

ACCEPTED PAPER · MAIN TEXT

Engineering a biocompatible 3D model of the bone marrow niche for drug screening against KMT2A-rearranged acute myeloid leukaemia cell line MOLM-13

Paper version: Accepted Paper

Accepted Papers are manuscripts accepted for publication, encompassing all changes made following the peer review process, along with a standard cover page indicating the paper version and an “Accepted Paper” watermark, but excluding any other editing, typesetting or other changes made by AccScience Publishing and/or authors post-acceptance.

Article ID: IJB026190172

Citation: Hope L, Sanchez-Rubio A, Berry C, Salmeron-Sanchez M, Copland M, Wheadon H. Engineering a biocompatible 3D model of the bone marrow niche for drug screening against KMT2A-rearranged acute myeloid leukaemia cell line MOLM-13. *Int J Bioprint*. 2026. doi: 10.36922/IJB026190172

Copyright: © 2026 Author(s). This is an Open Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher’s Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

AccScience Publishing

RESEARCH ARTICLE

Volume X Issue X (2026)

doi: 10.36922/IJB026190172

Engineering a biocompatible 3D model of the bone marrow niche for drug screening against KMT2A-rearranged acute myeloid leukaemia cell line MOLM-13

Running title: Bioprinted leukaemia model for drug screening

Lauren Hope¹, Alvaro Sanchez-Rubio², Catherine Berry², Manuel Salmeron-Sanchez^{2,3,4}, Mhairi Copland¹, and Helen Wheadon¹

¹Paul O’Gorman Leukaemia Research Centre, School of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom

²Centre for the Cellular Microenvironment, School of Molecular Biosciences, University of Glasgow, Glasgow, United Kingdom

³Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute for Science and Technology (BIST), Barcelona, Spain

⁴Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

(This article belongs to the *Special Issue: Bioprinting in Tumor Modeling, Diagnostics, and Therapy*)

***Corresponding author:**

Lauren Hope (Lauren.Hope@glasgow.ac.uk)

Citation: Hope L, Sanchez-Rubio A, Berry C, Salmeron-Sanchez M, Copland M, Wheadon H. Engineering a biocompatible 3D model of the bone marrow niche for drug screening against KMT2A-rearranged acute myeloid leukaemia cell line MOLM-13. *Int J Bioprint*. 2026. doi: 10.36922/IJB026190172

Received: May 5, 2026

Revised: June 16, 2026

Accepted: June 25, 2026

Published online: June 26, 2026

Copyright: © 2026 Author(s). This is an Open-Access article distributed under the terms of the Creative Commons Attribution License, permitting distribution, and reproduction in any medium, provided the original work is properly cited.

Publisher's Note: AccScience Publishing remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

ACCEPTED PAPER

Abstract

3D bioprinting technology has the potential to fabricate highly reproducible advanced biocompatible 3D in vitro models. It has been used successfully to emulate aspects of the bone marrow microenvironment to study leukaemia, including Acute Myeloid Leukaemia (AML). AML is an aggressive haematological cancer which arises due to the accumulation of genetic mutations in haematopoietic stem and progenitor cells (HSPCs) within the bone marrow, leading to production of Leukaemic Stem Cells (LSCs). Interactions between LSCs and bone marrow stroma induce LSC quiescence, protecting them from chemotherapy. In this study, we engineered a 3D-bioprinted co-culture bone marrow model containing MOLM-13 AML cells in co-culture with two main supportive cell types: bone marrow stroma (HS5) and osteoblast-like cells (CAL-72). This system was used to explore the efficacy of the Cyclin Dependent Kinase (CDK) 2/9 inhibitor fadraciclib alone and in combination with current AML therapies. Characterisation of Alginate/Gelatin hydrogels revealed a highly porous matrix structure, with a hydrogel stiffness within the range of human bone marrow. These hydrogels sustained MOLM-13 cells and HS5 spheroid viability for up to seven days, highlighting the suitability of this system to model bone marrow. Next, the efficacy of fadraciclib alone and in combination with current chemotherapies were assessed in 2D culture and in the final 3D bioprinted model. These data revealed that fadraciclib induced MOLM-13 cell death in both 2D and 3D culture, systems with a reduced level of cell death observed in 3D culture. Together, these data reveal a reproducible human bone marrow niche system, with potential for screening of novel single and combination therapies.

Keywords: 3D bioprinting; Additive manufacturing; Bioengineering; Leukaemia; Bone marrow; Chemotherapy; Hydrogels; Spheroids

1. Introduction

Acute Myeloid Leukaemia (AML) is the most common acute leukaemia in adults, with an average worldwide incidence rate of 1.73 cases per 100,000 people, with incidence rate increasing with age¹⁻³. AML arises due to the acquisition of genetic mutations within haematopoietic stem and progenitor cells (HSPCs) within the bone marrow, which can lead to development of leukaemic stem cells (LSCs)⁴. LSCs interact with bone marrow stromal cells such as mesenchymal stem cells (MSCs), fibroblasts and osteoblasts which can result in LSC quiescence, as well as remodelling of the bone marrow niche to support LSCs over HSPCs⁵⁻¹⁰. Consequently, these interactions protect LSCs from chemotherapy, leading to treatment refractoriness and relapse¹¹. Understanding and circumventing this protective microenvironment is highly important in treating AML.

Preclinical AML studies rely on 2D human cell studies, genetically modified animal models, and patient-derived xenograft models in immunocompromised mice. However, 2D cell culture is far removed from the *in vivo* environment, resulting in differences between *in vivo* and *in vitro* such as gene expression and cell behaviour¹². Animal models provide a complete multi-organ system to study, however, there are many physiological and genetic differences between these animals and leukaemia patients, and primary leukaemia cells display inconsistent engraftment in mice, varying significantly with AML subtype¹³. 3D *in vitro* bone marrow niche systems have gained popularity due to their closer similarity to the human *in vivo* environment compared to animal models and 2D culture models¹⁴⁻¹⁹. A frequently used component of these are hydrogels. Hydrogels are polymers made from synthetic or naturally occurring materials, that have been crosslinked to form a hydrophilic gel²⁰⁻²². Hydrogels provide a 3D environment more analogous to the *in vivo* extracellular matrix compared to traditional 2D culture^{20,23,24}. Additionally, aspects such as diffusion kinetics are more similar to *in vivo* tissue compared to 2D culture²⁵. Polymers used to make hydrogels can often be adapted for 3D bioprinting, to make scaffolds that are more complex, yet highly reproducible and cost effective²⁶.

Recently, 3D bioprinting has emerged as a technique with the potential to produce high throughput, highly reproducible systems for tissue engineering²⁷, disease modelling^{28,29} and drug screening^{30,31}. 3D bioprinting involves embedding one or more cell types within a biomaterial, such as a hydrogel, resulting in a bioink which can then be used to print structures containing these

cells. Previously, 3D bioprinting has been utilised to produce models which emulate the bone marrow microenvironment in healthy haematopoiesis³², as well as haematological malignancies including AML¹⁴, Chronic Lymphocytic Leukaemia^{33,34} and Multiple Myeloma³⁵. Together, this highlights that 3D bioprinting has the potential to manufacture reproducible humanised bone marrow models for the study of AML.

However, the stresses exerted on the cells through the printing process such as shear stress, tension and compression forces can affect cell viability³⁶. Hence, the choice of hydrogel is influenced by its gelation, crosslinking capabilities and viscosity, as it is important to select a gel that will print well, and will protect the cells against these stresses³⁷. Alginate/gelatin (Alg Gel) hydrogels are frequently used as bioinks due to their good printability, good cell viability post-printing, and tuneable stiffness^{20,26,38,39}. Alginate is bioinert^{40,41}; however, it can be combined with gelatin, which contains cell adhesion sites and therefore improves cell viability⁴². Gelatin alone at physiological temperature (37 °C) is in a liquid state⁴³, therefore the addition of alginate provides structural advantages and makes Alg Gel suitable for 3D bioprinting⁴⁴. Despite the wide use of Alg Gel as a bioink^{20,26,44}, it has not been used to develop an AML model, highlighting a key area for further research.

Therefore, the aim of this study was to use Alg Gel bioinks to develop a reproducible humanised 3D bioprinted AML model, incorporating selected components of the bone marrow microenvironment. Our system is optimised for printing directly into a 96 well plate. This model was then used to explore the efficacy of a novel Cyclin Dependent Kinase (CDK) 2-and-9 inhibitor known as fadraciclib. Previous studies evaluating the efficacy of fadraciclib in AML cell lines, AML primary samples and *in vivo* studies have revealed that fadraciclib is an effective and potent inhibitor of AML cells, and combines synergistically with current AML therapies such as azacitidine, cytarabine and venetoclax^{45,46}. Here, our model was used to assess fadraciclib alone and in combination with current AML therapies azacitidine, cytarabine and venetoclax.

2. Materials and Methods

2.1. Alginate/Gelatin Hydrogel Preparation

Sodium alginate (Sigma Aldrich) and gelatin type A (Sigma Aldrich) powders were used to make 1.5X hydrogel stocks of 1% Alginate/8%Gelatin (1%Alg 8%Gel), 1.5% Alginate/8%Gelatin

(1.5%Alg 8%Gel) and 2%Alginate/8%Gelatin (2%Alg 8%Gel). Under sterile conditions, alginate was added to PBS and the bottle was incubated at 37°C, mixing occasionally. When the alginate had dissolved, gelatin was added under sterile conditions. The gel mixture was incubated at 37°C, mixing occasionally, until the gelatin powder had fully dissolved. Alginate/gelatin hydrogel stocks were stored at 4 °C until use.

Prior to use, gel stocks were incubated at 37 °C for at least one hour. In cell-free experiments, the stock was diluted in Roswell Park Memorial Institute 1640 (RPMI; Gibco) to make a 1X gel solution. In cell-based experiments, the gel was diluted by one sixth in cell-specific media (RPMI or 50:50 RPMI/Dulbecco's Modified Eagle Medium (DMEM; Gibco)), then diluted by a further sixth using media containing cells (MOLM-13: final concentration of 2×10^6 cells/mL of hydrogel; HS5 spheroids: final concentration of 3500-4000 spheroids/mL of hydrogel depending on spheroid yield). Alg Gel hydrogels were crosslinked in 150 mM calcium chloride (Sigma Aldrich; dissolved in dH₂O) solution for 8-10 minutes. The gels were rinsed twice with media and incubated at 37°C.

2.2. Oscillatory Rheology

Hydrogels were produced as described above and cast into Polydimethylsiloxane (PDMS) moulds of 15 mm diameter. After crosslinking, gels were incubated in media at 37 °C with 5% CO₂, for 1 hour, 1 day or for 2 days before measurements were taken. Rheology was completed using the Modular Compact Rheometer (MCR) 302 (Anton Paar) using a 15 mm parallel plate with the platform heated to 37°C to match physiological temperature. Strain sweeps were completed to identify the linear viscoelastic region, at an angular frequency of 10 radian/s and a normal force of 0.5-1 N. Loss Factor (ratio of Loss Modulus to Storage Modulus) was calculated using equation 1 and the Young's Modulus (E) was calculated using equation 2.

$$\tan\delta = G''/G'$$

Equation 1: Loss factor (tanδ) calculation. G'': Loss Modulus; G': Storage Modulus.

$$E = G' \times 2(1 + \nu)$$

Equation 2: Young's Modulus (E) calculation. G': Storage Modulus; ν: Poisson's Ratio (hydrogels : ν=0.5). Completed at strain 0.01-0.1% and angular frequency 10 rad/s.

2.3. Scanning Electron Microscopy

The gels were cast as described and incubated in media for 1 hour or 2 days. Gels were dehydrated by submerging in liquid nitrogen for approximately 30-60 seconds then freeze-drying at -80 °C for approximately 24 hours at 0 Bar (Labconco). Freeze-dried gels were sliced and coated with gold-palladium prior to microscopy. Scanning Electron Microscopy (SEM) was completed using JEOL IT 100 SEM running at 10kV, and images were captured at 30X, 300X and 2000X magnification using INTOUCH Scope software version 1.05. These images were analysed using Fiji. Approximate pore areas were calculated using equation 3:

$$Area_{Ellipse} = \pi ab$$

Equation 3: Area of an ellipse. Area of an ellipse where ***a*** represents the radius of the semi-major axis and ***b*** represents the radius of the semi-minor axis.

