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METHOD ARTICLE

A detailed methodology for developing and applying a 3D polyHIPE scaffold to model myeloma cell dormancy *in vitro*

[version 1; peer review: 1 approved]

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Abstract

Background

Multiple myeloma is characterised by the outgrowth of malignant plasma cells in the bone marrow (BM). Disease relapse is driven by the reactivation of dormant myeloma cells (DMCs) that interact with osteoblastic lineage cells within the BM to evade therapy. Current two-dimensional (2D) *in vitro* culture systems lack the physiological and spatial complexity to replicate the BM microenvironment, while animal models offer limited control over microenvironmental components for systematic investigation of cell-to-cell interactions relevant to myeloma dormancy and are resource-intensive. Therefore, a more physiologically relevant and controllable *in vitro* model is needed to study dormancy mechanisms and microenvironmental regulation of drug responses.

Methods

We developed a reproducible and scalable three-dimensional (3D) polymerised High Internal Phase Emulsion (polyHIPE) scaffold model that mimics key features of the osteoblastic bone niche, where myeloma dormancy occurs. The osteoblast-supported model enables the investigation of dormancy induction, maintenance, and targeting within an endosteal-like environment. This protocol involves scaffold synthesis, sterilisation, osteoblast and myeloma cell seeding, and dormancy tracking using membrane dye retention.

Open Peer Review

Approval Status

1

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[view](#)

1. Elham Saberian¹, Pavol Jozef Šafárik
University, Kosice, Slovakia

Any reports and responses or comments on the article can be found at the end of the article.

Results

Using multiple osteoblastic (MG63, hFOB 1.19, and MC3T3) and myeloma (U266, JJN3, and 5TGM1) cell lines, we demonstrated the interchangeability of the model. PolyHIPE scaffolds facilitated osteoblast and myeloma cell infiltration and interaction over time and altered the drug sensitivity of cells in the 3D niche compared to conventional 2D cultures. Troubleshooting tips and time considerations were established to enhance the reproducibility.

Conclusions

While primarily intended for researchers studying myeloma dormancy, this 3Rs-aligned approach is adaptable and has the potential for broader use across the myeloma and cancer dormancy research. The model provides a controllable *in vitro* alternative for early-stage discovery and hypothesis-generating studies to minimise reliance on *in vivo* myeloma models, aligns with the 3Rs principles, and provides a platform to investigate dormancy-associated drug resistance and microenvironmental control of myeloma behaviour prior to targeted *in vivo* validation.

Keywords

Multiple myeloma, dormancy, 3D culture, polyHIPE scaffold, bone microenvironment, osteoblasts, 3Rs, drug response.



This article is included in the **NC3Rs** gateway.

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Research highlights

Scientific benefit(s):	• Allows study of osteoblast and myeloma cell interactions overtime (+3 weeks) • Allows study of myeloma cell dormancy induction • Allows study of bone marrow stromal cell influences on myeloma dormancy • Allows study of drug effects on whole and dormant myeloma cell populations
3Rs benefit(s):	• Provides an <i>in vitro</i> platform to minimise the use of animal models for the purposes of preclinical drug testing targeting myeloma cell dormancy • Potential to replace small pilot <i>in vivo</i> studies (typically 10–20 mice), reducing reliance on animal experiments in early-stage drug testing, supporting reduction in total animal numbers used per year
Practical benefit(s):	• Inexpensive materials needed to produce polyHIPE scaffolds • Modifiable scaffold composition – allows customisation to fit a range of cell types • Compatible with human and murine osteoblast and myeloma cell lines, adaptable to be used with readily available cells in laboratories without the necessity to buy specific cells • Scalable production – large batches of scaffolds (>200) can be produced in a short window of time (<2 weeks)
Current applications:	• Suitable for observing myeloma and osteoblast interactions • Suitable for generating dormant myeloma cells • Suitable for drug assessments on whole and segmented cell populations
Potential applications:	• Offers a platform to study myeloma cell interaction with other bone marrow stromal cells • Assessing drug sensitivity of dormant myeloma cells • Studying dormancy and drug response of other cancers (e.g. breast or prostate cancer) that become dormant within the bone marrow

Introduction

Multiple myeloma is a plasma cell malignancy within the bone marrow (BM).^{1,2} It is the third most common haematological cancer in the UK, and many current therapies, such as chemotherapy, immunotherapy, and targeted treatments, have helped make significant strides in treating the disease.^{3,4} However, a significant challenge in myeloma treatment is disease relapse, with approximately 66% of myeloma patients relapsing within four years of first remission.⁵ A major clinical challenge in relapse prevention is the persistence of chemotherapy-resistant cells, including dormant myeloma cells (DMCs), during minimal residual disease (MRD).^{6–8} DMCs are non-proliferative cells that are resistant to conventional treatments and reside in specialised endosteal niches within the BM, making them difficult to target.^{6,7} Much of what is known about DMC biology comes from preclinical studies, both *in vitro* and *in vivo*,^{9,10} where it has been demonstrated that their dormant status can be controlled and influenced by interactions with niche-resident cells, including osteoblast-lineage cells and osteoclasts.^{6,7,10} Consequently, DMCs may escape growth arrest and repopulate the BM, leading to disease relapse. Therefore, developing therapies that specifically target DMCs is critical for improving the long-term survival outcomes of patients with myeloma.

Several models have been employed to study dormant cancer cells, including *in vitro* and *in vivo* systems; these have been used to further our understanding of dormancy mechanisms and to investigate drug effects. One of the best-characterised murine models of myeloma is 5TGM1, where murine myeloma 5TGM1 cells are injected via the tail vein into C57BL/KaLwRijHsd mice and home to the BM.^{11–14} This model has been used to demonstrate that DMCs respond differently to chemotherapy agents, exhibiting resistance to melphalan and bortezomib.⁶ Additionally, AXL inhibitors such as cabozantinib have been shown to release DMCs from endosteal niches in this model.⁷

While *in vivo* models have been fundamental for testing the effects of therapeutic agents on DMCs and their endosteal niche interactions, they have significant limitations. They require a large number of animals to be used, which raises ethical concerns and limits drug testing capabilities owing to time and cost constraints. Additionally, the severity of these models is often defined as moderate under the Animals Scientific Procedures Act 1986 because of the tumour burden placed on the animals. Moreover, *in vivo* models often use only a single cell line for tumour inoculation, limiting their ability to capture the high heterogeneity of myeloma. Therefore, this places a large limitation on the interpretation of data generated from animal studies.

In contrast, *in vitro* models offer more ethical and cost-effective alternatives. They have been used to understand the molecular mechanisms driving dormancy. For instance, *in vitro*, DMCs can be observed in monocultures with low prevalence, but their prevalence can be increased by co-cultures such as osteoblasts through both direct cell-to-cell

contact and indirect signalling and reduced through indirect effects of mesenchymal stem cells.^{6,7,10,15} Established myeloma cell lines as well as primary myeloma cells from patients can be used to identify key markers of dormancy. *In vitro* models can overcome some limitations of *in vivo* models by incorporating a more heterogeneous sample pool and better replicating the complexity of the BM microenvironment. However, drug response data collected from two-dimensional (2D) *in vitro* models often fail to match the clinical outcomes.^{16,17} Furthermore, although technically simpler, 2D *in vitro* systems fail to recreate a three-dimensional (3D) bone microenvironment, which is crucial for *in vivo* myeloma cell dormancy. This limitation in the models can lead to contrasting data when drug candidates are considered, thereby slowing treatment development.

To address these challenges, we developed a novel *in vitro* model that mimics *in vivo* osteoblast and myeloma cell interactions in a 3D environment. This model provides a platform for initial drug screening against DMCs and mechanistic studies prior to the use of more focused preclinical *in vivo* studies, thereby significantly reducing the total number of animals used in myeloma research. This is because, on average, 50 mice were used in each myeloma *in vivo* study, and ~ 140 papers were published annually. By providing a controllable *in vitro* system for early drug response studies, this model has the potential to replace small pilot *in vivo* studies typically involving 10–20 mice per study, which are often used before larger efficacy *in vivo* studies.

Our model utilises polycaprolactone (PCL), which is commonly used in tissue engineering applications, to provide a 3D scaffold foundation that offers structural support similar to that of *in vivo* bone.^{18,19} PCL is a synthetic biodegradable polymer that has previously been validated extensively in human bone regeneration, making it a favourable material that is cost-effective and readily available.^{20,21} A key advantage of using PCL is that it eliminates the need to use animal-derived products, unlike similar 3D bone models such as those built on Matrigel, which can be unreliable owing to batch-to-batch variability.

While *in vitro* 3D models have been used to study myeloma, none have used PCL or specifically assessed DMCs due to various limitations. For example, most 3D models rely heavily on imaging techniques to examine cells within the model because of the limited retrieval of cells. The use of flow cytometry analysis is often restricted for 3D models because of the necessary steps required to isolate cells that can alter cellular characteristics.

By manufacturing the scaffold, the base material is processed to produce a PCL-based polymerised High Internal Phase Emulsion (polyHIPE) scaffold structure.^{18,20,22} This provided a highly porous, interconnected internal architecture that facilitated cell migration and simulated the BM niche. The polyHIPE structure can also be highly attuned to generate a refined scaffold that supports cell growth and cellular dormancy. Importantly, cells can be isolated from scaffolds through enzymatic digestion steps similar to those used in standard 2D culture methods.

To make our model easily achievable and adaptable, few additional components were added to the scaffold base. First, osteoblast-lineage cells can be cultured directly on the scaffold material to facilitate their ingrowth throughout the pores to mimic the endosteal niche. Following this, myeloma cells can be incorporated onto the scaffold, and their proliferation is monitored through the retention of a cell membrane dye, extensively used by others as a method to track dormant cells.^{6,7} As a result, our model offers a more robust and customisable system to replicate key aspects of the *in vivo* endosteal niche, offering potential insights into dormancy mechanisms.

In this article, we provide an overview of how we have developed and optimised this model, how the model (henceforth termed polyHIPE scaffold) can be prepared for *in vitro* systems, and how it can be applied to answer various research questions. We will also provide a step-by-step protocol covering the manufacturing of the PCL base, culturing osteoblast-lineage cells and myeloma cells on the scaffold, and tracking DMCs through several experimental procedures.

