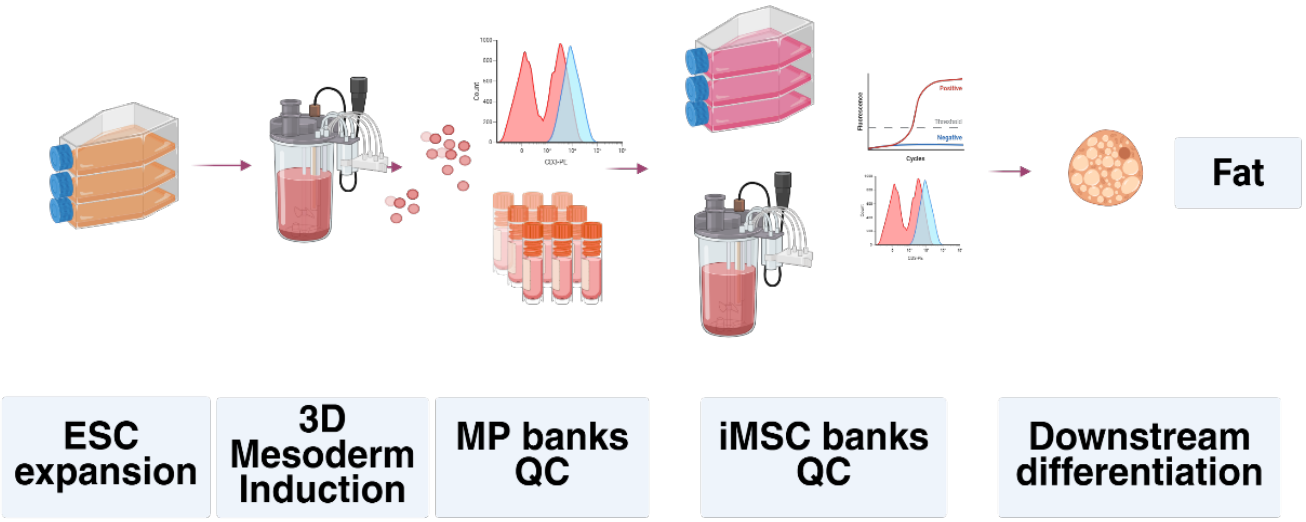


Figure 2. Derivation and phenotypic characterisation of bovine embryonic stem cell–derived mesenchymal stem cells. Pluripotent bovine embryonic stem cells (ESCs) were expanded and induced toward mesodermal fate under three-dimensional (3D) culture conditions, generating mesodermal progenitor (MP) populations that were subsequently expanded and cryopreserved as MP banks. Controlled lineage specification from MP stages yielded induced mesenchymal stem cells (iMSCs), which were further expanded to establish iMSC cell banks following quality control (QC) assessment. Molecular and phenotypic characterisation of banked iMSCs was performed using RT-qPCR and flow cytometry to confirm acquisition of mesenchymal identity. Functional qualification of iMSC banks was achieved by demonstrating the capacity for downstream adipogenic differentiation, supporting their suitability as a renewable mesenchymal cell source for scaffold-based culture applications.

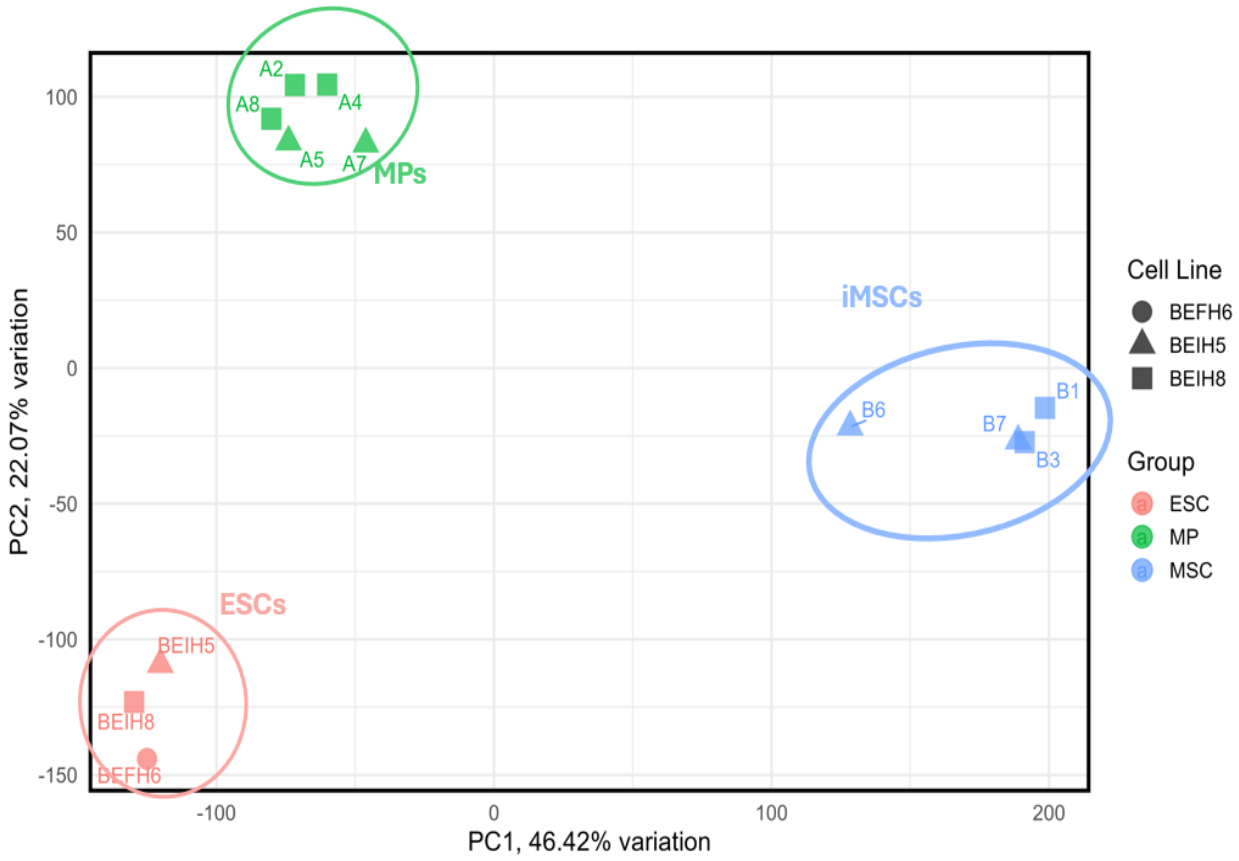


Figure 3. Transcriptomic profiling of bovine embryonic stem cell–derived mesenchymal stem cell differentiation. Transcriptomic profiling of bovine embryonic stem cells (ESCs), mesodermal progenitors (MPs), and induced mesenchymal stem cells (iMSCs) was performed by RNA sequencing and visualised using principal component analysis (PCA). The analysis shows distinct clustering of samples according to differentiation stage, supporting transcriptional divergence during lineage progression from pluripotent ESCs through MP intermediates to iMSCs.