2.4. Cell Lines

All cells were cultured at 37 °C with 5% CO₂, in growth media supplemented with L-glutamine (2 mM), penicillin (100 U/mL) and streptomycin (100 g/mL) (all Gibco). The human AML MOLM-13 cell line (DSMZ), which contains the *KMT2A-AF9* fusion gene⁴⁷, was cultured in RPMI media with 20% FBS (Gibco). HS5 bone marrow fibroblast cells (ATCC) were cultured in DMEM with 10% FBS (Gibco) and 20% HS5-conditioned media. CAL-72 osteosarcoma cells (ATCC) were cultured in DMEM supplemented with Insulin Transferrin Selenium (1X) (ITS; Gibco) and were cultured on Nunclon plates (Thermo Fisher Scientific) to aid adherence.

2.5. Production of HS5 spheroids using AggreWell™ plates

GFP-HS5 spheroids were generated using 24-well (1,200 microwells) low-adherence AggreWell™ plates (Stem Cell Technologies) as per manufacturer's instructions. 100 GFP-HS5 cells were seeded per spheroid, and the AggreWell™ plate was incubated at 37 °C for 24 hours to allow formation of spheroids. Spheroids were harvested by gently resuspending each well and passing the contents through a 37 µm strainer to remove single cells (average spheroid diameter: 86 µm).

2.6. 3D Bioprinting of Alginate/Gelatin hydrogels

2%Alg 8%Gel containing MOLM-13 cells and HS5 spheroids was loaded into the cartridge (Nordson Engineering Fluid Dispensing) and a 22 G needle (Nordson Engineering Fluid Dispensing) added to the tip of the cartridge. The cartridge was then loaded into the 3D bioprinter

(BIOX6 CELLINK), which maintained the cartridge at 37 °C until printing. The constructs were designed using DNA studio V3.3.2+7 (CELLINK). Bioprinted gels were directly cast into a 96 well plate containing 200 µL of 150 mM calcium chloride solution per well, at one hydrogel per well. Pressure exerted on the gel was between 8-12 kPa with a time of 1.25 seconds per droplet of gel. The gels were incubated at 37 °C for 24 hours prior to drug treatment.

For the final 3D model, CAL-72 cells were seeded into a 96-well Nunclon™ Delta treated plate (Thermo Fisher Scientific) and incubated for 24 hours prior to printing. 3D bioprinting was completed as outlined above. Following the production of bioprinted gels, the media on the CAL-72 cells was aspirated and replaced with 50:50 media supplemented with ITS. Bioprinted gels were then transferred into the wells containing CAL-72 cells. Gels were then incubated at 37 °C for 24 hours prior to drug treatment.

2.7. Viability staining

NucBlue® Live stain (Ex/Em: 360/460 nm) from the ReadyProbes™ Cell Viability Imaging Kit (ThermoFisher) and Ethidium homodimer-1 (Ex/Em: 517/617 nm) from the LIVE/DEAD™ Viability Kit (ThermoFisher) were added to warm cell-specific media as per manufacturer's instructions, and then hydrogels were submerged in the mixture. The plate was incubated at 37°C for 15-30 minutes protected from light. Gels were imaged using the EVOS M7000 Imager (Invitrogen) or the Zeiss Axiovert. Images were processed using Fiji and QuPath, whereby the number of alive (NucBlue® positive) and number of dead (Ethidium homodimer-1 positive) MOLM-13 cells were counted, and average MOLM-13 cell viability was calculated on Microsoft Excel.

2.8. Drug treatment of MOLM-13 cells in 2D monoculture

10 mM stocks of fadraciclib (CYC-065, Cyclacel) and azacitidine (Selleck Chem) were prepared in DMSO and stored at -20 °C and -80 °C respectively. Venetoclax (Selleck Chem) 10 mM stocks were prepared in DMSO and stored at -80°C, with 1 mM stocks stored at -20°C. 10 mM stocks of cytarabine (Sigma Aldrich) were prepared in water and stored at -20°C. Stocks were diluted in RPMI before treatment of MOLM-13 cells. The cells were incubated with chemotherapy treatments at 37 °C for 48 hours, before completion of downstream experiments.

2.9. Drug treatment of MOLM-13 cells in 2D co-culture with HS5 bone marrow fibroblasts

HS5 cells were seeded at 3.125×10^4 cells/cm² in six-well plates (Greiner Bio) and cultured for 24 hours before MOLM-13 cell seeding and drug treatment were completed (see above). Co-cultured cells were maintained in 20% RPMI and 10% DMEM with 20% HS5-conditioned media (in a 50:50 ratio).

2.10. Annexin V/DAPI staining of MOLM-13 cells

In co-culture experiments, surface marker staining was completed first to distinguish MOLM-13 cells (CD33⁺ CD73⁻) from HS5 cells (CD33⁻ CD73⁺) or CAL-72 (CD33⁻ CD73⁻). Cells were then stained in Hank's Balanced Salt Solution (HBSS) with calcium and magnesium, containing Annexin V antibody (BD Biosciences) and DAPI stain (BD Biosciences), and incubated at room temperature protected from light for 15-20 minutes before flow cytometry analysis (BD FACSCanto™ II, BD Biosciences). Flow cytometry data was analysed using FlowJo software.

2.11. Multiplex qRT-PCR using Fluidigm Biomark

First, MOLM-13 cells (CD33⁺ CD73⁻) were isolated from HS5 (CD33⁻ CD73⁺) co-culture using Fluorescence Activated Cell Sorting (FACS) based on cell surface markers. RNA was extracted using either the RNEasy Micro kit (Qiagen) or the Arcturus Picopure RNA extraction kit (Thermo Fisher Scientific). cDNA was produced using the High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific). Sample Target Amplification was completed on all samples, followed by multiplex quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) using the Fluidigm Biomark system. Primer sequences can be found in Supplementary Figure 4. The $2^{-\Delta\Delta Ct}$ method was used to determine the expression of selected target genes⁴⁸. First the average threshold cycle (Ct) for all replicates was calculated, then subtracted from the housekeeping gene average (*GUSB*, *ATP5B*, *ENOX2*, *TYW1*, *TBP*, *B2M*) (ΔCt). This value was then subtracted from the No Drug Control (NDC) average ΔCt value ($\Delta\Delta Ct$), and the fold change calculated using $2^{\Delta\Delta Ct}$.

2.12. Statistics

Unless otherwise stated, all data are presented as the average of three replicates with standard deviation as error bars. Raw data was processed using Microsoft Excel, then statistical analyses were performed using Prism 10 by GraphPad. Shapiro-Wilk normality testing was completed to assess the distribution of the data. Parametric tests included t-tests, One Way ANOVAs with Multiple Comparisons and Two Way ANOVAs with Multiple Comparisons. Mann Whitney U tests

and Kruskal-Wallis test with Dunn's Multiple Comparisons post-test were performed on datasets which were not normally distributed. Significance was depicted using asterisks as follows: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Analysis was completed within and between groups – non-significance is indicated via the absence of asterisks. Heat maps representing the multiplex qRT-PCR results were produced using Prism by GraphPad 10. $2^{-\Delta\Delta C_t}$ method was used to calculate fold change relative to NDC, then fold changes were $\log_{10}$ transformed to produce the values included in the heatmap.

3. Results

3.1. Characterisation and optimisation of Alginate/Gelatin hydrogels for use in a 3D bioprinted AML model

Bone marrow is reported to have a stiffness ranging 0.3-50 kPa^{49,50}, with the central marrow being the softest region (0.1-1 kPa) and the endosteal niche being the stiffest (25-50 kPa). Rheology was used to assess the viscoelastic properties of these hydrogels, their stiffness and therefore suitability for use as a bone marrow model. Rheology was carried out to assess the mechanical properties of hand-cast 1%Alg 8%Gel, 1.5%Alg 8%Gel, and 2%Alg 8%Gel, with and without MOLM-13 AML cells (Figure 1; Supplementary Figure 1, 2). Except for the 1%Alg 8%Gel cell-laden condition, the Young's Modulus significantly decreased with time in culture (Figure 1A-D). The Young's Modulus of 1%Alg 8%Gel and 2%Alg 8%Gel hydrogels decreased from 3 kPa and 10.7 kPa at day 0, to 1 kPa and 3.4 kPa, respectively, by day 2. On Day 0, significant differences were found between the Young's moduli of cell-laden and cell-free conditions for 1% and 1.5%Alg 8%Gel, with the cell-free hydrogels displaying a higher Young's Modulus compared to cell-laden (Figure 1A-D). However, at later timepoints, there was no significant difference in Young's Modulus between cell-free and cell-laden hydrogels (Figure 1A-D). The 2%Alg 8%Gel hydrogels were selected for future experiments as this hydrogel composition exhibited the highest Young's Modulus, and therefore may be more suitable for emulating the stiffer endosteal bone marrow niche.

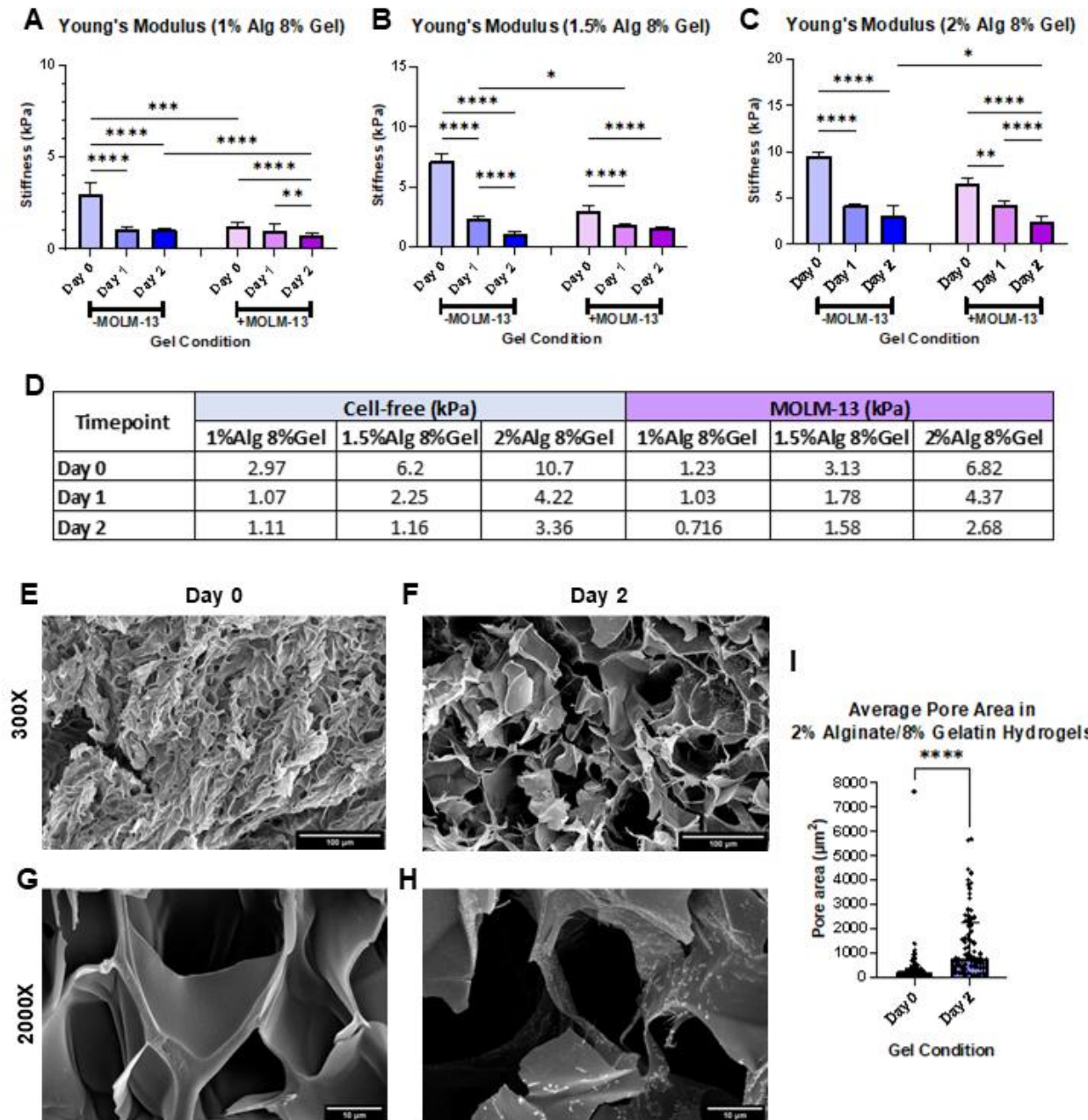


Figure 1: Characterisation of AlgGel hydrogel mechanical and structural properties. A-C: Young's Modulus as calculated using the Storage Modulus (Equation 1) for A: 1%Alg 8%Gel; B: 1.5%Alg 8%Gel; C: 2%Alg 8%Gel. Results were compared using a Kruskal-Wallis test with Dunn's Multiple Corrections post-tests. D: Average Young's Modulus for each condition (kPa). E-H: Representative Scanning Electron Microscopy images of 2%Alg 8%Gel hydrogels. E: Day 0, 300X magnification (scale bar: 100 μm); F: Day