We aimed for this model to be used by myeloma researchers focused on drug testing against DMCs, but it could also be applied more broadly as a versatile 3D model to study the biology and therapeutic responses of myeloma and niche cells. Moreover, it could be applied more generally to researchers investigating tumour–bone interactions, stromal niche biology, or early-stage therapeutic responses within 3D microenvironments. We propose that this model offers an easier alternative to gel-based models owing to its simplicity and reproducibility.

Materials and methods

Materials and equipment

The supplier and reference ID of all the reagents and equipment used in this study are listed in [Table 1](#). The methods described here focus on a small selection of myeloma and osteoblastic cells, but the model can be readily adapted to accommodate other cell lines/types.

Table 1. List of materials and equipment.

Chemicals and reagents		
Reagent	Source	Identifier*
RPMI 1640 Medium, GlutaMAX™ Supplement	GIBCO, Life Technologies	Cat#61870-010
Fetal Bovine Serum (FBS)	ThermoFisher	Cat#10500064 (Lot: 2575614H)
MEM Non-Essential Amino Acids Solution	Fisher Scientific	Cat#25-025-CIR
Sodium Pyruvate	ThermoFisher	Cat#11360-070
Penicillin-Streptomycin (pen/strep)	ThermoFisher	Cat#15140-122
DMEM/F-12, GlutaMAX™	ThermoFisher	Cat#10565018
Geneticin™ Selective Antibiotic (G418)	ThermoFisher	Cat#10131035
Oxoid™ Phosphate Buffered Saline Tablets (PBS)	ThermoFisher	Cat#BR0014G
Vybrant™ DiD Cell-Labeling Solution (DiD)	ThermoFisher	Cat#V22887
AlamarBlue™ Cell Viability Reagent	ThermoFisher	Cat#DAL1100
Paraformaldehyde, 4% in PBS	ThermoFisher	Cat#J61899
LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit	ThermoFisher	Cat#L34955
DAPI Solution	ThermoFisher	Cat#62248 (Lot: XD3562132)
Bright Cryo-M-Bed (OCT)	Bright Instruments	Cat#53581
Andwin Scientific Cryomould	Fisher Scientific	Cat#NC9511236
Epredia™ SuperFrost Plus™ Adhesion Slides	Fisher Scientific	Cat#12312148
Accutase® Cell Detachment Solution	Innovative Cell Technologies	Cat#AT104
Trypsin-EDTA	ThermoFisher	Cat#25300062
Hypermer B246 (surfactant)	Croda	https://www.crodaindustrialspecialties.com/en-gb/product-finder/product/531-hypermer_1_b246
PCLMA	In house	https://licensing.sheffield.ac.uk/product/polycaprolactone-methacrylate-pclma
2,4,6-Trimethylbenzoyl Phosphine Oxide/2-Hydroxy-2- Methylpropiophenone (Photoinitiator)	Sigma Aldrich	Cat#405663
Chloroform	Sigma Aldrich	Cat#02487
Toluene	Sigma Aldrich	Cat#89680
Experimental models: Cell lines		
Cell name	Source	Identifier
OPM2-GFP-Luc	DSMZ (lentiviral transduced in house with GFP-Luc)	RRID:CVCL_1625
JJN3-GFP-Luc	DSMZ (lentiviral transduced in house with GFP-Luc)	RRID:CVCL_2078
hFOB 1.19	ATCC	RRID:CVCL_3708
hFOB-RFP-Luc	This paper	N/A

Table 1. *Continued*

Experimental models: polyHIPE		
Item	Source	Identifier
PCL polyHIPE discs	TUoS	N/A
Software		
Software	Source	Identifier/Link
FlowJo v10	FlowJo, LLC	https://www.flowjo.com/solutions/flowjo RRID:SCR_008520
GraphPad Prism v9.0	GraphPad	https://www.graphpad.com/ RRID:SCR_002798
ImageJ Fiji	NIH	https://imagej.nih.gov/ RRID:SCR_003070
Equipment		
Equipment	Manufacturer	Identifier
EnSight Multimode Plate Reader	PerkinElmer	
Leica CM1900 Cryostat	Leica	Cat#CM1900
ZEISS LSM 980 with Airyscan 2	ZEISS	
BD® LSR II Flow Cytometer	BD Biosciences	
Integralslice Vibrating Microtome (Vibratome)	World Precision Instruments	Cat#WPI-CA-5100MZ-PLUS

*Batch/lot numbers are provided where available. All other reagents were used from multiple lots over the course of the study; exact batch numbers are not available.

Preparation of polyHIPE scaffolds

PolyHIPE scaffolds were prepared in-house as previously described.^{20,22} Briefly, surfactant was dissolved by heating in a mixture of 4-arm polycaprolactone methacrylate, then cooled prior to the addition of a photoinitiator and a solvent blend (40% toluene, 60% chloroform). Full details of the quantities used for a single batch are provided in Protocol 1 below; however, these can be scaled proportionally as required by users. Using a magnetic stirrer, the mixture was mixed at 400 rpm for 3 min at 37 °C, and then 2 ml water was added dropwise over 3 min. The resulting emulsion was mixed for an additional 5 min and then cured in a syringe for 5 min on both sides to produce polyHIPE tubes. This was then washed in 100% methanol for three days, then in water for a further day in order to remove contaminants. The washed polyHIPEs were then dried in a vacuum oven at room temperature overnight prior to sectioning to the desired thickness using a vibratome and then maintained in a dry environment at room temperature until required (Fig. 1). We observed that a thickness of 250 µm was ideal; however, this thickness could be tailored by users.

Cell culturing on polyHIPE scaffolds

Three myeloma cell lines (U266, JJN3, and 5TGM1) and three osteoblastic cell lines (MG-63, hFOB 1.19, and MC3T3) were used (Table 2). Myeloma cell lines JJN-3 (DSMZ, Germany), U266 (LGC Standards, UK), and 5TGM1¹¹ were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin (P/S), 1% non-essential amino acids (NEAA; Corning, UK), and 1% sodium pyruvate (SP).

MG-63 (ATCC) and MC3T3-E1 subclone 4 (ATCC) cells were maintained in minimum essential medium (MEM) alpha supplemented with 10% FBS and 1% P/S. Human fetal osteoblasts (hFOB 1.19; ATCC) were maintained in Dulbecco's Modified Eagle's medium/nutrient mixture F-12 medium supplemented with 10% FBS, 1% P/S, and 0.3 mg/ml G418 sulphate.

All cells were maintained at 37 °C with 5% CO₂ in a humidified incubator (95 ± 5% relative humidity), except hFOB 1.19 monocultured cells, which were maintained at 34 °C. The co-cultures were maintained in complete RPMI medium for myeloma cell maintenance.

All cells were routinely tested for mycoplasma contamination prior to use and were confirmed to be mycoplasma-free. Human cell lines were authenticated by short tandem repeat (STR) profiling using ATCC profiling services. Murine cell lines were obtained from established academic or commercial sources. Cells were expanded from early passage stocks and used within 30 passages, relative to the passage number provided by the supplier, to minimise phenotypic drift.

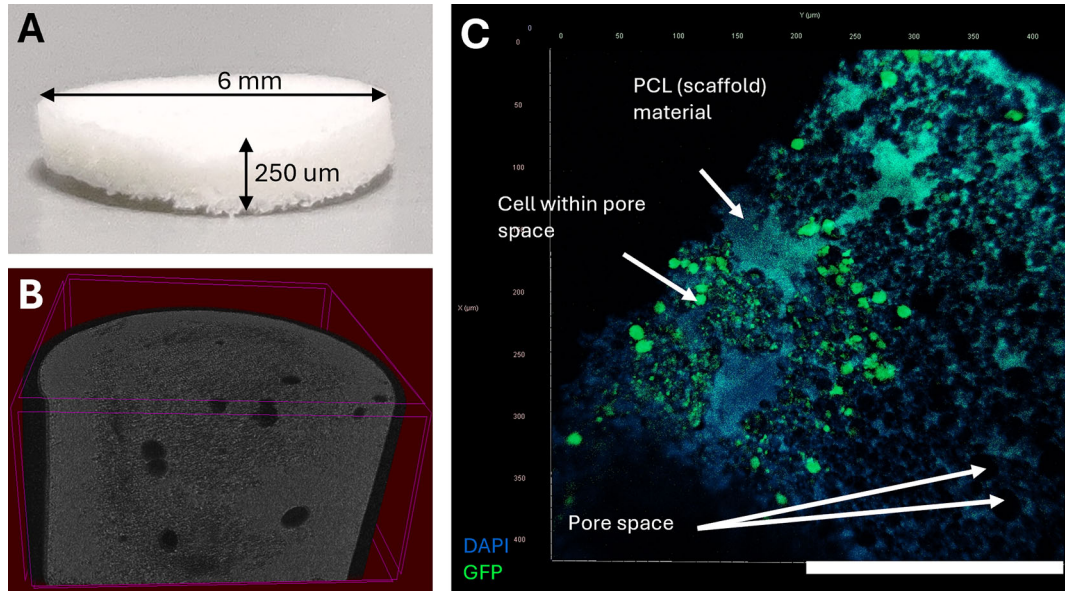


Figure 1. PolyHIPE scaffold architecture. (A) Photograph of a PolyHIPE scaffold with sponge-like appearance post processing cut to shape, with parameters of 6 mm diameter and 250 μm thickness. The highly porous structure and interconnectivity can be observed by (B) CT imaging to generate a whole 3D structure and (C) fluorescent imaging to capture a higher magnification cross section of a scaffold.

Unless stated otherwise, cells were maintained under standard culture conditions and used during the logarithmic phase of growth.

Scaffolds were sterilised by immersion in 100% methanol for 24 h, followed by washing in either phosphate-buffered saline (PBS) or complete media for 24 h, with a minimum of three changes. The scaffolds were then transferred to 48-well plates, and excess liquid was removed. Osteoblast cells were seeded onto the surface of scaffolds in 20 μl of RPMI media, at a density of 50,000 cells, and then incubated for 2 h at 37 $^{\circ}\text{C}$ to allow cell attachment. Following incubation, media was

Table 2. Cell line characteristics.