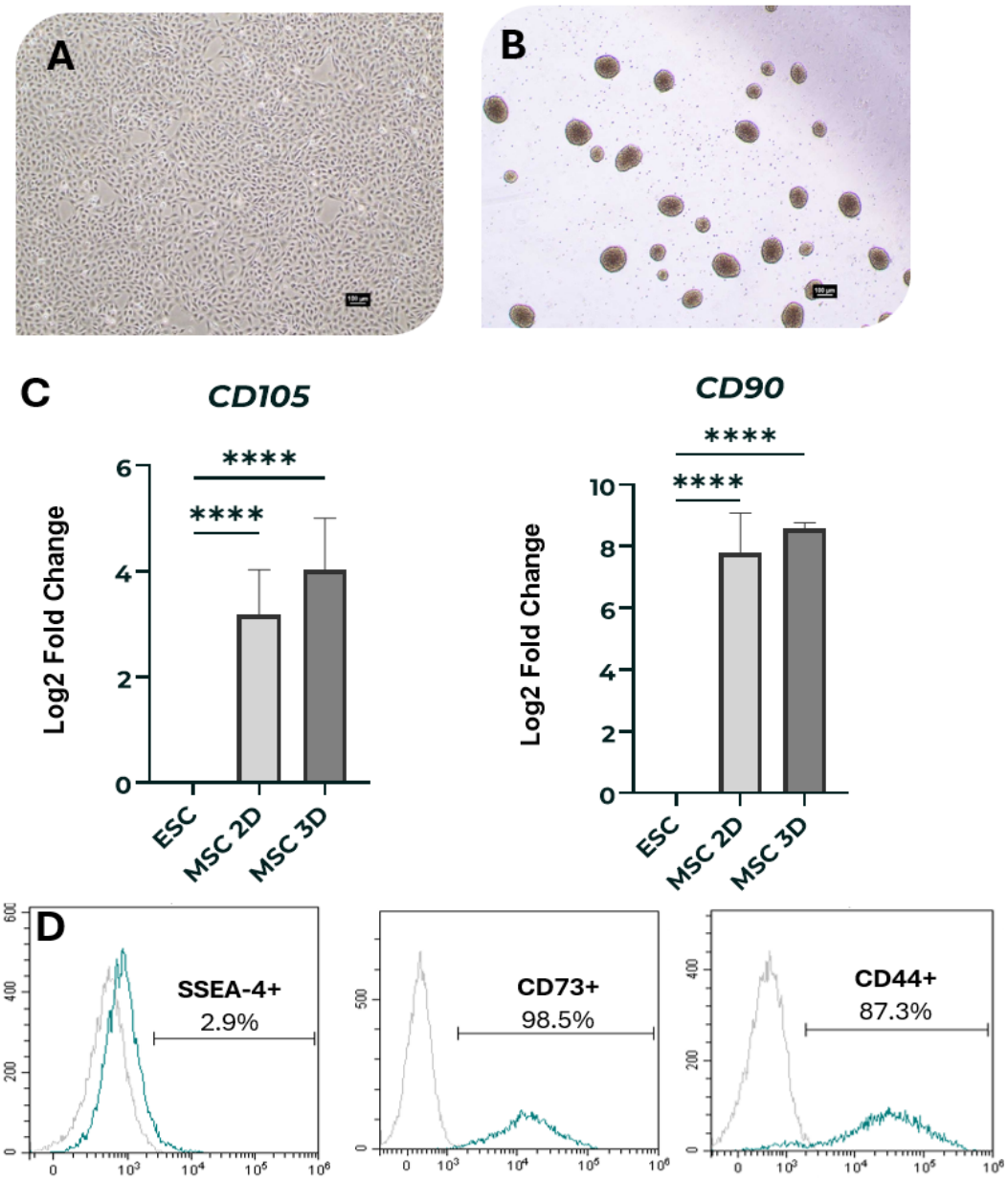


Figure 4. Phenotypic confirmation of bovine embryonic stem cell–derived mesenchymal stem cells in two- and three-dimensional culture. Bovine induced mesenchymal stem cells (iMSCs) were evaluated following expansion in both two-dimensional adherent culture and three-dimensional culture formats. Representative cell morphology is shown for iMSCs maintained under 2D (A) and 3D conditions (B). Molecular characterisation by RT–qPCR assessed expression of mesenchymal stem cell–associated markers relative to undifferentiated embryonic stem cells (n=3, ****=p<0.0001). Flow cytometric analysis was used to assess surface marker expression and confirm loss of pluripotency-associated antigens following differentiation, alongside acquisition of canonical mesenchymal surface markers (D) documenting the phenotypic characteristics of iMSCs expanded under planar and three-dimensional culture conditions. Scale bar = 100 µm.