0: 2000X magnification (scale bar: 10 μm); G: Day 2: 300X magnification (scale bar: 100 μm); H: Day 2: 2000X magnification (scale bar: 10 μm). I: Average pore area was calculated using Equation 3. Results were compared using a Mann-Whitney U test. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the average of three technical replicates with standard deviation depicted as error bars. Preliminary data from ref. ²⁶ (rheology data for cell-free Alg Gel hydrogels; SEM and pore area calculations for Day 0).

Bone marrow is a highly porous tissue, therefore, materials which aim to emulate this environment must also be suitably porous. Scanning electron microscopy (SEM) was therefore used to elucidate the microarchitecture of 2%Alg 8%Gel hydrogels over two days in culture (Figure 1E-I). The average pore area after two days had significantly increased compared to day 0, and the standard deviation had also increased (Figure 1I). This indicates that the ions within the media could have disrupted the calcium crosslinking, resulting in increased pore size and increased heterogeneity of the hydrogel structure. This concurs with the rheology data, which revealed a decrease in Young's Modulus after two days in culture, potentially due to the increased pore size as the hydrogel degrades ²⁶. Previous work has also highlighted that these hydrogels initially swell when immersed in media but then begin to degrade as the ions in the media interact with the ionic hydrogel network ⁵¹. Next, the ability of small molecules to diffuse through the hydrogels was assessed by immersing the hydrogels in media containing nanoparticles conjugated to FITC (Supplementary Figure 3). Here, it was observed that small molecules could diffuse through the network within two hours, with optimal diffusion after 24 hours.

To further determine whether 2%Alg 8%Gel hydrogels were suitable as a bone marrow model, MOLM-13 cell and HS5 spheroid viability were assessed. MOLM-13 is an AML cell line which displays a *KMT2A* rearrangement. Previous research has shown that it is sensitive to fadraciclib⁴⁵, making it ideal to test on this proof-of-concept AML bone marrow model ^{45,46}. Spheroids were selected instead of 2D adherent cell monolayers because spheroids display increased deposition of extracellular matrix proteins compared to 2D culture, as well as more relevant oxygen, nutrient and cytokine gradients compared to 2D monolayers^{52,53}. There were few significant differences observed between MOLM-13 cells which had been cast within hand-cast and 3D bioprinted hydrogels, indicating that bioprinting does not negatively impact MOLM-13 viability (Supplementary Figure 4C, D, F). Additionally, similar results were observed with HS5

spheroids, which remained over 85% viable over seven days (Supplementary figure 4F). No significant differences were observed between MOLM-13 in monoculture compared to co-culture with HS5 spheroids in the hydrogels (Supplementary Figure 4E).

MOLM-13 cells within 3D bioprinted hydrogels exhibited a cell viability of approximately 70-75% at day 0, and there was no significant decrease in viability across all timepoints for the monoculture condition (Supplementary Figure 4B, C, E). Aside from day 7, there were no significant differences between the monoculture and co-culture conditions, however, through F-actin staining, there was a significant increase in MOLM-13 cluster size in monoculture compared to co-culture, particularly at later timepoints (Supplementary Figure 5A, B, G, H). Clustering of leukaemia cells has previously been observed in hydrogels^{26,54,55}. This indicates that the hydrogel supports viability and proliferation of MOLM-13 cells. Spheroids produce oxygen, nutrient and cytokine gradients more analogous to those found *in vivo*; therefore, this reduced clustering indicates that the presence of HS5 spheroids may be reducing the rate of MOLM-13 proliferation, hence reducing the cluster size. HS5 spheroids in both hand cast and 3D bioprinted conditions also remained round throughout all timepoints, indicating that the cells within the spheroid do not migrate into the surrounding hydrogel matrix, and that 3D bioprinting does not disrupt HS5 spheroid morphology (Supplementary Figure 5A, B, E, F). Overall, these results show that 2%Alg 8%Gel hydrogels can support MOLM-13 cells and HS5 spheroids for up to seven days, and that the 3D bioprinting process does not affect cell viability or spheroid morphology.

3.2. CDK inhibitor fadraciclib in combination with azacitidine effectively induces apoptosis within MOLM-13 2D cell culture

To assess the final 3D bioprinted model, single and combination treatments using selected chemotherapies were first completed in 2D MOLM-13 monoculture and HS5 co-culture (Figure 2A). In this study, HS5 cells were selected as they exhibit similar expression patterns and surface marker expression to MSCs and can enhance the survival of leukaemia blasts *in vitro*⁵⁶⁻⁶⁰, with reduced batch-to-batch variation compared to Mesenchymal Stem Cells^{61,62}.

For this assessment of our 2D and 3D models, three drugs were selected in combination with fadraciclib based on previous work⁴⁵: azacitidine, a hypomethylating agent⁶³; cytarabine, which prevents DNA replication⁶⁴, and venetoclax, which is a B-Cell Lymphoma 2 (BCL-2)

inhibitor⁶⁵. All three of these are current chemotherapies which have been utilised effectively as combination therapies in AML^{66,67}. Previous work revealed a promising therapeutic window through combination therapy of fadraciclib with azacitidine⁴⁵. First, the EC50 for each drug was determined at 24, 48 and 72 hours (Supplementary Figures 6, 7A-D, 8A-D, 9A-D; Supplementary Table 1). Fadraciclib was then combined with the selected chemotherapies at a range of concentrations (0.5 x EC50, 1x EC50 and 2x EC50), to determine optimal concentrations and timepoints (Supplementary Figures 7E, F; 8E, F; 9E, F). Here, it revealed that the optimal timepoint for drug treatment was 48 hours, therefore for future experiments this timepoint was selected. MOLM-13 cells in monoculture and co-culture were treated with the 48-hour EC50s of fadraciclib (350 nM) and azacitidine (1600 nM) as single and combination therapies (Supplementary Figure 10A-C). Annexin V/DAPI and trypan blue staining indicated that the combination therapy induced more MOLM-13 cell death compared to either single drug treatment. This was observed to a lesser extent with co-culture, with fewer cells progressing to late apoptosis, indicating a protective effect by the HS5 cells (Figure 2B, C). For example, in the monoculture fadraciclib/azacitidine combination-treated condition 4.8×10^4 cells (36%) were in late apoptosis, whilst only 2.13×10^4 combination-treated cells in HS5 co-culture (12.1%) had entered late apoptosis, with most of the cells remaining viable or in early apoptosis (Figure 2B, C). Similar results were obtained for fadraciclib combined with other selected AML therapies cytarabine and venetoclax (Figure 2C, Supplementary Figure 10D-I).

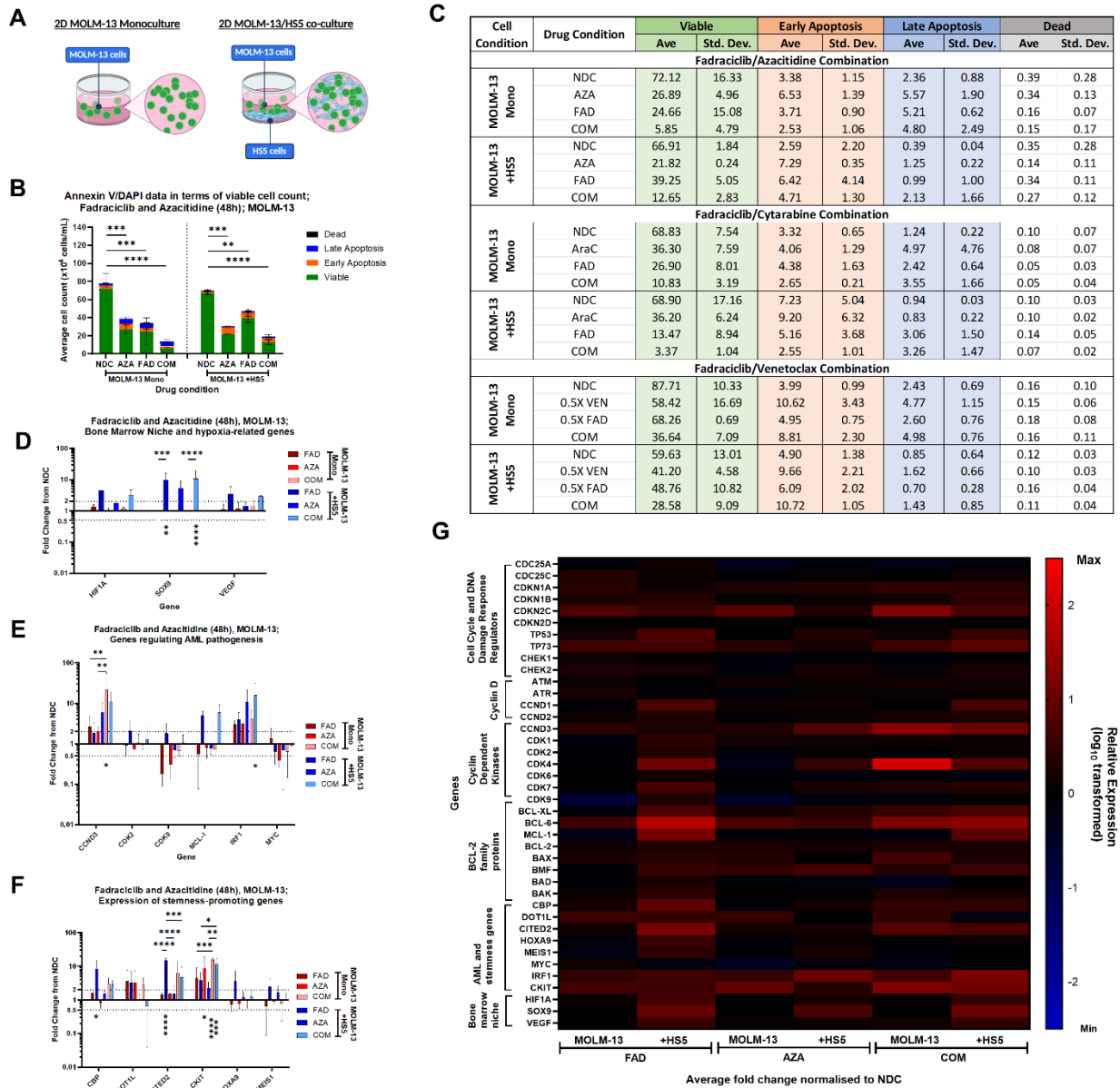


Figure 2: Efficacy of fadraciliclib and azacitidine in 2D monoculture and HS5 co-culture. A: A schematic of MOLM-13 cells in 2D monoculture and co-culture. Created in BioRender. Hope, L. (2026) <https://BioRender.com/4hj9gpi> B: Annexin V/DAPI data expressed in terms of cell counts for MOLM-13 cells treated with fadraciliclib (FAD), azacitidine (AZA) or combination (COM) therapy in monoculture (MOLM-13 Mono) (left) and co-culture (MOLM-13 +HS5) (right). C: A table outlining the average number of cells in each stage of apoptosis for all three combination therapy datasets, in monoculture and co-culture (cell number is $\times 10^4$); D: Heat map displaying differences in gene expression for FAD/AZA treated

MOLM-13 cells in monoculture and HS5 co-culture. Blue: downregulated compared to NDC; red: upregulated compared to NDC. E: Bar chart of selected genes affected by drug treatment. F: Bar chart of selected bone marrow niche-related genes. Two-way ANOVAs with multiple comparisons were used to detect significant differences between conditions in E, F. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the mean of three independent biological replicates with standard deviation depicted as error bars. Analysis was completed within and between groups – non-significance is indicated via the absence of asterisks. Asterisks below the x axis indicate a significant difference compared to NDC.