Cell name*	Species	Cell type	Description
U266-GFP-Luc	Human	Myeloma	Stably transduced to express luciferase (luc)-2A-green fluorescent protein (GFP). Doubling time ~ 50-60 h. Derived from peripheral blood of a 53-year-old male with refractory multiple myeloma. (RRID:CVCL_0566)
JJN3-GFP-Luc	Human	Myeloma	Cells stably express luciferase (luc)-2A-green fluorescent protein (GFP). Doubling time ~ 20-35 h. Derived from primary site tumour within the bone marrow of a female 57-year-old plasma cell leukaemia patient. (RRID: CVCL_2078)
5TGM1-GFP-Luc	Mouse	Myeloma	Cells stably express luciferase (luc)-2A-green fluorescent protein (GFP). Derived from murine 5T33 cells after repeated cycles of injection and harvesting from the bone marrow of C57BL/KaLwRij mice. (RRID:CVCL_VI66)
hFOB 1.19-RFP-Luc	Human	Foetal osteoblasts	Human foetal osteoblasts derived from foetal bone. Doubling time ~ 36 h. (RRID:CVCL_3708)
MG-63	Human	Osteosarcoma	Human osteosarcoma cell line derived from a 14-year-old male. Fibroblastic morphology described to have an immature osteoblast-like phenotype. (RRID: CVCL_0426)
MC3T3	Mouse	Pre-osteoblast	Murine pre-osteoblast cell line with the capability of differentiating into osteoblasts and osteocytes. Described to have a mature osteoblast-like phenotype. (RRID: CVCL_0D74)

*All cells were routinely tested for mycoplasma contamination. Human cell lines were authenticated by ATCC STR profiling. Cells were expanded from early-passage stocks and used within 30 passages of the passage number provided by the supplier.

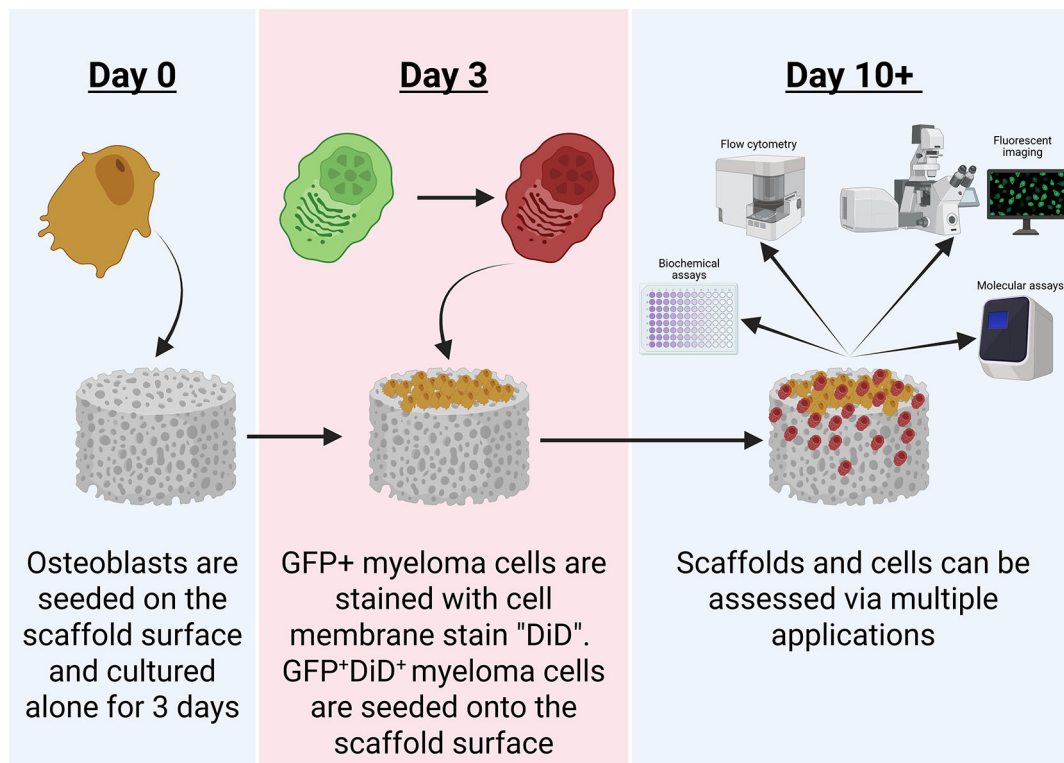


Figure 2. Model workflow. Osteoblasts are first applied to the scaffold, then allowed to adhere and migrate from the scaffold surface over 3 days of "pre-seeding". Following this, myeloma cells are stained with a cell membrane label (DiD) and seeded onto the scaffolds. Scaffolds are maintained up to day 10+ prior to further downstream assessments. Figure created in [BioRender](#).²⁴

added to the wells containing scaffolds, and the models were maintained as required. Osteoblasts were cultured for 3 days, then the scaffold was transferred to a new 48-well plate, and excess liquid was removed. Myeloma cells were subsequently seeded onto the polyHIPE scaffold surface in the same manner as osteoblasts (Fig. 2).

Cell localisation assessment by confocal microscopy

Osteoblastic (MC3T3) and myeloma (5TGM1) cells were seeded onto scaffolds as described above and co-cultured for 21 days post-myeloma cell seeding. Scaffolds were then washed in room temperature PBS x3, fixed in 4% PFA for 15 min at room temperature, then washed in PBS x3. Since the scaffolds are auto-fluorescent when imaged using a 405 nm laser, to increase the fluorescent signal of the material, scaffolds were then stained with DAPI (1:1000) for 10 min at room temperature and washed in PBS x3. The scaffolds were then dipped in OCT and placed into a cryomold containing a layer of OCT. This was then submerged in liquid nitrogen for 15 s and quickly transferred to a steel chuck in a cryostat chamber set to -20°C . Sections were cut to a thickness of 10–20 μm , with 10 μm representing the minimum thickness required to preserve structural integrity, and 20 μm the maximum thickness to ensure sufficient optical resolution and signal clarity for confocal microscopy. Sections were mounted on SuperFrost Plus glass slides at room temperature and air-dried for a minimum of 3 h.

Fluorescent imaging of the scaffolds was performed using a Zeiss LSM980 confocal microscope. Images were captured at x10 or x20 magnification using Plan-Apochromat 10x/0.3 M27 air or 20x/0.8 M27 air objectives, respectively. DAPI, GFP, and DiD channels were excited with 405, 488, and 639 nm lasers, respectively. The laser power, detector gain, and pinhole settings were optimised to maximise the signal while avoiding detector saturation and were kept consistent across samples. Images were acquired using Zeiss Zen 3.5 (blue edition) software.

Drug treatment assays

Osteoblastic (MG-63 or hFOB) and myeloma (JJN3 or U266) cells were seeded onto scaffolds as described above, 24-hours post myeloma cell seeding a drug was added to the culture well and scaffolds cultured for a further 3 days. For JJN3 co-cultures, melphalan (5.8 μM), bortezomib (1.5 nM), or lenalidomide (5 μM) were used at concentrations

representing 2D IC₂₅ values. For U266 co-cultures, bortezomib (3 nM), lenalidomide (50 µM), or a combination of both were used at concentrations representing 2D IC₅₀ values. The study design incorporated consistent seeding densities and repeated biological replicates to ensure reproducibility. Randomisation and blinding were not applied in this workflow, as all handling and measurements were performed using standardised protocols to maintain consistency across samples. Randomisation and blinding were implemented for scaffold-based flow cytometry assays, where individual scaffolds were processed and measured separately.

AlamarBlue® assessment

For JJN3 cultures, mixed-population cell viability was assessed using the alamarBlue® assay (Fig. 4A). 2D controls were maintained and treated in a similar manner in 6-well plates. Following the seeding of cells on scaffolds, at the indicated time points, scaffolds were moved to new wells containing fresh medium. The movement of scaffolds to new wells was necessary to ensure that measurements were reflective only of cells growing on and within the polyHIPE scaffolds, rather than those that failed to attach/fall off the scaffold. Once removed, 10% (v/v) alamarBlue® reagent was added to wells, and the scaffolds were incubated at 37 °C for 4 h. Following this, scaffolds were removed from the wells, and the fluorescence produced by the reduction of the alamarBlue® reagent in the remaining media was measured using an EnSight Multimode Plate Reader. Relative fluorescence intensity, commonly used as a proxy for cell viability in drug response assays, was used as an indicator of cell metabolic activity. Values were normalised to those of vehicle controls and expressed as percentages.

Flow cytometry assessment

For U266 co-cultures, cells were enzymatically removed from scaffolds following a regimen of 10 min wash in PBS, followed by 30 min incubation at room temperature on a shaker with accutase, and 10 min incubation with trypsin-EDTA at 37 °C on a shaker. PBS, accutase, and trypsin suspensions were collected after each step, and the cells were collected by centrifugation at 800xg for 5 min. As a further step to maximise the total number of cells isolated from scaffolds, the scaffolds were cut into segments and centrifuged in PBS at 800xg for 5 min.

Whole myeloma cell and DMC (DiD^{Hi}) cell population viability was assessed by flow cytometry (LSRII flow cytometer) following 30 min of incubation with live/dead violet at room temperature.

Ethics statement

This study did not involve human participants or human tissue samples requiring ethics approval. All human cell lines used in this study were obtained from commercial or academic sources and were used in accordance with institutional guidelines.

Statistical analysis

Unless otherwise stated, all experiments were performed in which a single scaffold represented an experimental unit (n). Data were analysed using GraphPad Prism v9.0 (GraphPad Software, USA) and are expressed as the mean ± standard deviation. Pairwise comparisons were performed using two-tailed Student's t-tests. Statistical significance was defined as $P < 0.05$.

Protocols

Below is a step-by-step guide for developing, applying, and troubleshooting the polyHIPE model for dormancy assessment. It is broken down into five protocols with a sixth section focused on troubleshooting.

1. Scaffold preparation

PolyHIPE scaffolds were synthesised according to the protocols of Aldemir Dikici *et al.*¹⁹ and Jackson *et al.*,²⁰ by forming a water-in-oil high internal phase emulsion (HIPE), with a 40:60 aqueous-to-oil phase ratio to ensure high porosity, which is then photopolymerised to a polyHIPE. Solvents and uncured materials were extracted prior to sectioning the discs and sterilisation for cell culture.

Steps:

1. Prepare PCL-M emulsion (~ 30 minutes)
 - a. In a glass vial, 0.40 g of PCL-M and 0.04 g of surfactant were mixed together. The surfactant was gently heated and allowed to cool to room temperature.

- b. Add 0.03 g Photoinitiator and 0.60 g of solvent blend (60 wt. % chloroform: 40 wt. % toluene) was added to the cooled mixture. Keep protected from light.
- c. A magnetic stirrer (20 mm × 7 mm) was used to stir the mixture at 400 rpm and 37 °C for 3 min to ensure that a homogeneous oil phase was produced.
- d. Add 2 ml of deionised water dropwise over 3 minutes while maintaining constant stirring.
- e. Mix for an additional 5 minutes to stabilise the emulsion.