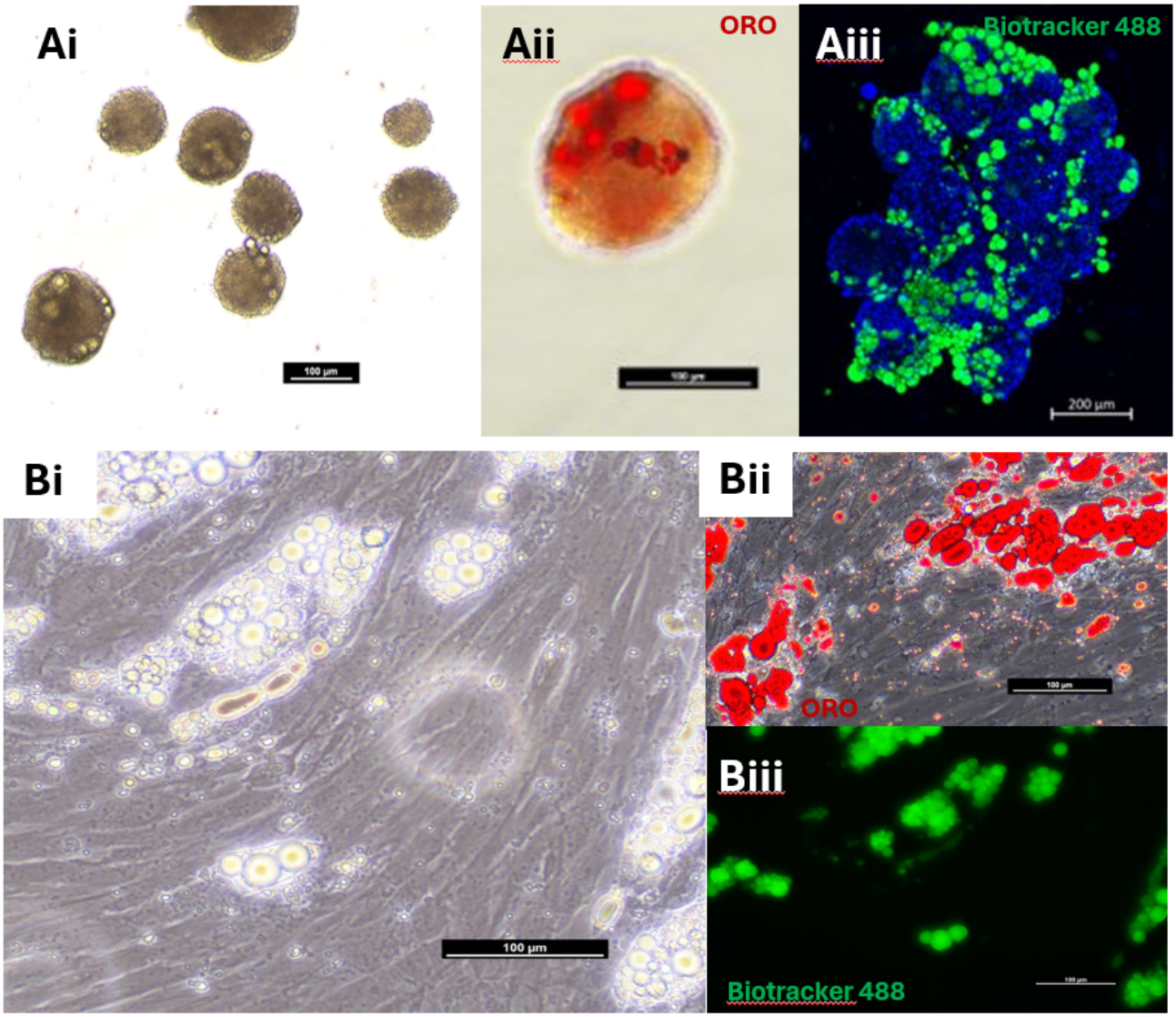


Figure 5. Adipogenic differentiation of bovine embryonic stem cell–derived mesenchymal stem cells in two- and three-dimensional culture.
Bovine induced mesenchymal stem cells (iMSCs) were subjected to adipogenic differentiation under suspension and adherent culture conditions. Representative morphology of differentiated iMSCs is shown following 14 days of induction in suspension culture and 6 days of induction in adherent culture (A,B). Intracellular lipid accumulation was visualised by brightfield microscopy and confirmed using Oil Red O staining and fluorescent lipid labelling with Biotracker dye (i–iii), demonstrating adipogenic differentiation in both culture formats. Scale bar = 100 µm.

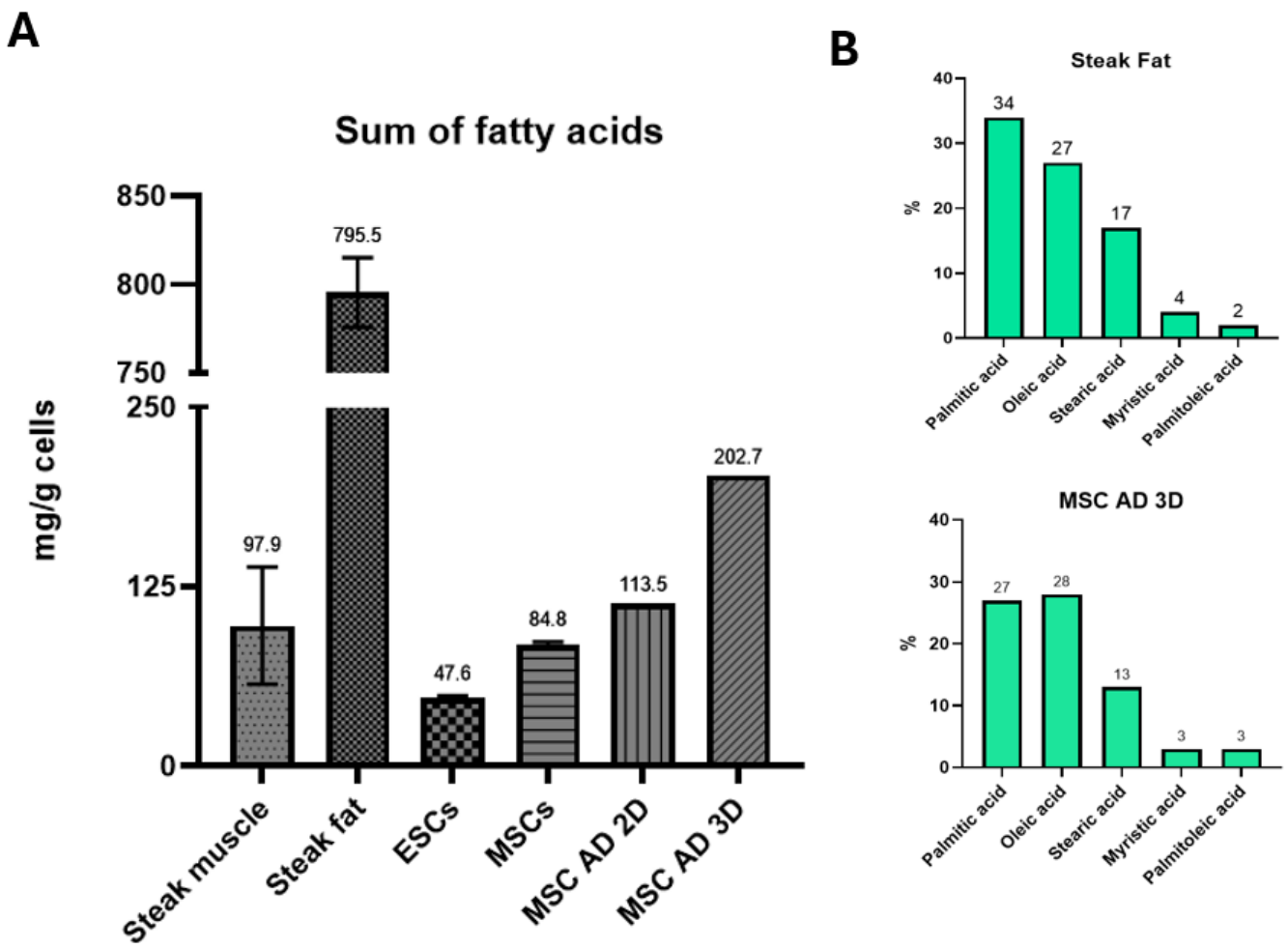


Figure 6. Fatty acid abundance and composition in differentiated bovine mesenchymal stem cell cultures. Fatty acid profiles were analysed in differentiated and undifferentiated bovine cell populations and compared with conventional bovine tissue samples. Total fatty acid abundance is shown for differentiated adipocyte cultures relative to undifferentiated embryonic stem cells and mesenchymal stem cells, alongside supermarket beef muscle and fat samples (A). Fatty acid composition is presented as the relative abundance of the five most prevalent fatty acids, enabling comparison between conventional steak fat and 3D differentiated iMSC adipocyte cultures (B).