This protective effect was further assessed using multiplex qRT-PCR. Almost global downregulation of genes within 2D monoculture MOLM-13 cells and upregulation of several genes in 2D MOLM-13/HS5 co-culture was observed (Figure 2D). Genes affected by fadriciclib either directly or indirectly, which include *CDK-2*, *CDK-9*, *MCL-1*, *HOXA9* and *MEIS1*, were downregulated in monoculture but upregulated in HS5 co-culture (Figure 2E). Genes relating to HS5-mediated protection of MOLM-13 cells were also examined (Figure 2F). *SOX9* displayed significantly increased expression in co-cultured MOLM-13 cells compared to monoculture. *SOX9* encodes a transcription factor which has been associated with cell proliferation^{68,69}, cell cycle regulation^{69,70} and stem cell homeostasis⁷¹. Transcription of *SOX9* is stimulated by interleukin (IL)-6, which is secreted by HS5 cells^{72,73}. Together, this indicates that upregulation of *SOX9* in MOLM-13/HS5 co-culture could be induced by cytokines released from the HS5 cells, and could show that *SOX9* has a role in self-renewal and maintenance of MOLM-13 cells, as observed in other cancers^{74–76}. Similar trends were also observed with the cytarabine and venetoclax-treated conditions (Supplementary Figure 11).

To model the endosteal niche, an osteoblastic component is required, therefore CAL-72 cells were selected. CAL-72 is an osteosarcoma cell line which resembles primary osteoblastic cells more closely than other osteosarcoma cell lines⁷⁷. Previous studies have revealed that CAL-72 co-culture with primary AML blasts results in increased blast proliferation, and increased secretion of angiogenic factors such as IL-8, Ang-1 and VEGF^{78–80}. Initial 2D co-culture experiments were conducted in MOLM-13/CAL-72 2D co-culture (Figure 3A). Absolute MOLM-13 cell counts decreased upon treatment with fadriciclib and azacitidine, with a further, significant decrease in combination therapy (Figure 3B, Supplementary Figure 12). Correspondingly, a large

proportion of drug-treated cells progressed toward early and late apoptosis (Figure 3B, C). These results indicated that the treatments remained effective at inducing apoptosis of MOLM-13 cells despite the presence of the CAL-72 monolayer. Overall cell counts appeared to be lower, even in the NDC (72.12x10⁴ viable cells in monoculture compared to 43.06x10⁴ cells), which shows reduced cell proliferation in co-culture, and therefore contrasts with previous findings^{78–80}.

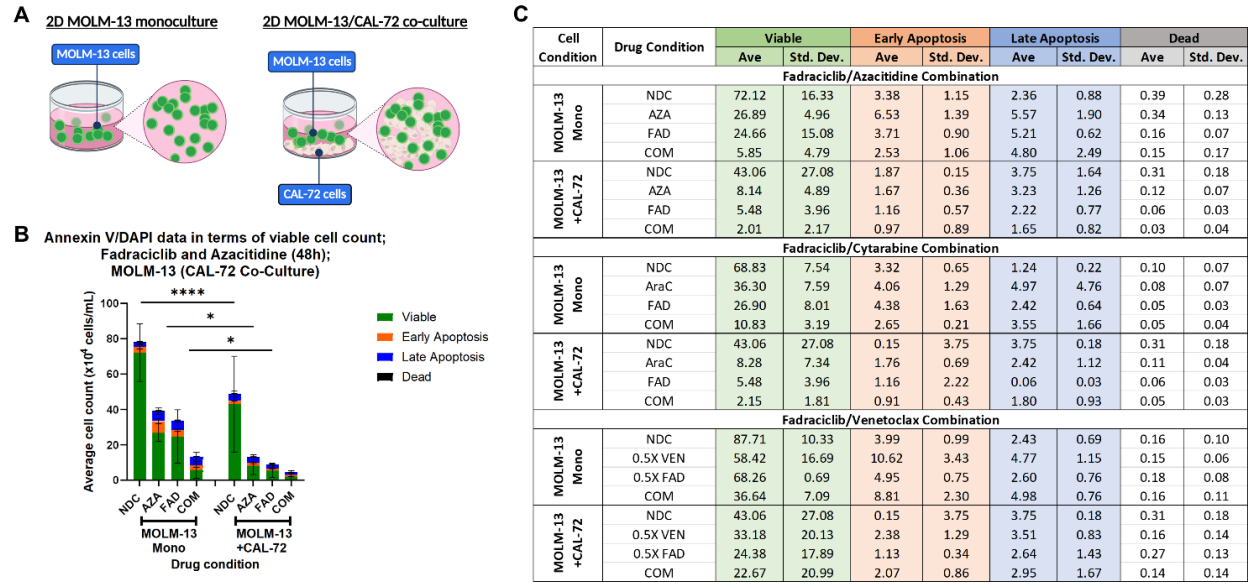


Figure 3: Assessment of fadracilicb and azacitidine efficacy in 2D CAL-72 co-culture. A: A schematic of MOLM-13 cells in 2D monoculture and CAL-72 co-culture. Created in BioRender. Hope, L. (2026) <https://BioRender.com/nggmc79> B: Annexin V/DAPI data expressed in terms of cell counts for MOLM-13 cells treated with fadracilicb (FAD), azacitidine (AZA) or combination (COM) therapy in monoculture (left) and CAL-72 co-culture (right). C: A table outlining the average number of cells in each stage of apoptosis for all three combination therapy datasets, in monoculture and CAL-72 co-culture. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the mean of three independent biological replicates with standard deviation depicted as error bars. Analysis was completed within and between groups, therefore non-significance is shown via the absence of asterisks.

3.3. The 3D bioprinted model displays greater chemoprotection in AML compared to the 2D monoculture and co-culture models

MOLM-13 cells were then bioprinted within 2%Alg 8%Gel hydrogels with or without the presence of HS5 spheroids (Figure 4A). Spheroids were selected due to their more physiologically relevant

oxygen, nutrient and cytokine gradients compared to 2D monolayers^{52,53}. Bioprinted hydrogels containing MOLM-13 cells in monoculture of HS5 spheroid co-culture were treated with fadraciclib alone and in combination with the selected chemotherapies. Significant differences were observed between NDC (76% viability in monoculture and co-culture) and all treated conditions (ranging 57.5% to 62% viability), however, no significant differences were seen between the single and combination therapies (Supplementary Figure 13).

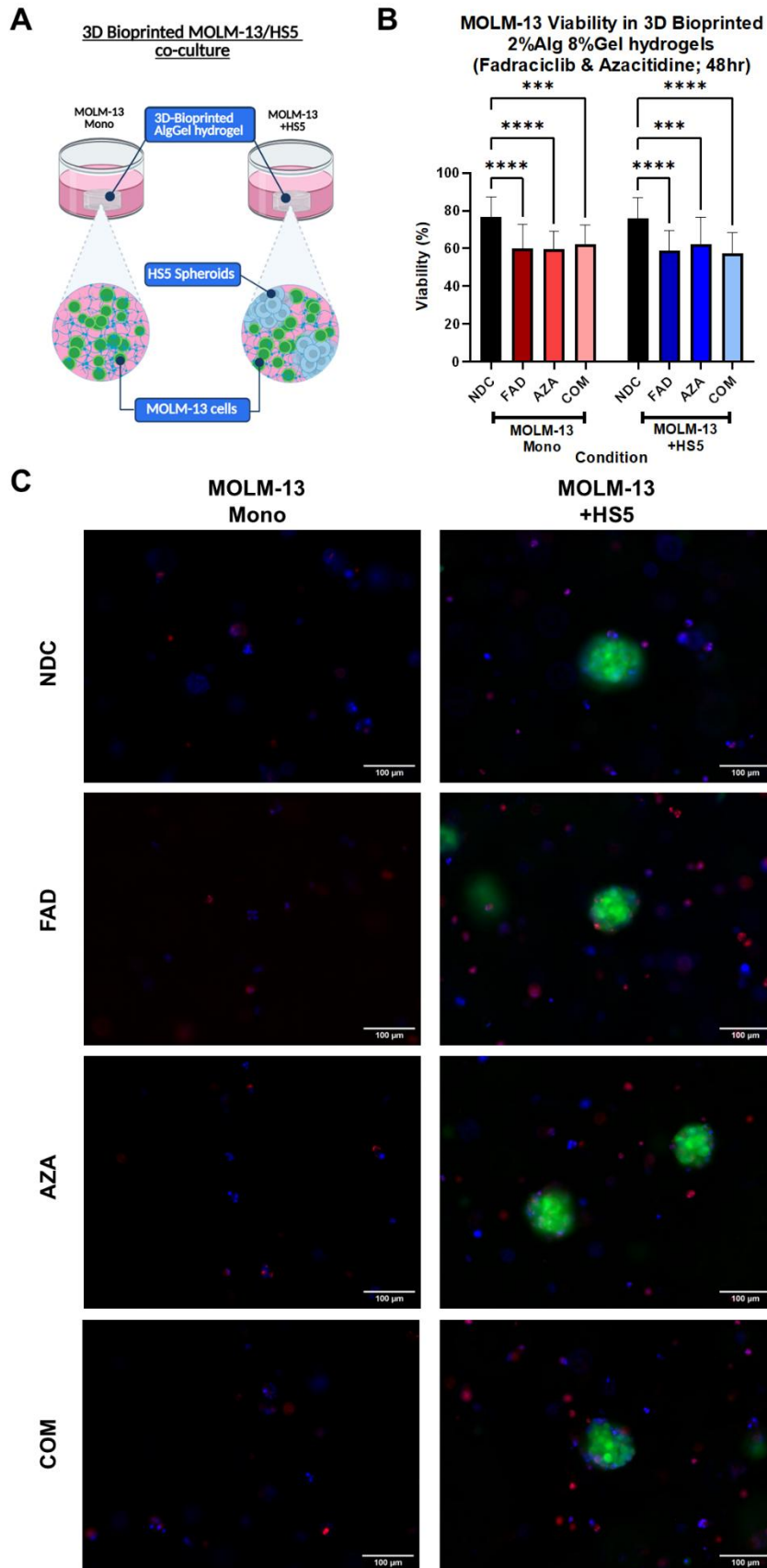


Figure 4: MOLM-13 cells in monoculture and HS5 spheroid co-culture within 3D bioprinted 2%Alg 8%Gel hydrogels, treated with fadraciclib and azacitidine. A: Schematic of 3D bioprinted MOLM-13/HS5 model. Created in BioRender. Hope, L. (2026) <https://BioRender.com/128bdxn> B: MOLM-13 cell viability within the 3D bioprinted hydrogels. One-way ANOVAs with multiple comparisons were used to find significant differences between conditions in B. C: Representative images of 3D bioprinted MOLM-13 cells in monoculture (MOLM-13 Mono) and HS5 co-culture (MOLM-13 + HS5). Images taken at 20X using the M7000 Imager (scale bar 100 μ m). Green: HS5 cells (GFP-HS5 cells); blue: live cells; red: dead cells. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the mean of three biological replicates from independent experiments which each had three technical replicates, with standard deviation depicted as error bars. Analysis was completed within and between groups, therefore non-significance is indicated via the absence of asterisks.