CRITICAL: Maintain temperature and stirring speed throughout to ensure reproducible pore architecture, lower stirring speeds will produce larger pores.

2. Polymerise the emulsion (~15 minutes)

- a. Load the emulsion into a 2 ml syringe (of ~6 mm diameter).
- b. Cure using an OmniCure Series 1000 system for 5 min on each side (18 W/cm², spectral output of 250–600 nm).
- c. Remove the polymerised polyHIPE tube from the syringe.

NOTE: We use 2 ml syringes to produce an ideal diameter for 48-well plates. Silicone moulds can also be used to cure the emulsion but may interfere with later processing steps when a glue is required.

3. Washing and drying (6 days)

- a. Submerge polyHIPE tubes in 100% methanol for three days, replacing methanol every 24 h.
- b. The mixture was washed in deionised water for 3 days, and the water was changed every 24 h to remove excess surfactant, solvent, and residual monomer.
- c. The scaffolds were dried overnight in a vacuum oven at room temperature until they were fully solvent-free.
- d. Scaffolds can be stored in this dry state long term (6 months).

4. Sectioning and sterilisation (2–3 days)

- a. Using a scalpel blade, dried polyHIPE tubes (~1 cm tall) were cut into smaller trunks to facilitate easier cutting in the next step.
- b. Using a small dot of quick drying glue, attach the polyHIPE tube standing on one of its circular ends to a vibratome steel chuck, and let the glue dry.
- c. Using a vibratome (frequency of 80 Hz, amplitude of 1.00 mm and speed of 0.1–0.3 mm/s), section polyHIPE trunks into scaffold discs of 250 µm thickness.
- d. Dip discs in deionised water or PBS and lay flat on a sealable container (e.g., petri dish) and allow to air-dry overnight. Failure to do this may result in curled discs.
- e. Store dry until required for experiments.
- f. For sterilisation, immerse discs in 100% methanol for 24 h (preferred) or 70% ethanol (alternative), followed by three PBS washes for a minimum of 1.5 h (ideally over 24 h to ensure complete removal of alcohol).
- g. Store scaffolds submerged in sterile PBS or media at room temperature for no longer than 24 h or proceed directly for cell seeding.

NOTE: Do not press the polyHIPE trunk into the glue with too much force as this may result in glue absorbing into the polyHIPE.

NOTE: The first cut into a polyHIPE trunk should be used to level off the top of the sample so that a straight edge is produced, this first cut should therefore be discarded.

NOTE: The given quantities in this protocol are appropriate to generate 200–300 scaffolds of 250 µm.

NOTE: We used UHU 3–62686 Super Glue Ultra-Fast Liquid to attach polyHIPE tubes to steel chucks but believe any fast-acting glue would be sufficient.

2. Osteoblast seeding

Osteoblast cells (hFOB 1.19) were pre-seeded onto the scaffolds to generate the osteoblast component of the 3D model. Pre-seeding prior to myeloma cells facilitates sufficient attachment and migration into the scaffolds such that myeloma cells do not outcompete osteoblasts because of their shorter doubling times. Osteoblasts were cultured for three days prior to the addition of myeloma cells, but osteoblasts could be cultured for longer periods (we cultured osteoblasts alone for up to 28 days).

Steps:

1. Scaffold preparation (~15 minutes)
 - a. Scaffolds were removed from the wash medium 10–15 minutes prior to seeding hFOB 1.19 cells onto scaffolds, then placed into individual wells of a 48-well tissue culture plate.
 - b. Excess medium carried over with the scaffold was removed from the wells, and the scaffolds were placed so that the attachment of osteoblasts onto the scaffolds was not affected by excess liquid.
 - c. Scaffolds were maintained at room temperature or placed in an incubator at 37 °C until the cells were ready to be seeded. This facilitated better liquid retention when cells were added to the scaffold, aiding cell attachment.
2. Osteoblast cell preparation (~20 minutes)
 - a. Culture hFOB 1.19 cells at 34 °C until ~80% confluence in a T75 tissue culture flask.
 - b. Remove media and wash the cell surface with 10 ml PBS twice.
 - c. Add 1–2 ml trypsin-EDTA (0.25%) to the T75 flask and incubate at 34 °C for 5 min.
 - d. Add 8–9 ml of complete medium to quench the trypsin and resuspend the cells.
 - e. Count cells using a hemocytometer or automated cell counter with trypan blue exclusion to ensure high viability (>90%) before seeding onto scaffolds.
 - f. Centrifuge cells at 300xg for 5 min and resuspend at a concentration of 2.5×10^6 cells/ml such that 20 µl contains 50,000 cells.
3. Seeding onto scaffolds (2 hours)
 - a. Seed cells on top of the scaffolds by carefully pipetting a 20 µl droplet onto the centre of the surface of each scaffold to promote attachment to the scaffold rather than the plastic surface of the well.
 - b. In the spare wells surrounding the scaffolds, PBS was added to prevent the scaffolds from drying out, and the scaffolds were placed in an incubator set to 34 °C for 2 h to allow cells to attach to the scaffold.

NOTE: This seeding strategy is necessary for adherent cell lines, where a proportion of cells may otherwise adhere to the well plate surface. The high localised density of cells in a small volume is important to improve scaffold-specific seeding/attachment efficiency.

4. Post-seeding culture (3–28 days)

- a. After the initial 2-hour incubation to facilitate hFOB 1.19 cell attachment, 500 µl of complete medium was gently added to the wells to fully submerge the scaffolds. Adding media along the inner edge of the wells minimises the disruption of cells on the scaffolds.
- b. Scaffolds should be maintained at 34 °C with 5% CO₂ for 72 h until required for subsequent use.

NOTE: Do not use 96 well plates for culture since the medium volume is not sufficient for the cell number seeded onto scaffolds. Similarly, scaffolds can be seeded into larger well plates but will float in the excess medium within these wells. Therefore, we used 48 well plates and replaced the media every 1–2 days as required.

NOTE: Here, scaffolds are maintained at 34 °C since this is the optimum maintenance temperature for hFOB 1.19 cells, if other osteoblast cells are used the temperature should be altered to match the cell's required temperature.

3. Myeloma cell labelling to track dormancy prior to seeding onto scaffolds

Vybrant DiD cell labelling solution (DiD) is used to indirectly track cell proliferation, since non-proliferative cells retain a strong dye signal, whereas proliferating cells do not. Therefore, we used DiD to identify DMCs (e.g. JJN3), since these cells retain a strong DiD signal. We used DiD, but other similar dyes could also be used. The myeloma cells were labelled prior to seeding onto scaffolds; we did not observe DiD transfer onto the polyHIPE material, but it is possible for DiD to transfer to osteoblasts.

Steps:

1. Myeloma cell preparation (~15 minutes)

- a. JJN3 cells were maintained at a density of 0.4×10^6 cells/ml at 37 °C in a T75 tissue culture flask and passaged upon reaching 1×10^6 cells/ml to maintain logarithmic growth.
- b. Myeloma cells can be counted in suspension directly from their culture flask using a hemocytometer or automated cell counter with trypan blue exclusion to ensure high viability (>90%) before seeding onto scaffolds.
- c. The required number of cells was centrifuged at 500xg for 5 min in a universal tube and the cell pellet was retained.
- d. The cell pellet was resuspended in a volume of serum-free media at a concentration of 1×10^6 cells/mL.

NOTE: We recommend staining double the number of cells required since loss of cells is commonly observed during the following steps, and an excess of cells is required as a reference point marker of the highest DiD mean fluorescent intensity (MFI) for flow cytometric analyses.

NOTE: The next steps followed the manufacturer's instructions but should be optimised for each cell line used.

2. DiD staining (1 hour)

- a. DiD reagent was added to the cell suspension at a volume of 5 µl per 1 ml of serum-free medium (or 5 µl per 1×10^6 cells).
- b. The cells were incubated with DiD at 37 °C for 20 min. The tube was gently shaken after the first 10 min to avoid cells settling at the bottom of the tube, resulting in nonuniform staining.
- c. Following incubation, the cells were centrifuged at 600xg for 5 min and the supernatant was discarded to remove excess unbound DiD dye.
- d. The cell pellet was washed in serum containing medium (1×10^6 cells/mL) and centrifuged for 5 minutes at 600xg.
- e. Repeat step 8 a total of three times to ensure that all excess DiD is removed.

- f. Following the last wash, the cells were resuspended in an appropriate volume of serum medium and counted.
- g. The volume of media needed to suspend cells was calculated to achieve a concentration of 2.5×10^6 cells per ml (5×10^4 cells per 20 μ l).
- h. Seed DiD-labelled myeloma cells onto scaffolds as described below.

NOTE: We increased the centrifugation speed following DiD labelling as the dye reduced the pelleting of cells at lower speeds.

NOTE: It is not necessary to perform these steps in a universal tube, but the flat bottom facilitates standing in an incubator and centrifugation without the need to transfer the cells between containers.

4. Co-culture setup with myeloma cells

Following osteoblast pre-seeding on the scaffold for three days, (DiD labelled) myeloma cells (J2N3) were added to the model system. We have cultured myeloma cells with osteoblasts for up to 21 days and advise leaving myeloma cells for at least 24 h for shorter study periods.

Steps:

1. Scaffold preparation (~10 minutes)
 - a. After 3 days of incubation with osteoblasts in monoculture, the scaffolds were transferred into clean wells of a fresh 48-well plate to avoid contamination from osteoblasts that had adhered to the original well plastic surface.
 - b. Scaffolds were moved 10 min before myeloma cells were seeded. Excess medium was removed from the wells, and scaffolds were placed back into an incubator at 37 °C until DiD-labelled myeloma cells were added.
2. Myeloma cell preparation and seeding onto scaffolds (2 hours)
 - a. DiD labelled myeloma cells should be at a concentration of 2.5×10^6 cells/ml such that 20 μ l contains 5×10^4 cells (Protocol 3).
 - b. The same method used to seed osteoblasts was used; myeloma cells were placed on top of the scaffold by carefully pipetting a 20 μ l droplet onto the centre of the scaffold surface to promote attachment to the scaffold rather than to the plastic surface of the well.
 - c. In spare wells surrounding those with scaffolds, PBS was added to prevent the scaffolds from drying out, and then the scaffolds were incubated at 37 °C for 2 h to allow cells to attach to the scaffold.
3. Post-seeding culture (1–21 days)
 - a. Following 2 h of incubation, 500 μ l complete medium was added to the individual wells to submerge the scaffold by gently pipetting the media down the side of the well. Scaffolds were then incubated at 37 °C, 5% CO₂ until required for the next experimental step.
 - b. Where necessary, media should be replaced every 3–4 days by careful pipetting to remove old media and then replaced with fresh media.