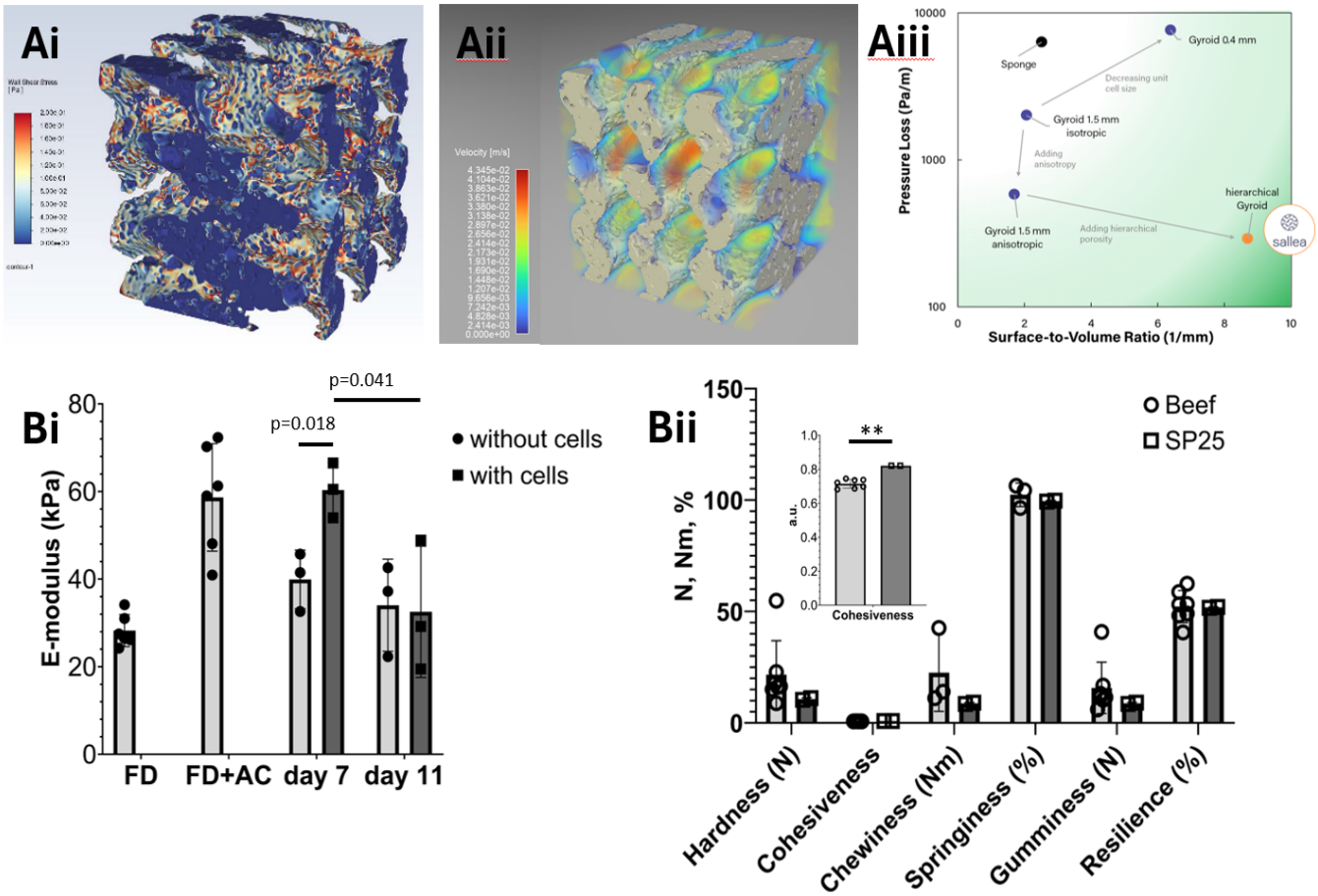


Figure 7. Shear environment and mechanical properties of edible protein-based scaffolds used for cell culture. A) Shear stress characterization of sallea scaffolds. i) Wall shear stress, ii) Flow velocity through the channels, iii) Pressure drop loss as a function of scaffold architecture. B) Mechanical characterization of sallea scaffolds. i) Emodulus quantification of freeze dried (FD), autoclaved (FD+AC) and cultured scaffolds with and without cells after 7 and 11 days, ii) TPA comparison between beef and soybased scaffolds (SP25). No statistical differences were observed except for cohesiveness (p=0.0009) although scaffolds showed lower average values for all parameters (n=7 for beef and n=2 for SP25).