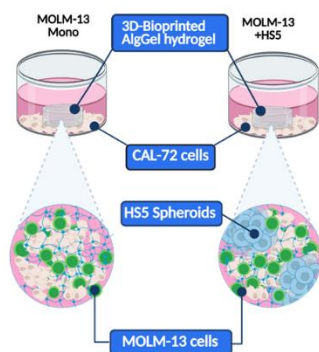
A CAL-72 cell monolayer was then cultured with bioprinted hydrogel containing MOLM-13 alone and MOLM-13 in co-culture with HS5 spheroids (Figure 5A). These cells were added as a monolayer instead of within the hydrogel system to structurally mimic osteoblast structure within the bone marrow, as these typically line the bone itself⁵¹. No significant differences were found between MOLM-13 cells in monoculture and HS5 co-culture in any treatment condition (Figure 5B; Supplementary Figure 14), similar to results obtained without the presence of CAL-72 cells (Figure 4B). Interestingly, MOLM-13 cells within the final tri-culture model displayed a decrease in viability of 5% when compared to azacitidine alone and 8% when compared to fadraciclib alone (Figure 5B). Whilst this was not statistically significant, AML LSCs make up only 0.1-1% of AML cells, therefore whilst this is a relatively small percentage it may be important⁸¹.

Clustering of MOLM-13 cells was observed in 3D bioprinted hydrogels (Figure 4C, Figure 5C) which could have led to increased cell numbers and therefore could influence the percentage of viable cells recorded. Therefore, the reduced clustering of MOLM-13 cells in HS5 spheroid co-culture could indicate that the HS5 cells are slowing the proliferation of MOLM-13 cells, creating a more quiescence-like phenotype, similar to the results obtained in 2D co-culture. However, the results obtained with and without the CAL-72 monolayer were not significantly different, which indicates that the addition of the CAL-72 monolayer may not have added protection to the MOLM-

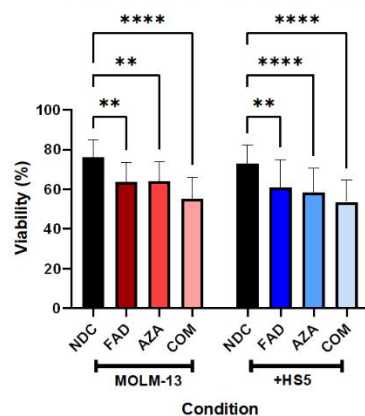
13 cells compared to HS5 spheroids alone (Supplementary Figure 15). This could be due to a lack of direct cell-cell contact, as found in AML and other leukaemias^{57,82}.

ACCEPTED PAPER

A 3D Bio-printed MOLM-13/HS5/CAL-72 tri-culture



B MOLM-13 Viability in 3D Bioprinted 2%Alg 8%Gel hydrogels +CAL-72 (Fadraciclib & Azacitidine; 48hr)



C

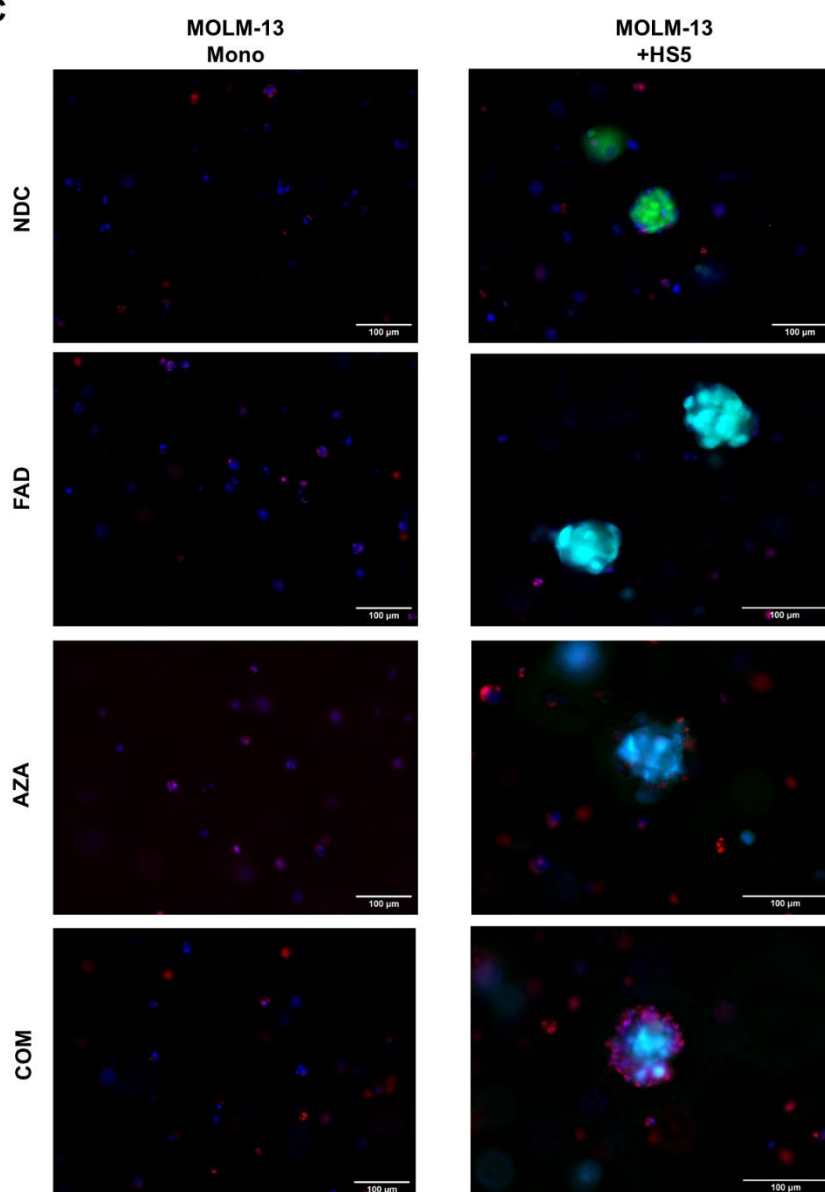


Figure 5: MOLM-13 cell viability within the final 3D bioprinted model following chemotherapy treatment. A: Schematic of 3D bioprinted MOLM-13/HS5 model with CAL-72 monolayer. Created in BioRender. Hope, L. (2026) <https://BioRender.com/o9ahyli> B: MOLM-13 cell viability within the final 3D bioprinted model. C: Representative images of 3D bioprinted MOLM-13 cells in monoculture and HS5 co-culture within the final model. Images taken at 20X using the M7000 Imager (scale bar 100 μ m). Green: HS5 cells (GFP-HS5 cells); blue: live cells; red: dead cells. One-way ANOVAs with multiple comparisons were used to detect significant differences between conditions in B. Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ **** $P \leq 0.0001$. Bars represent the average of three independent biological replicates each with three technical replicates, with standard deviation shown as error bars. Analysis was completed within and between groups – non-significance is indicated via the absence of asterisks.

Finally, the data obtained from the 3D niche systems were compared to the 2D monoculture and co-culture data (Supplementary Figure 15). In the 2D systems, the MOLM-13 viability in the NDC conditions was slightly higher than those in the 3D hydrogel systems, however there were larger decreases in cell viability for each single treatment arm and a more prominent decrease in combination arms in the 2D cultures (Figure 2B&C, Figure 3B&C, Supplementary Figure 15) when compared to those observed in 3D culture (Figure 4B&C, Figure 5B&C, Supplementary Figure 15). Correspondingly, this results in a higher normalised MOLM-13 cell viability in the 3D systems compared to the 2D systems (Supplementary Figure 15 B, D, F). Together, this indicates that the 3D culture system confers a greater protective effect on the MOLM-13 cells compared to 2D culture.

4. Discussion

3D bioprinting has emerged as a novel high-throughput technology for generating reproducible bone marrow models for several blood cancer types^{14,28,34,83}. Alg Gel hydrogels were selected as they have previously been shown to display high cell viability and good printability^{20,26,38,39,84,85}. SEM revealed that 2%Alg 8%Gel have a highly porous structure, with increasing pore size over time (Figure 1E-G). This increased pore size was reflected in a reduction in Young's Modulus (Figure 1A-D). Interestingly, the presence of MOLM-13 cells did not significantly affect the rheological properties of the hydrogels (Figure 1A-D). This could be because MOLM-13 cells are

unable to degrade the surrounding matrix as there are no cell-cleavable sites on the alginate polymers. Regardless, the stiffness of 2%Alg 8%Gel hydrogels remained within the range of bone marrow stiffness ^{49,50}, highlighting its utility as a bone marrow model. No significant differences in MOLM-13 viability were found between hand-cast and 3D bioprinted hydrogels, indicating the hydrogel matrix the cells are embedded in protect them from the shear forces and other stressors experienced during the printing process ^{86,87}. These data concur with previous research, whereby there were no significant differences in viability between hand cast and 3D bioprinted Alg Gel hydrogels containing adipose-derived stem cells ⁸⁸. Staining of F-Actin and cell nuclei showed that MOLM-13 cells formed clusters within the hydrogel over time, thus indicating they were able to proliferate. This mirrors previous studies culturing leukaemia cells within hydrogels ^{26,54,55}, and highlights that bioprinting does not induce significant adverse effects on MOLM-13 cells. Together, this indicates that 2%Alg 8%Gel hydrogels support the viability and proliferation of MOLM-13 cells within this hydrogel matrix. Further, HS5 spheroids maintained their shape and did not migrate into the hydrogel matrix, which could be because alginate lacks cell adhesion motifs and MMP-degradable sites required to promote the migration of HS5 cells out of the spheroid ^{40,41}. Cell spreading has been observed in hydrogel compounds with more cell binding motifs and MMP-cleavable sites ^{26,89,90}. Altogether, these data highlight the potential for 2%Alg 8%Gel hydrogels as the basis of this biocompatible bone marrow model.

Next, we determined the efficacy of our 3D bioprinted bone marrow model for drug screening. In 2D culture, significant differences in MOLM-13 viability were observed between single-treated and combination-treated arms, whereas no significant differences were observed between these arms in 3D culture (Supplementary Figure 15). This reflects that whilst the therapies were effective at inducing apoptosis of MOLM-13 cells, the combination therapy was less effective in 3D compared to 2D cultures. Therefore, the bone marrow niche model developed appears to provide a higher level of chemoprotection compared to 2D culture. This increased drug resistance in 3D models compared to 2D has been observed in several previous bone marrow *in vitro* models ^{34,91}, with resistance increased further when stromal cells such as MSCs are added to the 3D model ^{14,16}. Bray and colleagues developed a 3D *in vitro* tri-culture model, revealing that AML cell lines and primary samples displayed increased resistance to chemotherapies in 3D culture compared to 2D, with this resistance increasing further in 3D co-culture with MSCs and endothelial cells ¹⁶. Treatment with a combination of chemotherapy drugs at clinically relevant concentrations resulted

in a vast reduction in cell viability, similar to our findings whereby 3D culture reduced sensitivity to chemotherapy. Sbrana and colleagues developed a successful 3D bioprinted bone marrow model for modelling CLL using CELLINK bioinks, which could be cultured for up to 28 days³⁴. However, they did not include stromal cells, which could have strengthened their model. Further to this, Alhattab et al, developed a 3D bioprinted AML model comprising AML cells with endothelial cells and MSCs cultured within a peptide hydrogel, and found increased chemoresistance and AML cell quiescence in 3D culture compared to 2D culture¹⁴. In 3D culture, several genes associated with AML cell survival were upregulated, including *HIF1A*, *CCND1* and *MYC*. This further highlights the advantages associated with 3D culture over 2D culture: in 3D culture, AML cells survive in an environment which is closer to *in vivo* conditions, and this affects their gene expression, proliferation and viability⁹². As a result, this impacts the chemoresistance of AML cells, which could lead to more clinically translatable results compared to 2D culture. However, their hydrogels were made in-house, whilst alginate and gelatin are highly characterised and commercially available^{20,26}.