NOTE: Since myeloma cells grow in suspension, direct adherence to the scaffold is variable and influenced by the presence of osteoblasts and the success of seeding as a single droplet. Nevertheless, droplet-based seeding was effective for achieving distribution throughout the scaffold pores.

NOTE: The total media volume added to wells could be increased to 1 ml total if needed, ensuring the media is gently and slowly added down the well wall.

5. Post-culture scaffold processing

Several methods can be used to process the scaffolds for analysis. Below are examples of how we processed the scaffolds; however, this is not an exhaustive list.

1. Dissociation of cells from scaffolds (e.g. for flow cytometry)
 - a. The scaffolds were removed from the wells and cultured in fresh 48 well plate.
 - b. Wash scaffolds in 400 µl PBS for 5 min at room temperature on a shaker in order to wash FBS from scaffolds and to isolate cells that are weakly attached to scaffold discs.
 - c. The scaffolds were moved to a new well containing 300 µl of accutase solution and incubated at room temperature on a shaker for 30 min.
 - d. The scaffolds were moved to a new well containing 250 µl of trypsin-EDTA and incubated at 37 °C for 10 min.
 - e. Quench trypsin by adding 500 µl serum containing media to wells.
 - f. Combine PBS, accutase, and trypsin suspensions to 1.5 ml Eppendorf tubes.
 - g. Scaffolds were cut using scissors or a blade into quarters and placed into an extra Eppendorf tube containing 500 µl PBS.
 - h. Centrifuge all tubes (from steps f and g) at 800xg for 5 minutes.
 - i. Remove supernatant from tubes and resuspend pellets in 100 µl PBS.
 - j. Live/dead staining was added to each Eppendorf tube and incubated at room temperature for 30 min.
 - k. Centrifuge suspension at 600xg for 5 min and resuspend cells in PBS containing 10% FBS.
 - l. Run samples on a flow cytometer, gating for live/viable populations, and for DiD staining profiles.
2. Scaffold fixation
 - a. Scaffolds were removed from the wells post-culture and placed into fresh wells of a 48 well plate.
 - b. Gently wash scaffolds with 500 µl PBS for 10 minutes at room temperature.
 - c. Remove the PBS and repeat the above step a further two times (a total of three washes should be performed).
 - d. Remove PBS and add 4% PFA to wells, incubate at room temperature for 15 min.
 - e. PFA was removed and the scaffolds were washed in 500 µl PBS for 10 min at room temperature.
 - f. Remove the PBS and repeat the above step a further two times (three total washes should be performed).
 - g. PBS was removed and 500 µl DAPI solution (DAPI:PBS ratio 1:1000) was added to the wells, followed by incubation at room temperature for 10 min.
 - h. The DAPI solution was removed, and the scaffolds were washed in 500 µl PBS for 10 min at room temperature.
 - i. Remove the PBS and repeat the above step a further two times (three total washes should be performed).
 - j. The scaffolds were maintained in PBS at 4 °C and wrapped in foil until sectioning was performed (up to a month).

NOTE: To maintain representative architecture of cell growth, the scaffolds were handled carefully and reagents added gently to wells to minimise the disruption of cells on the scaffolds.

3. Scaffold frozen sectioning

- a. A cryostat chamber should be set to -20°C .
- b. Prepare a cryo-mold by placing enough OCT to fill the cryo-mold, ensuring that no bubbles are present in the OCT layer, as this may cause cracking of the OCT and scaffold in later steps.
- c. Previously fixed scaffolds (Protocol 5, step 2) were removed from the wells containing PBS, and excess liquid was removed by dabbing the edge of the scaffold onto a paper towel.

OPTIONAL: Scaffold can be cut in half to create 2 equally sized semi-circles or left as a whole disc.

- d. The scaffold was then dipped in OCT and placed in the prepared mold. If the scaffold was cut, each half was placed such that the straight edge was facing down. If the scaffold was not cut, it was placed flat on OCT.

NOTE: Each method produces scaffolds that are embedded in a different plane. This can also be achieved by sectioning the OCT block at different angles (see below). We suggest the scaffold should be kept whole as it allows better control of the positioning of the scaffold in the mould. If time to section a scaffold is the limiting factor, it is possible to place the semi-circles flat with one laid on top of the other, but once again this gives the user less control over the scaffolds.

- e. The OCT mold was immersed in an ethanol/dry ice bath or liquid nitrogen for 10–15 seconds, and then quickly transferred to the cryostat chamber.
- f. The solidified OCT block was removed from the mold and attached to a steel chuck in the required orientation by placing a drop of OCT onto a cooled steel chuck and then placing the OCT block onto it.
- g. The OCT block was allowed to stick to the chuck by placing the chuck in the cryostat chamber for 5 min or until the freshly laid OCT solidified.
- h. Cut into the block until the scaffold is located.
- i. Take sections of the scaffolds at $10\text{ }\mu\text{m}$ to $20\text{ }\mu\text{m}$ thickness.
- j. The section was flattened out using a brush and collected onto a glass slide by placing the slide over the cut section.
- k. Allow sections to air dry on scaffolds for 3 hours prior to imaging.

NOTE: Marking the top of the OCT mould with a pencil once it has been solidified helps the user to orientate the block.

NOTE: Using coloured OCT may help with the identification of the scaffold rather than white OCT since the scaffolds are also white.

NOTE: Aqueous mounting media can be used to fix a coverslip to the slide to preserve the sample longer.

6. Troubleshooting

1. Uneven cell distribution or poor seeding efficiency

Issue: Cells failed to adhere evenly or settle onto the plastic well plate instead of the scaffold.

Steps to troubleshoot:

- Ensure that the scaffolds are dry on the surface before seeding to help the droplets stay in place. This can be achieved by placing scaffolds at 37 °C if previously performed only at room temperature. Alternatively, the drying time could be increased.
 - Ensure that cells were seeded in a 10–20 µl droplet directly onto the centre of the scaffold to maximise coverage.
 - When incubating cells on the scaffolds initially, a pre-warmed incubator was used to avoid disturbing the plates during the initial 2-hour attachment period.
 - Scaffolds were moved to new wells to avoid the attraction of cells to those adhered to the well plate plastic rather than the scaffold.
2. Scaffolds floating on top of media layer in well plates

Issue: Scaffolds float because of disruption during media addition or incubation, resulting in poor cell contact or movement in the well.

Steps to troubleshoot:

- Add medium slowly down the side of the well after the initial 2-hour seeding phase.
 - Avoid agitation during media changes and move plates gently during transportation.
 - If scaffolds float after the addition of media, gently press the scaffold to the bottom of the well using the pointed end of a 200 µl sterile plastic pipette tip.
3. Loss of non-adherent cells (e.g. myeloma)

Issue: Suspension cells are lost or do not adequately attach to the scaffold.

Steps to troubleshoot:

- Cells were allowed to settle by initially seeding in a smaller volume (10 µl) before adding larger volumes of media.
 - Handle plates gently to avoid agitation of the scaffold.
 - The frequency of media changes was reduced to minimise the disruption of myeloma cells on scaffolds by increasing the total well volume to 1 ml.
 - Monitor suspension cells in supernatant as part of overall viability readouts.
4. Low cell recovery from scaffolds

Issue: Insufficient cell yield from digested scaffolds for flow cytometric or molecular analyses.

Steps to troubleshoot:

- Optimise enzymatic digestion protocols (e.g., use of collagenase/accutase or increased incubation length). This will be cell dependent, but the use of accutase at 30 min, followed by trypsin for 5 min, has shown no effect on cells in our hands.
- Use agitation (e.g. plate shaker) and centrifuge scaffolds to improve recovery.
- Pool multiple scaffolds for endpoint analyses that require higher cell input (e.g., RNA extraction).

5. Imaging difficulties due to scaffold autofluorescence

Issue: Fluorescent (confocal) images are unclear due to scaffold properties.

Steps to troubleshoot:

- Include scaffold-only controls to assess background autofluorescence.
- Avoid using fluorophores with emission in the blue range (e.g., DAPI, Alexa Fluor 405), as the scaffold autofluorescence is highest at shorter wavelengths. Therefore, prioritise using fluorophores with longer emission wavelengths (e.g., Alexa Fluor 647) to improve the signal-to-noise ratio in confocal imaging.
- Optimise laser intensity to reduce background noise.