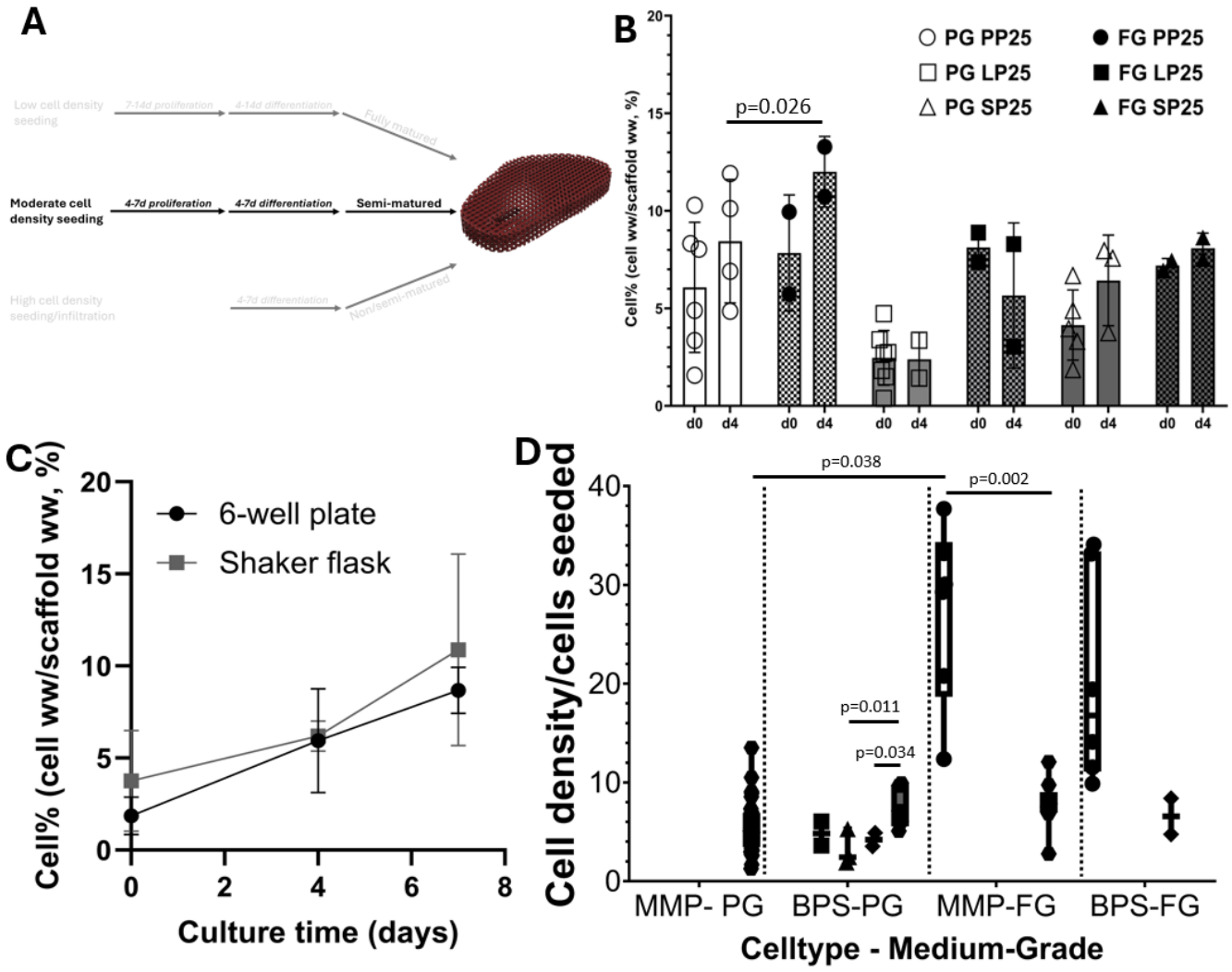


Figure 8. Evaluation of scaffold-based culture conditions across medium composition, scaffold material, and culture format. (A) Bioprocess development for scaffold cultures focusing on moderate cell density seeding (graphic produced on Biorender.com) (B) Scaffolds were compatible with both PharmaGrade (PG) and FoodGrade (FG) medium and peabased (PP25), lentilbased (LP25) or soybased (SP25) scaffolds with cells showing no difference in growth response in either medium composition. C) Scaling prototyping showed that comparable growth can be obtained in 125mL shaker flasks and 6 well cultures on a rocker (30rpm). D) ML analysis of a partial data set revealed optimization parameters for bioprocess development (●1M cells seeded, ■ 1.7M cells seeded, ▲ 2M cells seeded, ◆ 3.5M cells seeded, ⬢ 5M cells seeded. MMPPG = murine muscle progenitors in PharmaGrade medium, BPSPG = bovine primary satellites in PharmaGrade medium, MMPFG = murine muscle progenitors in FoodGrade medium, BPSFG = bovine primary satellites in FoodGrade medium.