To represent the bone marrow endosteal niche, CAL-72 osteosarcoma cells were included in the model. CAL-72 display many characteristics reminiscent of human primary osteoblastic cells and are frequently utilised as an osteoblastic model^{77,93}. On this basis, they were selected as part of the model. However, few significant differences were observed in 3D cultured MOLM-13 cells with and without the CAL-72 monolayer (Figure 5). This could be because AML cells require cell-cell contact to be fully protected from chemotherapeutic drugs. Previous studies using leukaemia cells cultured with stromal cells separated using Transwell inserts have indicated that direct cell-cell contact is necessary for increasing leukaemia cell chemoresistance^{57,82}. Therefore, the addition of CAL-72 cells as a monolayer may not be as effective as including them within the 3D hydrogel network.

Overall, the 3D bioprinted AML model developed here effectively models certain aspects of the 3D leukaemic bone marrow microenvironment, such as high porosity, mechanical properties and reduced sensitivity to chemotherapy. Additionally, it is optimised with a standard 96 well-assay format, and in general shows low variation between biological replicates (Supplementary Figure 16). This model, in conjunction with current disease detection and stratification methods,

could provide a method of assessing chemotherapies before administering to AML patients. Next Generation Sequencing (NGS) is often completed on patients' blood using a selected panel of genes commonly associated with myeloid disorders^{94,95}. These data are then used to inform the consequent treatment plan, however, the initial treatments may not always be effective, which can slow down recovery of the patient. Potentially, our bone marrow niche system could reduce the time used to find the most suitable treatments – administering selected targeted therapies to 3D bioprinted hydrogels containing cells from the patient could highlight which are more effective, and these could translate to improved patient recovery. If this is effective, this system could be adapted for use in other haematological and solid disorders^{20,87,96-98}. However, it is important to note that our model is a proof of concept and has been utilised only with one cell line. Further stratification with other cell lines and primary cells would be necessary before progressing toward preclinical testing.

Finally, this system could be adapted to model other diseases and conditions. Previously, Alg Gel hydrogels have been selected to model various cancers including non-small cell lung cancer⁸⁷ and breast cancer²⁰. This is likely due in part to the tuneable stiffnesses previously assessed using these hydrogels²⁶ and within this present study (Figure 1A-D), indicating that our model could be adapted to model other haematological and solid cancers. Additionally, these hydrogels are highly porous (Figure 1E-I), permitting the perfusion of media and chemotherapies (Figure 6, Supplementary Figure 3). Finally, the spheroids maintain their structure for at least seven days in culture and remain viable (Supplementary Figures 4 & 5). This highlights that this hydrogel formulation maintains spheroid structure due to the lack of cell-cleavable sites. Many hydrogels which are derived from natural sources, such as collagen, contain cleavable sites which are useful when studying biological processes such as migration, but do not maintain spheroid integrity whilst supporting cell viability; Alg Gel bioinks are more suitable (Supplementary Figures 4 & 5).

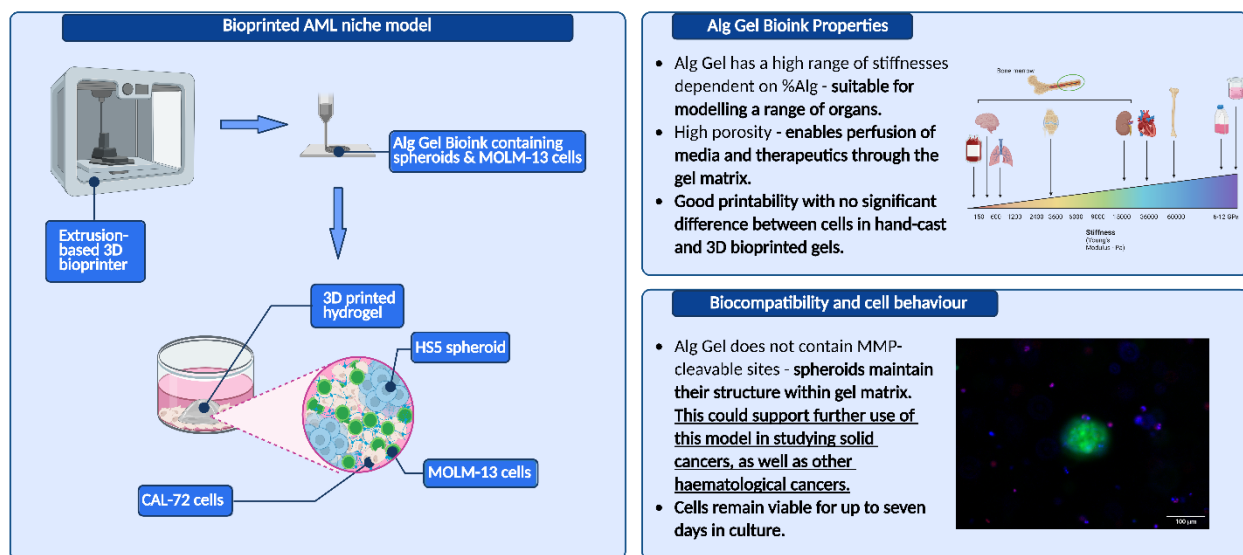


Figure 6: Description of this current model and an outline of features which could prove beneficial for modelling of other organs. Organ stiffness diagram was adapted from ref.⁹⁹ with additional information taken from^{49,50}. Image of viability stained MOLM-13 co-culture with GFP-HS5 spheroids within 2%Alg 8%Gel hydrogel was taken using the Zeiss Axiovert microscope at 20X magnification. Created in BioRender. Hope, L. (2026) <https://BioRender.com/njux9hk>

5. Conclusions

Here, a 3D bioprinted bone marrow niche model was produced which appears to have a chemoprotective effect on MOLM-13 AML cells. This model comprised of MOLM-13 cells and HS5 spheroids within a 2%Alg 8%Gel hydrogel, co-cultured with a CAL-72 cell monolayer. Whilst 3D bioprinted AML models have previously been completed¹⁴, this is the first 3D bioprinted bone marrow model to use alginate/gelatin composite hydrogels, with an endosteal niche component. Greater viability following administration of novel chemotherapeutic drug combinations was observed in the 3D model compared to 2D liquid culture, therefore producing results which more closely replicate human *in vivo* conditions compared to 2D culture. This model is also cost-effective, reproducible, and optimised for a standard 96-well plate format, and could potentially provide a robust model for drug screening in AML.

Acknowledgments

We would like to thank Jennifer Cassels for her support and expertise in flow cytometry, and we would like to thank Margaret Mullin for providing technical support and guidance in Scanning Electron Microscopy. We thank Daniella Zheleva and David Blake, formerly at Cyclacel Ltd for useful discussions. Finally, we would like to thank Dr Xenia Bubnova and Dr Caroline Busch for providing the GFP-HS5 cells they had developed, and Dr Emma Kelly for providing the fluorescent nanoparticles utilised in the drug diffusion study.

Funding

We acknowledge funding from the LifeTIME CDT (grant number: EP/S02347X/1), which was funded by the Engineering and Physical Sciences Research Council, Cyclacel Ltd, and Animal Free Research UK.

Conflict of interest

M.C. has received research funding from Cyclacel and Incyte, is/has been an advisory board member for Novartis, Incyte, Ascentage, Jazz Pharmaceuticals, Pfizer, Azurity, Crossbow, Servier and Mendus, and has received honoraria from Astellas, Novartis, Incyte, Pfizer, Janssen and Jazz Pharmaceuticals. The other authors have no conflicts of interest to disclose.

Author contributions

Conceptualization: All authors

Formal analysis: Lauren Hope

Funding acquisition: Helen Wheadon, Mhairi Copland, Catherine Berry, Manuel Salmeron-Sanchez

Investigation: Lauren Hope, Alvaro Sanchez-Rubio

Methodology: Lauren Hope, Alvaro Sanchez-Rubio

Supervision: Helen Wheadon, Mhairi Copland, Catherine Berry, Manuel Salmeron-Sanchez

Writing—original draft: Lauren Hope

Writing—review & editing: Helen Wheadon, Mhairi Copland, Catherine Berry, Alvaro Sanchez-Rubio

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data supporting this study can be found within the Results and Supplementary File, with further data available upon request. Preliminary data from Figure 1 was originally published in ref. ²⁶, and a more extensive data set which included this data reanalysed with additional data added (presence of MOLM-13 cells in hydrogels used for rheology, and Day 2 SEM analysis and images).

Further disclosure

Some data was presented as a poster at the European School of Haematology 6th International Conference – Acute Myeloid Leukaemia “Molecular and Translational”: Advances in Biology and Treatment, Portugal.

References

1. Jani CT, Ahmed A, Singh H, et al. Burden of AML, 1990-2019: Estimates From the Global Burden of Disease Study. *JCO Glob Oncol*. 2023;(9):e2300229. doi:10.1200/GO.23.00229
2. Chen P, Liu X, Zhao Y, Hu Y, Guo J, Wang H. Global, national, and regional burden of acute myeloid leukemia among 60–89 years-old individuals: insights from a study covering the period 1990 to 2019. *Front Public Health*. 2024;11. doi:10.3389/fpubh.2023.1329529
3. El Chaer F, Bewersdorf JP, Stahl M, Zeidan AM. The global epidemiology of acute myeloid leukaemia. *Nat Rev Clin Oncol*. Published online December 5, 2025;1-14. doi:10.1038/s41571-025-01099-7
4. Khwaja A, Bjorkholm M, Gale RE, et al. Acute myeloid leukaemia. *Nat Rev Dis Primers*. 2016;2(1):1. doi:10.1038/nrdp.2016.10
5. Allan DS, De Lisio M. Reversing pathological remodelling of the bone marrow microenvironment in acute myeloid leukemia. *Stem Cell Investig*. 2018;5(1):29. doi:10.21037/sci.2018.09.02
6. Barrera-Ramirez J, Lavoie JR, Maganti HB, et al. Micro-RNA Profiling of Exosomes from Marrow-Derived Mesenchymal Stromal Cells in Patients with Acute Myeloid Leukemia: Implications in Leukemogenesis. *Stem Cell Rev*. 2017;13(6):817-825. doi:10.1007/s12015-017-9762-0
7. Chandran P, Le Y, Li Y, et al. Mesenchymal stromal cells from patients with acute myeloid leukemia have altered capacity to expand differentiated hematopoietic progenitors. *Leukemia Research*. 2015;39(4):486-493. doi:10.1016/j.leukres.2015.01.013
8. Ishikawa F, Yoshida S, Saito Y, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nat Biotechnol*. 2007;25(11):11. doi:10.1038/nbt1350
9. Raaijmakers MHGP, Mukherjee S, Guo S, et al. Bone progenitor dysfunction induces myelodysplasia and secondary leukemia. *Nature*. 2010;464(7290):852-857. doi:10.1038/nature08851
10. Wacławiczek A, Hamilton A, Rouault-Pierre K, et al. Mesenchymal niche remodeling impairs hematopoiesis via stanniocalcin 1 in acute myeloid leukemia. *J Clin Invest*. 2020;130(6):3038-3050. doi:10.1172/JCI133187
11. Yilmaz M, Wang F, Loghavi S, et al. Late relapse in acute myeloid leukemia (AML): clonal evolution or therapy-related leukemia? *Blood Cancer Journal*. 2019;9(2):2. doi:10.1038/s41408-019-0170-3
12. Kapałczyńska M, Kolenda T, Przybyła W, et al. 2D and 3D cell cultures – a comparison of different types of cancer cell cultures. *Arch Med Sci*. 2018;14(4):910-919. doi:10.5114/aoms.2016.63743
13. Bubnova X, Hope L, Wheadon H. Evaluation of current mouse *in vivo* models and advanced *in vitro* models for leukaemia research. *Blood Reviews*. 2025;74:101318. doi:10.1016/j.blre.2025.101318
14. Alhattab DM, Isaioglou I, Alshehri S, et al. Fabrication of a three-dimensional bone marrow niche-like acute myeloid Leukemia disease model by an automated and controlled process using a robotic