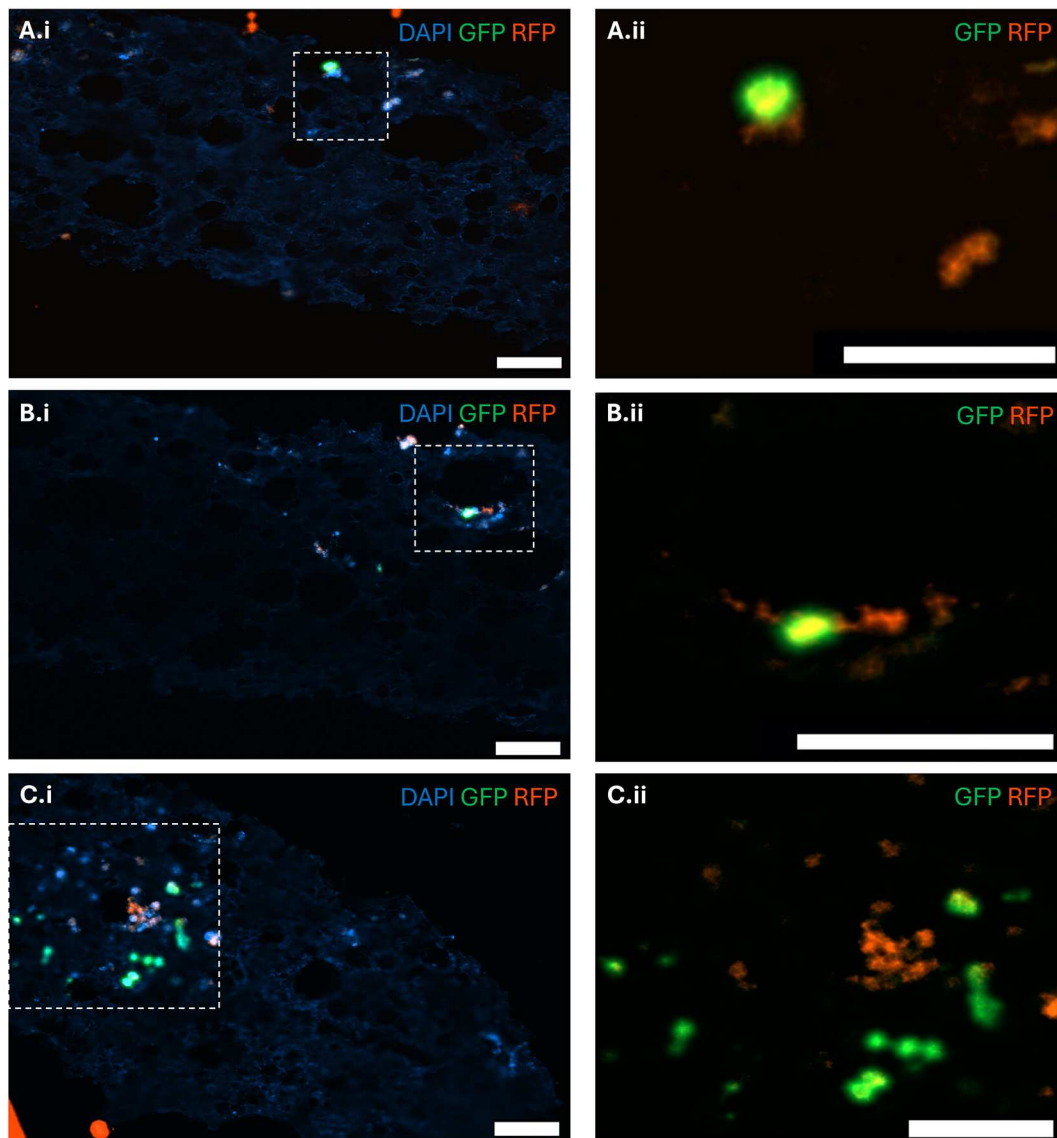


Figure 3. Example application of immunofluorescent assessment of cell locality on polyHIPE scaffolds. Fluorescent images of scaffold cross sections in which scaffolds were pre-seeded with RFP-tagged MC3T3 osteoblastic cells, then co-cultured with GFP-tagged 5TGM1 myeloma cells for 21 days. Scaffold structure was observed with DAPI counter stain. (A) Example of a single myeloma cell in direct contact with an osteoblast. (B) Example of a single myeloma cell neighbouring an osteoblast. (C) Example of clusters of myeloma cells and osteoblasts growing separately. Scale bar = 50 μ m.

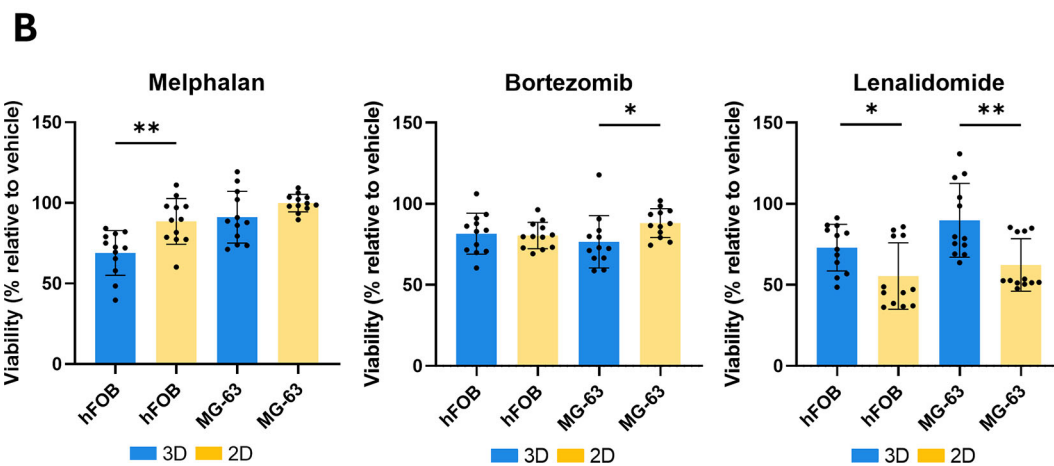
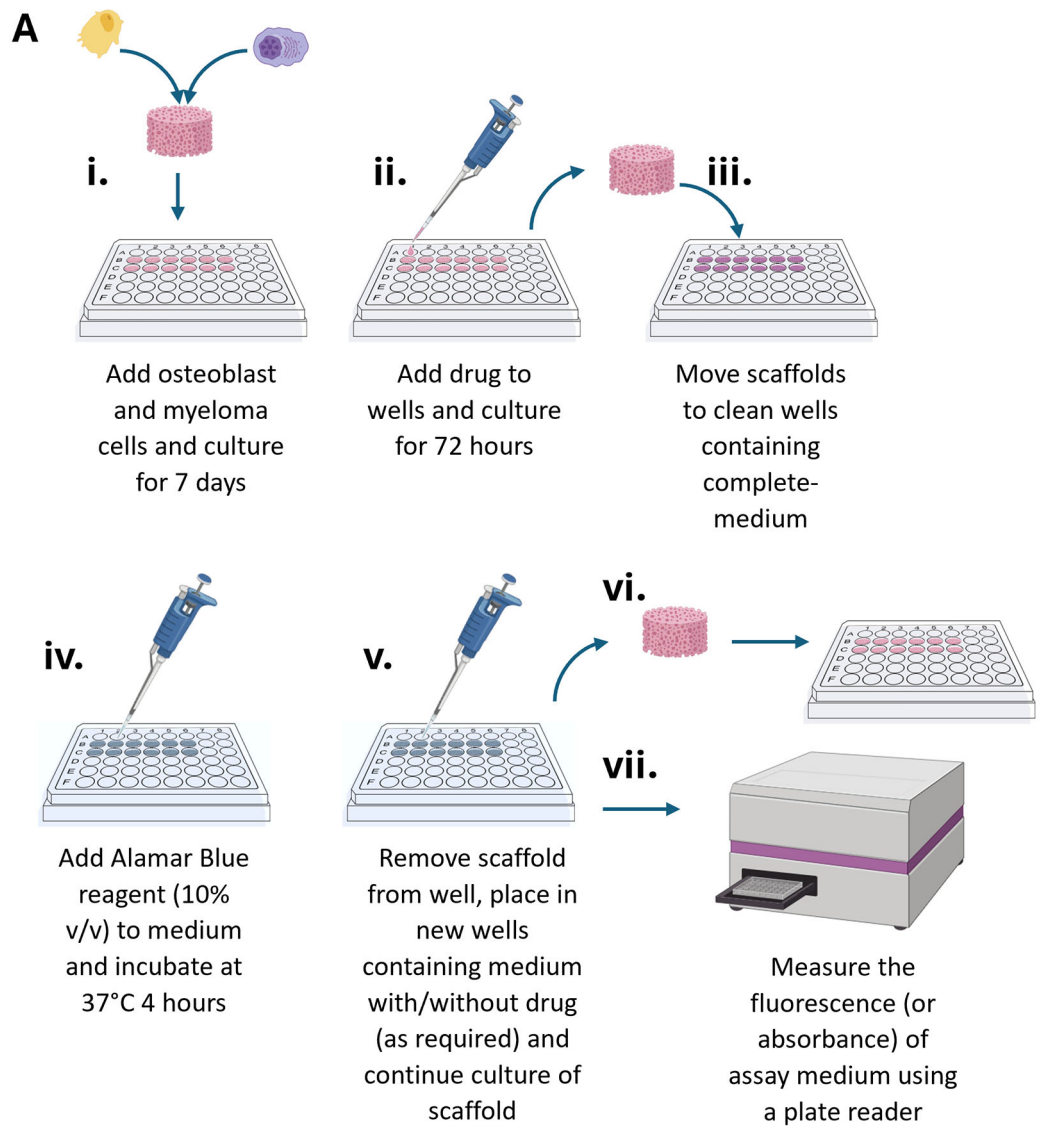


Figure 4. Caption continued on next page.

Results

Osteoblasts and myeloma cells are able to directly interact within polyHIPE scaffolds

Confocal imaging of the scaffold cross-sections confirmed that the scaffolds successfully supported the co-culture and spatial organisation of myeloma and osteoblast-lineage cells. After 21 days of co-culture of 5TGM1 and MC3T3 cells on the scaffolds, both cell types were able to establish networks throughout the scaffold structure. Myeloma cells grew in direct contact with osteoblasts (Fig. 3A), adjacent to osteoblast clusters (Fig. 3B), or near osteoblast populations without direct contact (Fig. 3C). These observations indicate that the scaffolds permit both direct contact-dependent and indirect paracrine interactions, resembling the cell-to-cell interactions observed within the endosteal niche *in vivo*.^{6,23}

Drug responses differ on polyHIPE scaffolds compared to 2D culture

Having confirmed the co-culture architecture within our scaffolds, we next assessed drug responses on the scaffolds and compared this with standard 2D *in vitro* cultures. JJN-3 myeloma cells were co-cultured with either hFOB 1.19 or MG-63 osteoblastic cells and treated with 2D IC25 concentrations of melphalan, bortezomib, or lenalidomide: three standard-of-care myeloma therapies with known mechanisms of action. Whole scaffold populations were assessed using alamarBlue® assays (Fig. 4A) and showed distinct responses between the osteoblast types and culture methods (Fig. 4B). In hFOB co-cultures, cells were significantly more sensitive to melphalan on our scaffolds (3D) compared to 2D, whereas MG-63 co-cultures displayed a similar but non-significant trend. Bortezomib treatment had greater cytotoxicity in 3D MG-63 co-cultures, while hFOB co-cultures showed no difference. In contrast, lenalidomide sensitivity was reduced in 3D cultures for both osteoblast co-cultures. Despite this, for any given drug and culture format (e.g., melphalan treatment in 2D), there were no significant differences in cytotoxicity between MG-63 and hFOB co-cultures. This suggests that the sensitivity of each drug was altered by microenvironmental cues, particularly 2D vs. 3D environments, whereas the osteoblast type contributes more subtle effects that become apparent only when comparing across culture formats. Notably, alamarBlue® measures the conversion of resazurin to resorufin in all viable cells (myeloma and osteoblasts); therefore, these experiments do not allow definitive conclusions to be drawn for a specific cell type.