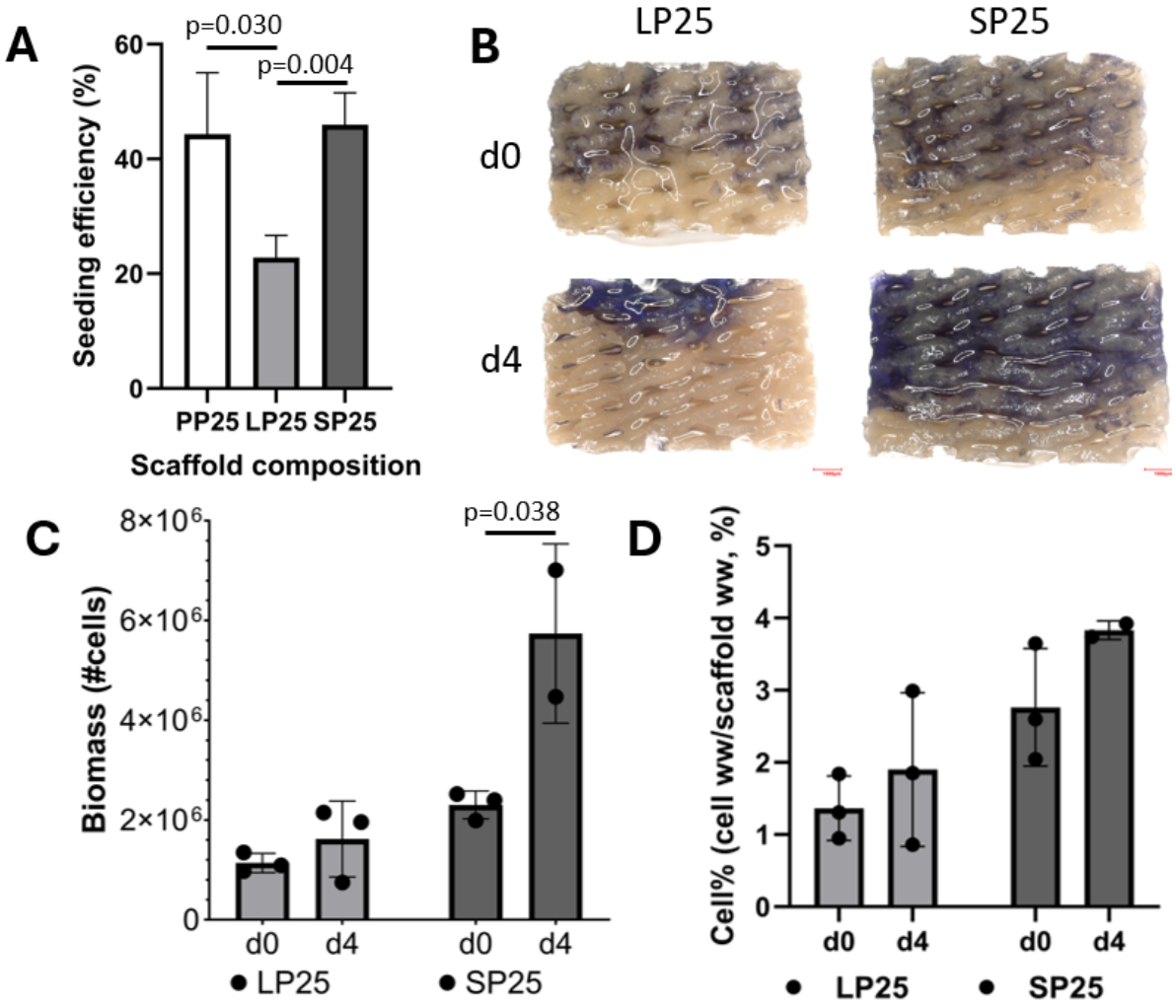


Figure 9. Compatibility of bovine induced mesenchymal stem cells with plant-based scaffold materials. Bovine induced mesenchymal stem cells (iMSCs) were evaluated for attachment and short-term growth on plant-derived scaffolds composed of pea- (PP25), lentil-(LP25), and soy-based protein (SP25) formulations. Initial seeding efficiency of iMSCs on the different scaffold materials is shown to compare early cell attachment across scaffold types (A). Cell distribution on lentil- and soy-based scaffolds following culture was visualised by MTT staining (B). Quantitative analysis of biomass accumulation and cell coverage was performed using MTT-based measurements to describe differences in total biomass and spatial distribution between scaffold materials (C,D)

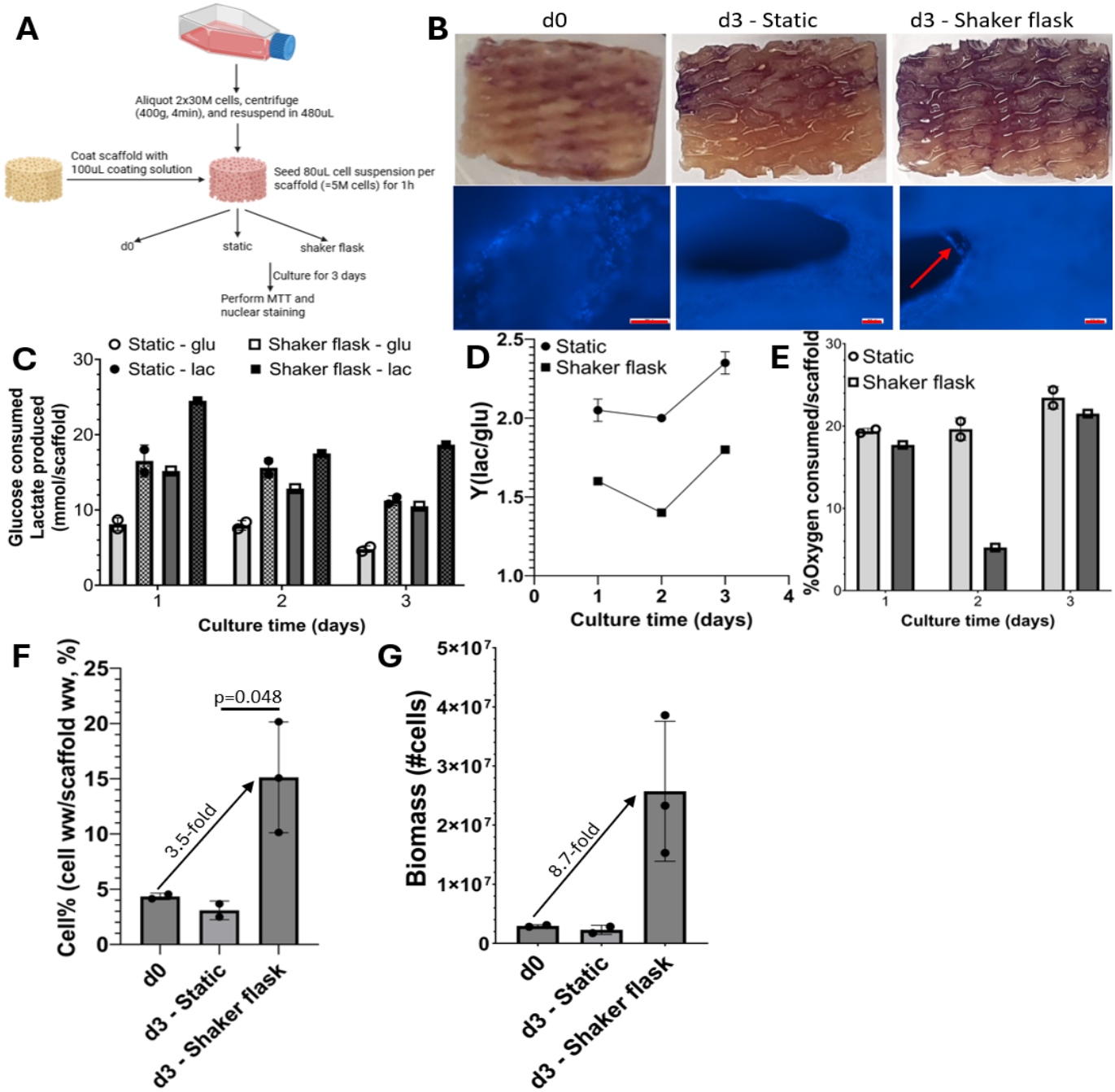


Figure 10. Dynamic and static scaffold-based culture of bovine induced mesenchymal stem cells. Bovine induced mesenchymal stem cells (iMSCs) were cultured on soy-based scaffolds under static and dynamic conditions to assess cell distribution, metabolic activity, and biomass accumulation. A schematic of the scaffold culture configuration and experimental strategy is shown (A). Following short-term culture, macroscopic and microscopic cell distribution on scaffolds was visualized by MTT staining and nuclear counterstaining, with representative images showing cell localisation and pore-spanning structures within the scaffold architecture (B). Metabolic activity during culture was assessed by measuring glucose consumption, lactate production, and oxygen uptake on a per-scaffold basis (C–E). Quantitative MTT-based analysis was used to estimate scaffold-associated cell coverage and total cell number following dynamic culture (F,G). Scale bar = 100 µm.