- multicellular bioprinting system. *Biomater Res.* 2023;27(111):1-28. doi:10.1186/s40824-023-00457-9
15. Borella G, Da Ros A, Porcù E, et al. Acute Myeloid Leukemia (AML) in a 3D Bone Marrow Niche Showed High Performance for in Vitro and In Vivo Drug Screenings. *Blood.* 2019;134:544. doi:10.1182/blood-2019-128382
 16. Bray LJ, Binner M, Körner Y, von Bonin M, Bornhäuser M, Werner C. A three-dimensional ex vivo tri-culture model mimics cell-cell interactions between acute myeloid leukemia and the vascular niche. *Haematologica.* 2017;102(7):1215-1226. doi:10.3324/haematol.2016.157883
 17. de Janon A, Mantalaris A, Panoskaltsis N. Three-Dimensional Human Bone Marrow Organoids for the Study and Application of Normal and Abnormal Hematoimmunopoiesis. *The Journal of Immunology.* 2023;210(7):895-904. doi:10.4049/jimmunol.2200836
 18. Gilchrist AE, Harley BAC. Engineered Tissue Models to Replicate Dynamic Interactions within the Hematopoietic Stem Cell Niche. *Advanced Healthcare Materials.* 2022;11(7):2102130. doi:10.1002/adhm.202102130
 19. Xiao Y, McGuinness ChanelleAS, Doherty-Boyd WS, Salmeron-Sanchez M, Donnelly H, Dalby MJ. Current insights into the bone marrow niche: From biology in vivo to bioengineering ex vivo. *Biomaterials.* 2022;286:121568. doi:10.1016/j.biomaterials.2022.121568
 20. Jiang T, Munguia-Lopez JG, Gu K, et al. Engineering bioprintable alginate/gelatin composite hydrogels with tunable mechanical and cell adhesive properties to modulate tumor spheroid growth kinetics. *Biofabrication.* 2019;12(1):015024. doi:10.1088/1758-5090/ab3a5c
 21. Lewis EEL, Wheadon H, Lewis N, et al. A Quiescent, Regeneration-Responsive Tissue Engineered Mesenchymal Stem Cell Bone Marrow Niche Model via Magnetic Levitation. *ACS Nano.* 2016;10(9):8346-8354. doi:10.1021/acs.nano.6b02841
 22. Lewis NS, Lewis EE, Mullin M, Wheadon H, Dalby MJ, Berry CC. Magnetically levitated mesenchymal stem cell spheroids cultured with a collagen gel maintain phenotype and quiescence. *J Tissue Eng.* 2017;8:2041731417704428. doi:10.1177/2041731417704428
 23. Anseth KS, Bowman CN, Brannon-Peppas L. Mechanical properties of hydrogels and their experimental determination. *Biomaterials.* 1996;17(17):1647-1657. doi:10.1016/0142-9612(96)87644-7
 24. Giuseppe MD, Law N, Webb B, et al. Mechanical behaviour of alginate-gelatin hydrogels for 3D bioprinting. *Journal of the Mechanical Behavior of Biomedical Materials.* 2018;79:150-157. doi:10.1016/j.jmbbm.2017.12.018
 25. Mazzocchi A, Soker S, Skardal A. 3D bioprinting for high-throughput screening: Drug screening, disease modeling, and precision medicine applications. *Applied Physics Reviews.* 2019;6(1):011302. doi:10.1063/1.5056188

26. Sanchez-Rubio A, Hope L, Barcelona-Estaje E, Jayawarna V, Williams J, Salmeron-Sanchez M. Gelatin vs GelMA in alginate-based bioinks as a platform for versatile 3D bioprintable *in vitro* systems. *Biomaterials Advances*. 2025;177:214408. doi:10.1016/j.bioadv.2025.214408
27. Agarwal T, Onesto V, Banerjee D, et al. 3D bioprinting in tissue engineering: current state-of-the-art and challenges towards system standardization and clinical translation. *Biofabrication*. 2025;17(4):042003. doi:10.1088/1758-5090/ade47a
28. Braham MVJ, Ahlfeld T, Akkineni AR, et al. Endosteal and Perivascular Subniches in a 3D Bone Marrow Model for Multiple Myeloma. *Tissue Engineering Part C: Methods*. 2018;24(5):300-312. doi:10.1089/ten.tec.2017.0467
29. Heinrich MA, Bansal R, Lammers T, Zhang YS, Michel Schiffelers R, Prakash J. 3D-Bioprinted Mini-Brain: A Glioblastoma Model to Study Cellular Interactions and Therapeutics. *Advanced Materials*. 2019;31(14):1806590. doi:10.1002/adma.201806590
30. Badge AK, Dawande P, Bankar NJ, Noman O. Three-dimensional Bioprinting Platform for Chemotherapy Evaluation in Leukemia Patients: An Innovative Approach. *Journal of Applied Hematology*. 2025;16(1):90. doi:10.4103/joah.joah_15_25
31. Rudzinska-Radecka M, Turos-Korgul L, Mukherjee D, Podsiwylow-Bartnicka P, Piwocka K, Guzowski J. High-throughput formulation of reproducible 3D cancer microenvironments for drug testing in myeloid leukemia. *Biofabrication*. 2024;17(1):015035. doi:10.1088/1758-5090/ad998d
32. Zhou D, Chen L, Ding J, et al. A 3D engineered scaffold for hematopoietic progenitor/stem cell co-culture in vitro. *Sci Rep*. 2020;10(1):1. doi:10.1038/s41598-020-68250-5
33. Ribezzi D, Pinos R, Bonetti L, et al. Design of a novel bioink suitable for the 3D printing of lymphoid cells. *Frontiers in Biomaterials Science*. 2023;2(1):1-12. doi:https://doi.org/10.3389/fbiom.2023.1081065
34. Sbrana FV, Pinos R, Barbaglio F, et al. 3D Bioprinting Allows the Establishment of Long-Term 3D Culture Model for Chronic Lymphocytic Leukemia Cells. *Frontiers in Immunology*. 2021;12(1):Article No. 639572. doi:10.3389/fimmu.2021.639572
35. Rodriguez C, Aleman J, George S, et al. Patient-Specific 3D Printed Multiple Myeloma Organoid Model for Niche Recapitulation and Prediction of Chemotherapy Response. *Clinical Lymphoma Myeloma and Leukemia*. 2019;19:S336. doi:10.1016/j.clml.2019.07.332
36. Nair K, Gandhi M, Khalil S, et al. Characterization of cell viability during bioprinting processes. *Biotechnology Journal*. 2009;4(8):1168-1177. doi:10.1002/biot.200900004
37. Kačarević ŽP, Rider PM, Alkildani S, et al. An Introduction to 3D Bioprinting: Possibilities, Challenges and Future Aspects. *Materials*. 2018;11(11):11. doi:10.3390/ma11112199
38. Y. Chung JH, Naficy S, Yue Z, et al. Bio-ink properties and printability for extrusion printing living cells. *Biomaterials Science*. 2013;1(7):763-773. doi:10.1039/C3BM00012E

39. Ouyang L, Yao R, Zhao Y, Sun W. Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells. *Biofabrication*. 2016;8(3):035020. doi:10.1088/1758-5090/8/3/035020
40. Fonseca K, Raquel Maia F, A. Cruz F, et al. Enzymatic, physicochemical and biological properties of MMP-sensitive alginate hydrogels. *Soft Matter*. 2013;9(12):3283-3292. doi:10.1039/C3SM27560D
41. Schulz A, Gepp MM, Stracke F, von Briesen H, Neubauer JC, Zimmermann H. Tyramine-conjugated alginate hydrogels as a platform for bioactive scaffolds. *Journal of Biomedical Materials Research Part A*. 2019;107(1):114-121. doi:10.1002/jbm.a.36538
42. Contessi Negrini N, Angelova Volponi A, Sharpe PT, Celiz AD. Tunable Cross-Linking and Adhesion of Gelatin Hydrogels via Bioorthogonal Click Chemistry. *ACS Biomater Sci Eng*. 2021;7(9):4330-4346. doi:10.1021/acsbiomaterials.1c00136
43. Xie D, Bian J, Ni C, Zhao P, Pu Z, Yue J. Tuning Room-Temperature Injectability of Gelatin-Based Hydrogels via Introduction of Competitive Hydrogen Bonds. *ACS Macro Lett*. 2025;14(3):313-319. doi:10.1021/acsmacrolett.5c00018
44. He Y, Yang F, Zhao H, Gao Q, Xia B, Fu J. Research on the printability of hydrogels in 3D bioprinting. *Sci Rep*. 2016;6(1):1. doi:10.1038/srep29977
45. Chantkran W, Hsieh YC, Zheleva D, Frame S, Wheadon H, Copland M. Interrogation of novel CDK2/9 inhibitor fadraciclib (CYC065) as a potential therapeutic approach for AML. *Cell Death Discov*. 2021;7(1):1. doi:10.1038/s41420-021-00496-y
46. Frame S, Saladino C, MacKay C, et al. Fadraciliclib (CYC065), a novel CDK inhibitor, targets key pro-survival and oncogenic pathways in cancer. *PLOS ONE*. 2020;15(7):e0234103. doi:10.1371/journal.pone.0234103
47. Matsuo Y, MacLeod R, Uphoff C, et al. Two acute monocytic leukemia (AML-M5a) cell lines (MOLM-13 and MOLM-14) with interclonal phenotypic heterogeneity showing MLL-AF9 fusion resulting from an occult chromosome insertion, ins(11;9)(q23;p22p23). *Leukemia*. 1997;11(9):1469-1477. doi:10.1038/sj.leu.2400768
48. Rao X, Huang X, Zhou Z, Lin X. An improvement of the $2^{-\Delta\Delta CT}$ method for quantitative real-time polymerase chain reaction data analysis. *Biostat Bioinforma Biomath*. 2013;3(3):71-85.
49. Jansen LE, Birch NP, Schiffman JD, Crosby AJ, Peyton SR. Mechanics of intact bone marrow. *Journal of the Mechanical Behavior of Biomedical Materials*. 2015;50:299-307. doi:10.1016/j.jmbbm.2015.06.023
50. Zhang P, Zhang C, Li J, Han J, Liu X, Yang H. The physical microenvironment of hematopoietic stem cells and its emerging roles in engineering applications. *Stem Cell Research & Therapy*. 2019;10(1):327. doi:10.1186/s13287-019-1422-7
51. Bartl R, Bartl C, Bonél HM, von Tresckow E. Anatomy and Physiology of the Bone–Bone Marrow System. In: Bartl R, Bartl C, Bonél HM, von Tresckow E, eds. *Bone Marrow Edema: Forms, Pathogenesis, Diagnosis, and Therapy*. Springer; 2025:1-15. doi:10.1007/978-3-662-72321-0_1