To investigate the effects of the drugs on distinct myeloma populations, U266 myeloma cells were treated in 2D and 3D co-cultures (with hFOB or MG-63 cells) with bortezomib, lenalidomide, or a combination of both (Fig. 5). Flow cytometric analysis again showed that in 3D cultures, viability was significantly reduced following bortezomib or combination treatment compared with vehicle controls, whereas no significant differences were observed in 2D cultures (Fig. 5A-D).

When DMCs (DiD^{Hi}) were analysed separately in 2D and 3D cultures, some differences were observed. In 2D hFOB co-cultures, combination treatment led to significantly fewer DiD^{Hi} cells compared to the vehicle (Fig. 5E), while no significant differences were observed in 2D MG-63 co-cultures (Fig. 5F). However, in 3D cultures, both hFOB and MG-63 co-cultures had significantly fewer DiD^{Hi} cells in bortezomib-treated samples compared with vehicle (Fig. 5G-H), demonstrating reproducibility on our scaffolds with different osteoblastic cells, although it should be noted that fewer cells were retrieved from hFOB co-cultures on scaffolds than MG-63 co-cultures.

Summary/discussion

Model overview and applications

The polyHIPE scaffold provided a 3D, highly porous environment suitable for modelling interactions between osteoblasts and myeloma cells under physiologically relevant conditions. Its architecture supports high-resolution imaging and fluorescent tracking of proliferative and DMCs, visualisation of osteoblast networks, and co-localisation studies using confocal microscopy. Sequential seeding allows investigation of dormancy induction and osteoblast-myeloma cell interactions within a bone-lining niche-like context. The scaffold also serves as a platform for functional assays, including pharmacological testing, thereby supporting early-stage drug evaluation. Its modular design allows adaptation to different cell types, co-culture combinations, and assay formats, bridging the gap between conventional 2D culture and *in vivo* models, while supporting early-stage therapeutic evaluation. Below are examples of how we used some of these methods in our study.

Figure 4. Cellular assessment using alamarBlue® assays. (A) Schematic of scaffold-based drug treatment workflow. Created using Biorender.com. Osteoblasts are seeded onto scaffolds, followed by myeloma cells and cultured for 7 days. Drug is then added for 72 hours. Scaffolds are subsequently transferred to fresh wells, alamarBlue® reagent is added and scaffolds are incubated for 4 hours, after which scaffolds are moved to new wells for continued culture. Fluorescence/absorbance of alamarBlue®-containing media is measured to assess cell viability. (B) Relative cell viability assessment of JJN3 cells co-cultured with hFOB 1.19 or MG-63 cells following treatment with melphalan (5.8 µM), bortezomib (1.5 nM), or lenalidomide (5 µM) in 3D scaffolds or 2D controls. Each scaffold (3D) or well (2D) was treated as a single experimental unit for statistical analysis; n = 12 experimental units per condition, comprising 3 independent runs with 4 technical replicates each. *P < 0.05, **P < 0.01.

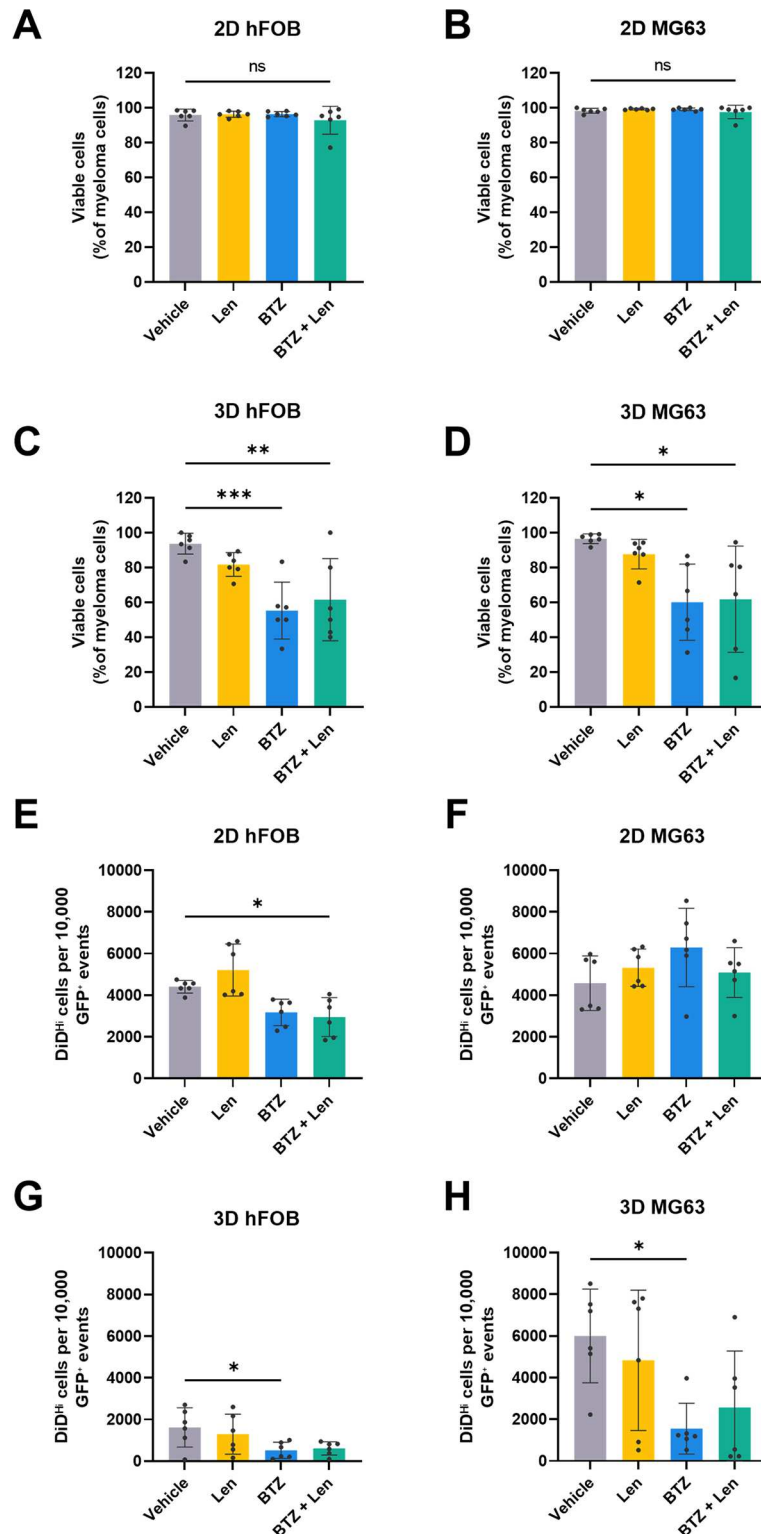


Figure 5. (Dormant) cell viability assessment by flow cytometry. U266 cells were co-cultured with hFOB or MG-63 cells and treated with bortezomib (3 nM), lenalidomide (50 μ M), or a combination of both for 72 hours. (A-D) Cells were then retrieved from scaffolds, and the cell viability of whole U266 myeloma cell populations was assessed by live dead staining (a 2D comparison was also run under the same conditions). (E-H) Dormant (DiD^{Hi}) U266 cell populations were also assessed following treatment. The number of DiD^{Hi} cells is presented normalised to 10,000 GFP⁺ cells to facilitate comparison between 2D and 3D. Each scaffold (3D) or well (2D) was treated as a single experimental unit for statistical analysis; n = 6 experimental units per condition, comprising 2 independent runs with 3 technical replicates each. * P < 0.05, ** P < 0.01, *** P < 0.001, ns P > 0.05.

This protocol describes the step-by-step use of a novel 3D scaffold model that replicates the key features of the BM microenvironment to study myeloma cell dormancy and drug responses. It offers a scalable, reproducible, and adaptable platform that minimises reliance on animal models in line with 3Rs principles. The validation of this model against existing *in vivo* data is ongoing. However, we demonstrated the compatibility of our model with human and mouse osteoblastic cells as well as human and mouse myeloma cells. We also demonstrate a range of downstream analysis methods using our model, which widens the potential applications of this model. For instance, confocal imaging allowed us to validate that the polyHIPE scaffolds were capable of recreating the spatial organisation, facilitating direct and indirect contact between osteoblasts and myeloma cells, which is characteristic of the BM niche. These interactions are important and difficult to model simultaneously in 2D *in vitro* systems. Thus, the ability to visualise these interactions on our scaffolds underscores their suitability for studying cell-to-cell interactions and relationships.

Through drug testing on our model compared to 2D *in vitro* cultures, we were able to confirm the influence of the 3D structure, which largely increased drug sensitivity. Importantly, the use of a range of cell lines in our model reduces the need for animal-derived materials and increases the adaptability of our model. Using a range of osteoblast cell lines for co-cultures, we were able to confirm the ability to derive similar conclusions across several osteoblast phenotypes, which are important to consider as they may influence therapeutic responses. The use of hFOB and MG-63 cells, which are two commonly used osteoblastic cell lines in research laboratories, was interchangeable, even with different myeloma cell lines. Importantly, the similarities observed on our scaffold when using different osteoblast types were not consistently replicated in 2D culture, highlighting the detrimental effect of 2D culture compared with 3D culture. At the cellular level, flow cytometry confirmed that DMCs could be targeted on our scaffolds, mirroring observations made previously using *in vivo* models⁶ and emphasises our model's capacity to study dormancy-associated resistance mechanisms. Ongoing work aims to further validate our 3D model using *in vivo* data. We hope to further explore the integration of patient-derived samples to enhance the clinical relevance of the model.

Compared with other 3D models, our polyHIPE scaffold offers several practical and biological advantages. Hydrogel-based or collagen scaffolds often lack the structural rigidity and pore interconnectivity needed for long-term co-culture or imaging, while microfluidic “bone marrow-on-a-chip” systems are technically complex and difficult to scale for parallel experiments. PolyHIPE scaffolds bridge this gap, offering a comparatively higher-throughput system that combines stability, tunability, and optical accessibility with compatibility for imaging, biochemical, and flow cytometric analyses. Thus, it is a suitable model for preclinical application. Importantly, the model aligns with NC3Rs 3Rs principles. By reproducing key aspects of the BM microenvironment *in vitro*, the scaffold enabled the evaluation of cellular dormancy and drug responses without relying on murine models.

Based on the work presented here, we suggest that scaffold-based assays could replace small pilot *in vivo* studies, typically with ~10–20 animals per study. Widespread adoption of this model has the potential to reduce the use of hundreds of animals annually across the field, minimising the experimental burden of research while maintaining translational relevance.