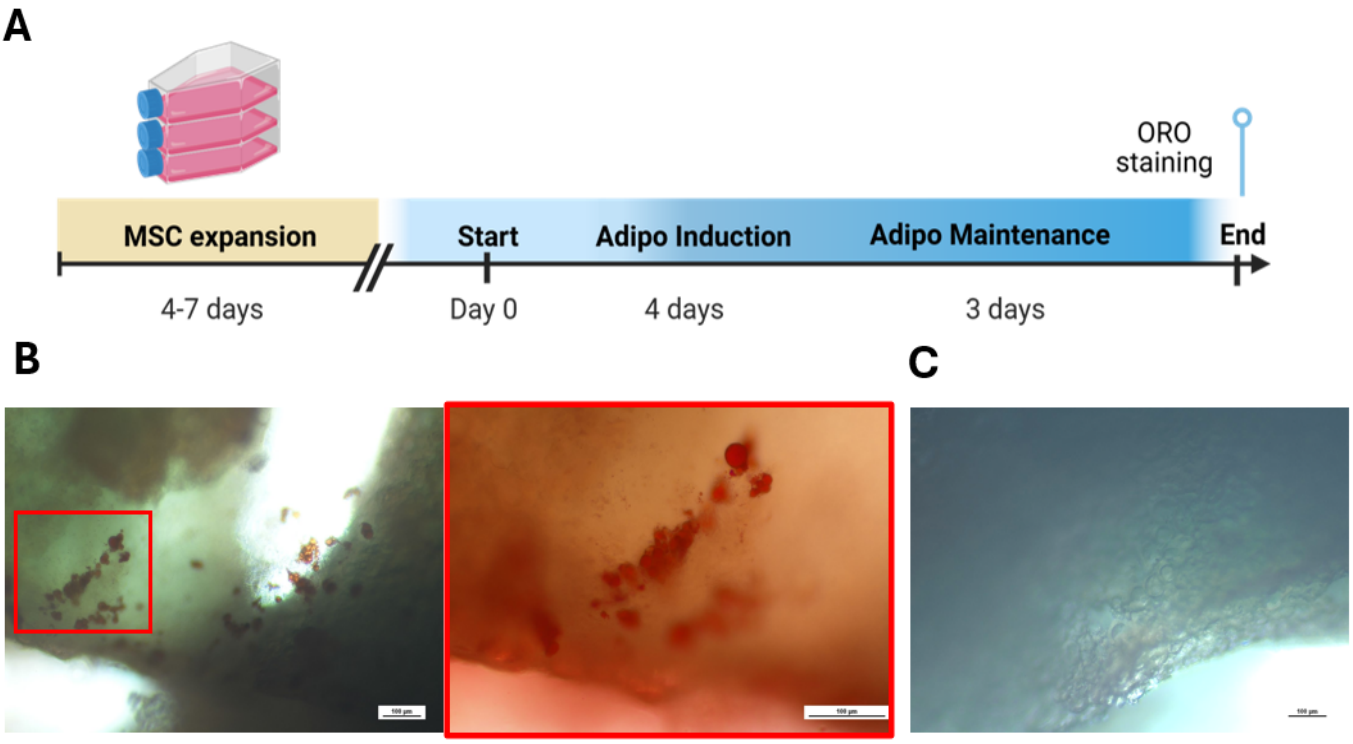
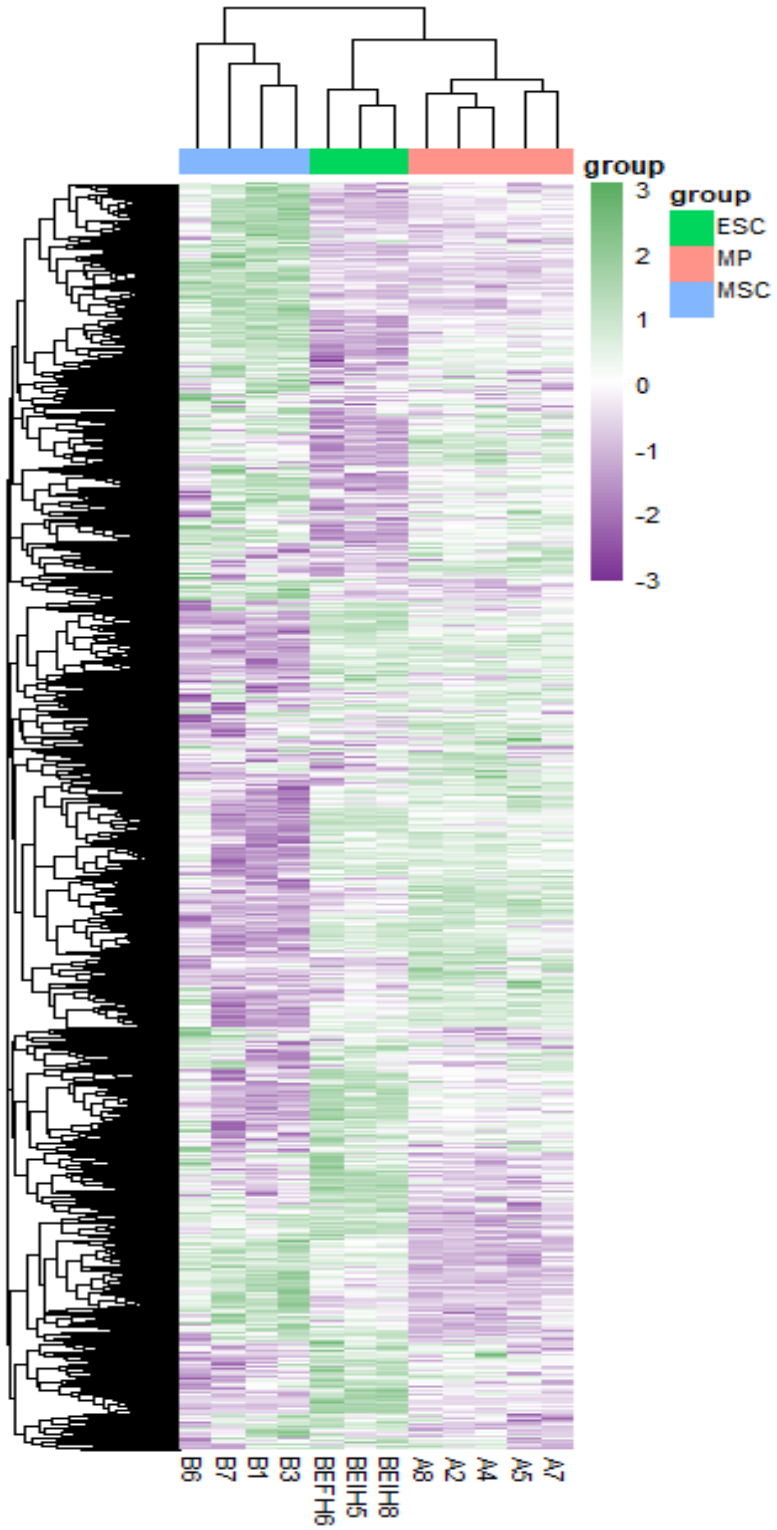
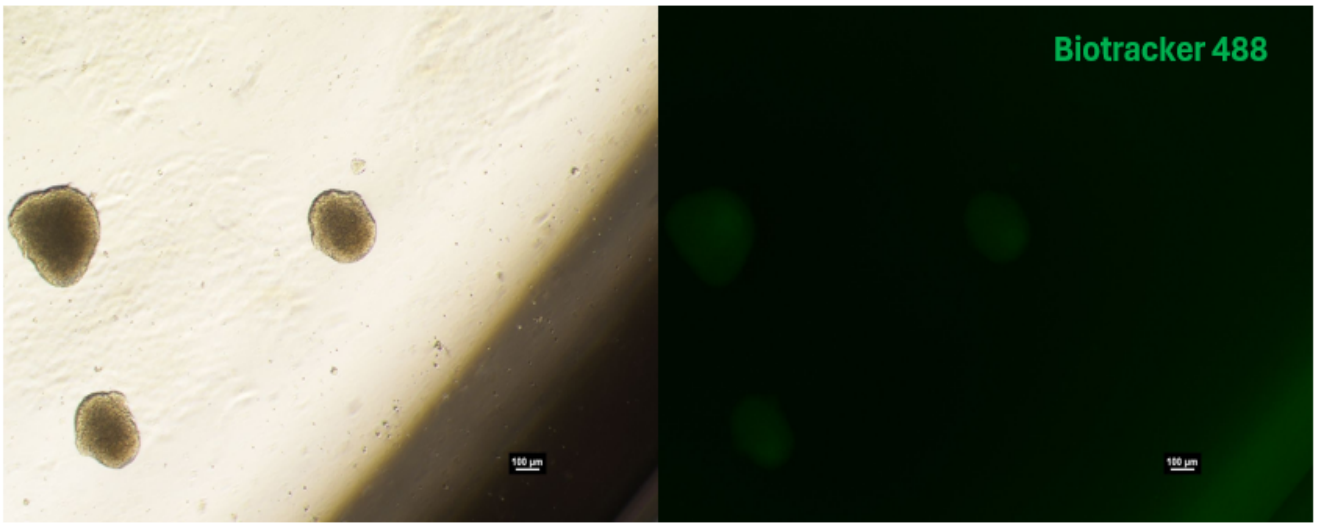