52. Tevis KM, Colson YL, Grinstaff MW. Embedded Spheroids as Models of the Cancer Microenvironment. *Advanced Biosystems*. 2017;1(10):1700083. doi:10.1002/adbi.201700083
53. Zandoni M, Pignatta S, Arienti C, Bonafè M, Tesei A. Anticancer drug discovery using multicellular tumor spheroid models. *Expert Opinion on Drug Discovery*. 2019;14(3):289-301. doi:10.1080/17460441.2019.1570129
54. Svozilová H, Plichta Z, Proks V, et al. RGDS-Modified Superporous Poly(2-Hydroxyethyl Methacrylate)-Based Scaffolds as 3D In Vitro Leukemia Model. *International Journal of Molecular Sciences*. 2021;22(5):5. doi:10.3390/ijms22052376
55. Vu TTT, Lim C, Lim M. Characterization of leukemic cell behaviors in a soft marrow mimetic alginate hydrogel. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*. 2012;100B(7):1980-1988. doi:10.1002/jbm.b.32765
56. Adamo A, Delfino P, Gatti A, et al. HS-5 and HS-27A Stromal Cell Lines to Study Bone Marrow Mesenchymal Stromal Cell-Mediated Support to Cancer Development. *Frontiers in Cell and Developmental Biology*. 2020;8(1):1-13. doi:https://doi.org/10.3389/fcell.2020.584232
57. Garrido SM, Appelbaum FR, Willman CL, Banker DE. Acute myeloid leukemia cells are protected from spontaneous and drug-induced apoptosis by direct contact with a human bone marrow stromal cell line (HS-5). *Experimental Hematology*. 2001;29(4):448-457. doi:10.1016/S0301-472X(01)00612-9
58. Hou D, Wang B, You R, et al. Stromal cells promote chemoresistance of acute myeloid leukemia cells via activation of the IL-6/STAT3/OXPHOS axis. *Ann Transl Med*. 2020;8(21):1346. doi:10.21037/atm-20-3191
59. Miari KE, Williams MTS. Stromal bone marrow fibroblasts and mesenchymal stem cells support acute myeloid leukaemia cells and promote therapy resistance. *British Journal of Pharmacology*. 2023;181(2):216-237. doi:10.1111/bph.16028
60. Mohammadi S, Nikbakht M, Sajjadi SM, et al. Reciprocal Interactions of Leukemic Cells with Bone Marrow Stromal Cells Promote Enrichment of Leukemic Stem Cell Compartments in Response to Curcumin and Daunorubicin. *Asian Pac J Cancer Prev*. 2017;18(3):831-840. doi:10.22034/APJCP.2017.18.3.831
61. Wang Z, Chai C, Wang R, et al. Single-cell transcriptome atlas of human mesenchymal stem cells exploring cellular heterogeneity. *Clinical and Translational Medicine*. 2021;11(12):e650. doi:10.1002/ctm2.650
62. Yin JQ, Zhu J, Ankrum JA. Manufacturing of primed mesenchymal stromal cells for therapy. *Nat Biomed Eng*. 2019;3(2):2. doi:10.1038/s41551-018-0325-8
63. Keating GM. Azacitidine. *Drugs*. 2012;72(8):1111-1136. doi:10.2165/11209430-000000000-00000
64. Faruqi A, Tadi P. Cytarabine. In: *StatPearls*. StatPearls Publishing; 2023. Accessed December 7, 2023. <http://www.ncbi.nlm.nih.gov/books/NBK557680/>

65. Bose P, Gandhi V, Konopleva M. Pathways and mechanisms of venetoclax resistance. *Leukemia & Lymphoma*. 2017;58(9):2026-2039. doi:10.1080/10428194.2017.1283032
66. Aldoss I, Yang D, Aribi A, et al. Efficacy of the combination of venetoclax and hypomethylating agents in relapsed/refractory acute myeloid leukemia. *Haematologica*. 2018;103(9):e404-e407. doi:10.3324/haematol.2018.188094
67. Cherry EM, Abbott D, Amaya M, et al. Venetoclax and azacitidine compared with induction chemotherapy for newly diagnosed patients with acute myeloid leukemia. *Blood Advances*. 2021;5(24):5565-5573. doi:10.1182/bloodadvances.2021005538
68. Grimm D, Bauer J, Wise P, et al. The role of SOX family members in solid tumours and metastasis. *Seminars in Cancer Biology*. 2020;67:122-153. doi:10.1016/j.semcancer.2019.03.004
69. Xu YR, Yang WX. SOX-mediated molecular crosstalk during the progression of tumorigenesis. *Seminars in Cell & Developmental Biology*. 2017;63:23-34. doi:10.1016/j.semcd.2016.07.028
70. Hu J, Li K, Li Z, et al. Sex-determining region Y box-containing genes: regulators and biomarkers in gynecological cancers. *Cancer Biol Med*. 2019;16(3):462-474. doi:10.20892/j.issn.2095-3941.2019.0062
71. Wang J, Wan X, Le Q. Cross-regulation between SOX9 and the canonical Wnt signalling pathway in stem cells. *Frontiers in Molecular Biosciences*. 2023;10:1-12. doi:doi.org/10.3389/fmolb.2023.1250530
72. Roecklein B, Torok-Storb B. Functionally distinct human marrow stromal cell lines immortalized by transduction with the human papilloma virus E6/E7 genes. *Blood*. 1995;85(4):997-1005. doi:10.1182/blood.V85.4.997.bloodjournal854997
73. Yu Z, Zhang W, Kone BC. Signal transducers and activators of transcription 3 (STAT3) inhibits transcription of the inducible nitric oxide synthase gene by interacting with nuclear factor κ B. *Biochemical Journal*. 2002;367(1):97-105. doi:10.1042/bj20020588
74. Fan Y, Li Y, Yao X, et al. Epithelial SOX9 drives progression and metastases of gastric adenocarcinoma by promoting immunosuppressive tumour microenvironment. *Gut*. 2023;72(4):624-637. doi:10.1136/gutjnl-2021-326581
75. Song S, Ajani JA, Honjo S, et al. Hippo Coactivator YAP1 Upregulates SOX9 and Endows Esophageal Cancer Cells with Stem-like Properties. *Cancer Research*. 2014;74(15):4170-4182. doi:10.1158/0008-5472.CAN-13-3569
76. Song S, Maru DM, Ajani JA, et al. Loss of TGF- β Adaptor β 2SP Activates Notch Signaling and SOX9 Expression in Esophageal Adenocarcinoma. *Cancer Research*. 2013;73(7):2159-2169. doi:10.1158/0008-5472.CAN-12-1962
77. Rochet N, Leroy P, Far DF, Ollier L, Loubat A, Rossi B. CAL72: a human osteosarcoma cell line with unique effects on hematopoietic cells. *Eur J Haematol*. 2003;70(1):43-52. doi:10.1034/j.1600-0609.2003.02766.x

78. Bruserud O, Ryningen A, Wergeland L, Glenjen NI, Gjertsen BT. Osteoblasts increase proliferation and release of pro-angiogenic interleukin 8 by native human acute myelogenous leukemia blasts. *Haematologica*. 2004;89(4):4. doi:10.3324/%x
79. Hatfield KJ, Hovland R, Øyan AM, et al. Release of angiopoietin-1 by primary human acute myelogenous leukemia cells is associated with mutations of nucleophosmin, increased by bone marrow stromal cells and possibly antagonized by high systemic angiopoietin-2 levels. *Leukemia*. 2008;22(2):2. doi:10.1038/sj.leu.2404985
80. Reikvam H, Nepstad I, Bruserud Ø, Hatfield KJ. Pharmacological targeting of the PI3K/mTOR pathway alters the release of angioregulatory mediators both from primary human acute myeloid leukemia cells and their neighboring stromal cells. *Oncotarget*. 2013;4(6):830-843.
81. Roboz GJ, Guzman M. Acute Myeloid Leukemia Stem Cells: Seek and Destroy. *Expert Rev Hematol*. 2009;2(6):663-672. doi:10.1586/ehm.09.53
82. Sison EAR, Rau RE, McIntyre E, Li L, Small D, Brown P. MLL-rearranged acute lymphoblastic leukaemia stem cell interactions with bone marrow stroma promote survival and therapeutic resistance that can be overcome with CXCR4 antagonism. *British Journal of Haematology*. 2013;160(6):785-797. doi:10.1111/bjh.12205
83. Bessy T, Itkin T, Passaro D. Bioengineering the Bone Marrow Vascular Niche. *Frontiers in Cell and Developmental Biology*. 2021;9. Accessed December 31, 2023. <https://www.frontiersin.org/articles/10.3389/fcell.2021.645496>
84. Aroguz AZ, Baysal K, Adiguzel Z, Baysal BM. Alginate/Polyoxyethylene and Alginate/Gelatin Hydrogels: Preparation, Characterization, and Application in Tissue Engineering. *Appl Biochem Biotechnol*. 2014;173(2):433-448. doi:10.1007/s12010-014-0851-0
85. Duan B, Hockaday LA, Kang KH, Butcher JT. 3D Bioprinting of heterogeneous aortic valve conduits with alginate/gelatin hydrogels. *Journal of Biomedical Materials Research Part A*. 2013;101A(5):1255-1264. doi:10.1002/jbm.a.34420
86. Cheng J, Lin F, Liu H, et al. Rheological Properties of Cell-Hydrogel Composites Extruding Through Small-Diameter Tips. *Journal of Manufacturing Science and Engineering*. 2008;130(0210141-5). doi:10.1115/1.2896215
87. Mondal A, Gebeyehu A, Miranda M, et al. Characterization and printability of Sodium alginate - Gelatin hydrogel for bioprinting NSCLC co-culture. *Sci Rep*. 2019;9(1):1. doi:10.1038/s41598-019-55034-9
88. Yao R, Zhang R, Luan J, Lin F. Alginate and alginate/gelatin microspheres for human adipose-derived stem cell encapsulation and differentiation. *Biofabrication*. 2012;4(2):025007. doi:10.1088/1758-5082/4/2/025007
89. Miao F, Liu T, Zhang X, et al. Engineered bone tissues using biomineralized gelatin methacryloyl/sodium alginate hydrogels. *Journal of Biomaterials Science, Polymer Edition*. 2022;33(2):137-154. doi:10.1080/09205063.2021.1980360

90. Zuo X, Zhang H, Zhou T, et al. Spheroids of Endothelial Cells and Vascular Smooth Muscle Cells Promote Cell Migration in Hyaluronic Acid and Fibrinogen Composite Hydrogels. *Research*. 2020;2020:Article No. 8970480. doi:10.34133/2020/8970480
91. Shang ZY, Sun H, Xiang HF, et al. Three-dimensional porous composite scaffolds for in vitro marrow microenvironment simulation to screen leukemia drug. *Biomed Mater*. 2020;15(3):035016. doi:10.1088/1748-605X/ab74e2
92. Jensen C, Teng Y. Is It Time to Start Transitioning From 2D to 3D Cell Culture? *Frontiers in Molecular Biosciences*. 2020;7(1):33. doi:https://doi.org/10.3389/fmolb.2020.00033
93. Peter B, Eisenwort G, Sadovnik I, et al. BRD4 degradation blocks expression of MYC and multiple forms of stem cell resistance in Ph+ chronic myeloid leukemia. *American Journal of Hematology*. 2022;97(9):1215-1225. doi:10.1002/ajh.26650
94. El Achi H, Kanagal-Shamanna R. Biomarkers in Acute Myeloid Leukemia: Leveraging Next Generation Sequencing Data for Optimal Therapeutic Strategies. *Front Oncol*. 2021;11. doi:10.3389/fonc.2021.748250
95. Myeloid gene panel analysis. Manchester University NHS Foundation Trust. Accessed March 26, 2026. <https://mft.nhs.uk/nwglh/test-information/cancer/haematology-oncology/national-genomic-test-directory-services-available/acute-myeloid-leukaemia/myeloid-gene-panel-analysis/>
96. Hiller T, Berg J, Elomaa L, et al. Generation of a 3D Liver Model Comprising Human Extracellular Matrix in an Alginate/Gelatin-Based Bioink by Extrusion Bioprinting for Infection and Transduction Studies. *International Journal of Molecular Sciences*. 2018;19(10):10. doi:10.3390/ijms19103129
97. Hong S, Song JM. 3D bioprinted drug-resistant breast cancer spheroids for quantitative *in situ* evaluation of drug resistance. *Acta Biomaterialia*. 2022;138:228-239. doi:10.1016/j.actbio.2021.10.031
98. Heltmann-Meyer S, Fleischer S, Kadam S, et al. Ink comparison for extrusion-based bioprinting in the context of breast cancer and melanoma models. *International Journal of Bioprinting*. 2025;11(2):249. doi:10.36922/ijb.5828
99. Handorf AM, Zhou Y, Halanski MA, Li WJ. Tissue stiffness dictates development, homeostasis, and disease progression. *Organogenesis*. 2015;11(1):1-15. doi:10.1080/15476278.2015.1019687