Finally, the adaptability of the model extends its potential beyond myeloma. By varying cell types, ECM coatings, or scaffold composition, it can easily be adapted for studying broader aspects of myeloma (aside from dormancy) or for studying other cancers such as breast or prostate dormancy. Adoption by other researchers can be aided by sourcing pre-made scaffolds from our in-house facility; however, the potential to manufacture the scaffolds within separate laboratories requires specialised equipment (e.g., vibratome), which could limit the uptake of the model.

Data availability

The datasets underlying the results presented in this study are openly available in Figshare:

Figshare. PolyHIPE scaffolds as a 3D model of myeloma dormancy – underlying datasets. <https://doi.org/10.15131/shef.data.31361887>.²⁴

This project contains the following underlying data:

- Figure 1B_CT_reconstruction.zip
- Figure 1C_raw_image.czi
- Figure 3A_raw_image.czi

- Figure 3B_raw_image.czi
- Figure 3C_raw_image.czi
- Figure 4B_raw_and_processed_data_1.xlsx
- Figure 4B_raw_and_processed_data_2.xlsx
- Figure 5_FCS_files.zip
- Figure 5A-D_processed_data.xlsx
- Figure 5E-H_processed_data.xlsx

All data are available under the terms of the [Creative Commons Attribution 4.0 international \(CC BY 4.0\) license](#).

No additional data are required to replicate the findings reported in this study.

Acknowledgements

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References

1. Bataille R, Chappard D, Marcelli C, *et al.*: **Mechanisms of bone destruction in multiple myeloma: the importance of an unbalanced process in determining the severity of lytic bone disease.** *J. Clin. Oncol.* 1989; **7**(12): 1909–1914.
[PubMed Abstract](#) | [Publisher Full Text](#)
2. Bolli N, Avet-Loiseau H, Wedge DC, *et al.*: **Heterogeneity of genomic evolution and mutational profiles in multiple myeloma.** *Nat. Commun.* 2014; **5**: 2997.
[Publisher Full Text](#)
3. Moreau P, Attal M, Facon T: **Frontline therapy of multiple myeloma.** *Blood.* 2015; **125**(20): 3076–3084.
[Publisher Full Text](#)
4. Dingli D, Ailawadhi S, Bergsagel PL, *et al.*: **Therapy for Relapsed Multiple Myeloma: Guidelines From the Mayo Stratification for Myeloma and Risk-Adapted Therapy.** *Mayo Clin. Proc.* 2017; **92**(4): 578–598.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
5. Podar K, Leleu X: **Relapsed/Refractory Multiple Myeloma in 2020/2021 and Beyond.** *Cancers (Basel).* 2021; **13**(20): 5154.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
6. Lawson MA, McDonald MM, Kovacic N, *et al.*: **Osteoclasts control reactivation of dormant myeloma cells by remodelling the endosteal niche.** *Nat. Commun.* 2015; **6**: 8983.
[Publisher Full Text](#)
7. Khoo WH, Ledergor G, Weiner A, *et al.*: **A niche-dependent myeloid transcriptome signature defines dormant myeloma cells.** *Blood.* 2019; **134**(1): 30–43.
[Publisher Full Text](#)
8. Reagan MR, Ghobrial IM: **Multiple myeloma mesenchymal stem cells: characterization, origin, and tumor-promoting effects.** *Clin. Cancer Res.* 2012; **18**(2): 342–349.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
9. Damen MPF, van Rheenen J, Scheele C: **Targeting dormant tumor cells to prevent cancer recurrence.** *FEBS J.* 2021; **288**(21): 6286–6303.
[Publisher Full Text](#)
10. Chen Z, Orlowski RZ, Wang M, *et al.*: **Osteoblastic niche supports the growth of quiescent multiple myeloma cells.** *Blood.* 2014; **123**(14): 2204–2208.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
11. Oyajobi BO, Munoz S, Kakonen R, *et al.*: **Detection of myeloma in skeleton of mice by whole-body optical fluorescence imaging.** *Mol. Cancer Ther.* 2007; **6**(6): 1701–1708.
[Publisher Full Text](#)
12. Asosingh K, Radl J, Van Riet I, *et al.*: **The 5TMM series: a useful in vivo mouse model of human multiple myeloma.** *Hematol. J.* 2000; **1**(5): 351–356.
[PubMed Abstract](#) | [Publisher Full Text](#)
13. Vlummens P, De Veirman K, Menu E, *et al.*: **The Use of Murine Models for Studying Mechanistic Insights of Genomic Instability in Multiple Myeloma.** *Front. Genet.* 2019; **10**: 740.
[Publisher Full Text](#)
14. Garrett IR, Dallas S, Radl J, *et al.*: **A murine model of human myeloma bone disease.** *Bone.* 1997; **20**(6): 515–520.
[Publisher Full Text](#)
15. Mehdi SJ, Ghatak K, Ling W, *et al.*: **Growth and dormancy control of myeloma cells by mesenchymal stem cells.** *Leuk. Res.* 2023; **133**: 107355.
[PubMed Abstract](#) | [Publisher Full Text](#)
16. Wei F, Wang S, Gou X: **A review for cell-based screening methods in drug discovery.** *Biophys. Rep.* 2021; **7**(6): 504–516.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
17. Abbas ZN, Al-Saffar AZ, Jasim SM, *et al.*: **Comparative analysis between 2D and 3D colorectal cancer culture models for insights into cellular morphological and transcriptomic variations.** *Sci. Rep.* 2023; **13**(1): 18380.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
18. Owen R, Sherborne C, Paterson T, *et al.*: **Emulsion templated scaffolds with tunable mechanical properties for bone tissue engineering.** *J. Mech. Behav. Biomed. Mater.* 2016; **54**: 159–172.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
19. Aldemir Dikici B, Sherborne C, Reilly GC, *et al.*: **Emulsion templated scaffolds manufactured from photocurable polycaprolactone.** *Polymer.* 2019; **175**: 243–254.
[Publisher Full Text](#)
20. Jackson CE, Ramos-Rodriguez DH, Farr NTH, *et al.*: **Development of PCL PolyHIPE Substrates for 3D Breast Cancer Cell Culture.** *Bioengineering (Basel).* 2023; **10**(5): 522.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
21. Aldemir Dikici B, Malayeri A, Sherborne C, *et al.*: **Thiolene- and Polycaprolactone Methacrylate-Based Polymerized High**

- Internal Phase Emulsion (PolyHIPE) Scaffolds for Tissue Engineering.** *Biomacromolecules*. 2022; **23**(3): 720–730.
[PubMed Abstract](#) | [Publisher Full Text](#)
22. Dikici S, Aldemir Dikici B, MacNeil S, *et al.*: **Decellularised extracellular matrix decorated PCL PolyHIPE scaffolds for enhanced cellular activity, integration and angiogenesis.** *Biomater. Sci.* 2021; **9**(21): 7297–7310.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
23. Kawano Y, Moschetta M, Manier S, *et al.*: **Targeting the bone marrow microenvironment in multiple myeloma.** *Immunol. Rev.* 2015; **263**(1): 160–172.
[Publisher Full Text](#)
24. Sprules A, Lawson M, Green A, *et al.*: *Raw data files supplemental to paper titled "A detailed methodology for developing and applying a 3D polyHIPE scaffold to model myeloma cell dormancy in vitro".* The University of Sheffield; 2026 [cited 2026Mar27].
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Elham Saberian 

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This manuscript describes the development and application of a three-dimensional (3D) polyHIPE scaffold model designed to study dormant myeloma cells within an osteoblast-supported microenvironment. The authors provide a detailed protocol covering scaffold fabrication, sterilization, osteoblast and myeloma cell seeding, dormancy tracking, imaging, flow cytometric analysis, and troubleshooting. The model addresses an important challenge in multiple myeloma research by providing a more physiologically relevant alternative to conventional two-dimensional culture systems while supporting the principles of Replacement, Reduction, and Refinement (3Rs) in animal research.

Overall Assessment

The manuscript is well written, scientifically relevant, and provides a comprehensive methodological framework that will be valuable to researchers studying myeloma dormancy, tumor-microenvironment interactions, and drug responses. The rationale for developing the model is clearly articulated, and the methodology is described in sufficient detail to facilitate implementation by other laboratories.

Major Comments

1. Source Data Availability

Although representative results and application examples are presented, the manuscript should explicitly state where all underlying raw data are available. The authors should provide links, repository accession numbers, or supplementary files containing:

- Raw flow cytometry datasets (FCS files).
- Quantitative measurements underlying graphs.
- Original microscopy images where feasible.
- Statistical analysis files.

This would further strengthen reproducibility and transparency.

1. Validation of Biological Relevance

The authors demonstrate successful scaffold fabrication and cellular localization; however, additional discussion regarding how closely the model recapitulates key biological features of the in vivo endosteal niche would strengthen the manuscript. Inclusion of known dormancy-associated molecular markers or comparison with previously established dormancy models could further support the biological relevance of the system.

1. Quantitative Performance Metrics

The manuscript would benefit from additional quantitative information regarding:

- Cell recovery efficiency following scaffold digestion.
- Inter-batch variability of scaffold fabrication.
- Long-term reproducibility across independent experiments.

Such information would help users assess the robustness of the platform.

Minor Comments

1. The troubleshooting section is particularly useful and should be retained.
2. The authors may consider including a schematic summary of the complete workflow with estimated timelines for each stage.
3. Some readers may benefit from additional discussion regarding potential limitations of the model, including the absence of vascular, immune, and extracellular matrix components that are present in vivo.
4. It would be helpful to provide recommendations regarding adaptation of the protocol for primary patient-derived myeloma cells.

Conclusion

This is a valuable and carefully developed methodological contribution that addresses an important need in multiple myeloma research. The protocol is technically sound, reproducible, and likely to be broadly useful for investigators studying cancer dormancy and tumor microenvironment interactions. Subject to clarification regarding source data availability and minor enhancements to the discussion of model limitations and validation, I recommend indexing.

Is the rationale for developing the new method (or application) clearly explained?

Yes

Is the description of the method technically sound?

Yes

Are sufficient details provided to allow replication of the method development and its use by others?

Yes

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Multiple Myeloma, Cancer Biology, Biomaterials, Tissue Engineering, Regenerative Medicine, Drug Delivery, Bone Tissue Engineering, 3D Cell Culture Models, Tumor Microenvironment, Biomedical Engineering.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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