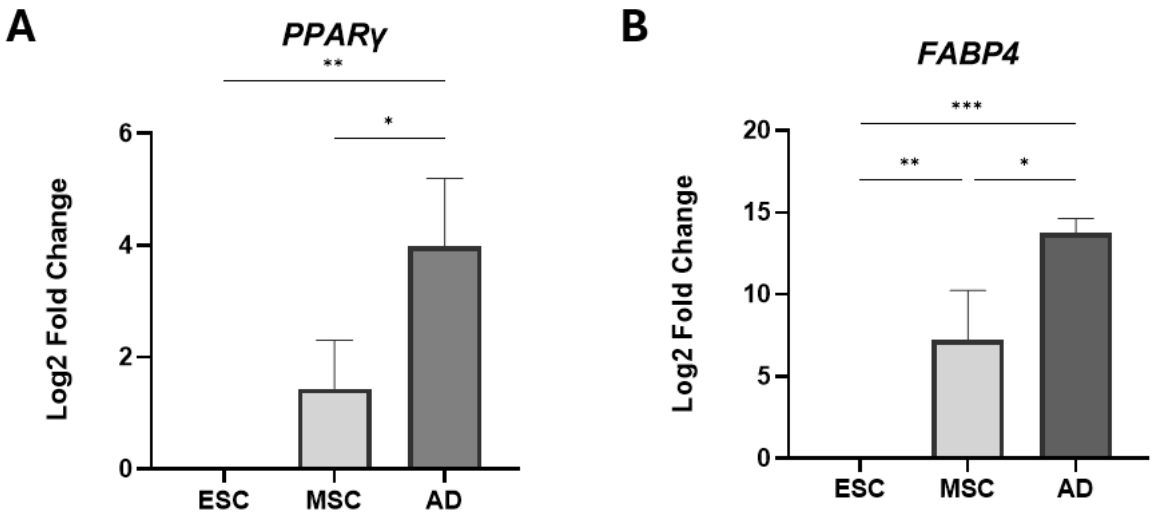
Figure 11. Adipogenic differentiation of bovine induced mesenchymal stem cells cultured on soy-based scaffolds. Adipogenic differentiation of bovine induced mesenchymal stem cells (iMSCs) cultured on edible protein-based scaffolds was evaluated following scaffold-based expansion. A schematic illustrating the timeline of iMSC differentiation toward adipocytes is shown (A). After culture in adipogenic induction and maintenance media, iMSCs on soy-based scaffolds exhibited intracellular lipid droplet formation and positive Oil Red O staining (B). In contrast, iMSCs cultured on scaffolds under proliferation conditions showed no detectable Oil Red O staining (C). Representative images are shown for each condition. Scale bar = 100 µm.



Supplementary Figure S1. Hierarchical clustering of transcriptomic profiles from bovine embryonic stem cell–derived differentiation stages. Unsupervised hierarchical clustering of RNA-sequencing data from bovine embryonic stem cells (ESCs), mesodermal progenitors (MPs), and induced mesenchymal stem cells (iMSCs). Expression values are shown as $\log_2$ counts per million ($\log_2$CPM), with gene-wise normalisation to z-scores. Rows (genes) and columns (samples) were clustered independently to visualize similarities in transcript abundance across differentiation stages, with colour indicating relative expression levels.



Supplementary Figure S2. Lipid staining of bovine induced mesenchymal stem cells in suspension culture. Bovine induced mesenchymal stem cells (iMSCs) cultured under suspension conditions were stained with a fluorescent lipid tracer to assess intracellular lipid accumulation. Representative fluorescence images show iMSCs labelled with BioTracker™ 488 lipid dye, with no detectable signal corresponding to lipid droplet formation. Scale bar = 100 µm.



Supplementary Figure S3. Adipogenic marker expression in differentiated three-dimensional bovine mesenchymal stem cell cultures. Expression of adipogenic marker genes was assessed in three-dimensional adipocyte cultures derived from bovine induced mesenchymal stem cells (iMSCs). Relative expression of *PPARγ* is increased compared to undifferentiated embryonic stem cells ($p<0.0033$) and iMSCs ($p<0.0265$) (n=3), (A), and *FABP4* is significantly increased compared to undifferentiated ESC ($p<0.0002$) and iMSCs ($p<0.0107$) (n=3) (B).