

A Bioresponsive Silica–Collagen Xerogel Platform for Local Bortezomib Delivery and Treatment of Multiple Myeloma Bone Lesions

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Abstract

Malignant plasma cells accumulating in the bone marrow trigger the development of lytic bone lesions in 80% of patients suffering from multiple myeloma. These lesions often lead to fractures and are notoriously difficult to treat surgically. Myeloma cells that remain viable after treatment within the protective milieu of these bone defects continue to disrupt normal bone metabolism, thereby preventing proper healing. Such residual activity likely contributes to the frequent disease recurrence observed even in cases where minimal residual disease (MRD) is undetectable. Biomaterials intended for direct application at the site of lesions could provide mechanical reinforcement, facilitate bone repair, and enable the local administration of anticancer drugs at high concentrations that would be intolerable if delivered systemically, helping to overcome acquired resistance. Silica-collagen xerogels (sicXer) together with bortezomib-releasing variants (boXer) were formulated for targeted intervention against osteolytic bone involvement and persistent MRD. Release profiles of bortezomib were determined in vitro as well as directly from tissue sections in vivo by means of ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) and time-of-flight secondary ion mass spectrometry (ToF-SIMS). Effects on bone-forming processes were studied in vitro by quantifying changes in osteoclast and osteoblast populations and their respective activities. In vivo evaluation involved applying the materials to drill-hole defects created in rat bones and in the 5T33-myeloma mouse model, with subsequent analysis performed via immunohistochemistry, UPLC-MS/MS, μ CT, and ToF-SIMS. The materials' capacity to eliminate myeloma cells was tested in 10 human myeloma cell lines (HMCLs) and 8 primary patient-derived myeloma samples, 4 of which originated from individuals who had not responded to systemic bortezomib therapy. Both sicXer and boXer provided initial structural stability equivalent to that of trabecular bone. Degradation rates were adjustable by varying granule dimensions and fabrication protocols, as reflected in the liberation patterns of silica, collagen, and bortezomib into the culture environment. In vitro testing demonstrated reduced osteoclast function without compromising osteoblast differentiation or activity. The resulting promotion of net bone deposition while preserving essential remodeling mechanisms was confirmed in a rat bone defect model, in which defects treated with boXer showed markedly superior bone formation compared with untreated controls. In addition, both materials induced apoptosis in myeloma cells from all HMCLs and all primary samples examined. Application in the 5T33-myeloma mouse model confirmed that the materials effectively stabilized the created defects and locally restrained the proliferation of malignant plasma cells. By simultaneously providing mechanical stability to fragile bone lesions, stimulating regenerative healing, and exerting potent anti-tumor effects, sicXer and boXer represent promising candidates for clinical trials within multimodal treatment frameworks combining systemic and localized approaches for multiple myeloma and certain non-malignant bone disorders.

Keywords: Bone substitute material, Bortezomib, Multiple myeloma, Osteolytic bone lesions, Local treatment, Minimal residual disease

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Introduction

Multiple myeloma is characterized by the accumulation of malignant plasma cells in the bone marrow [1-4], leading to lytic bone lesions in 80% of patients over the course of their illness. These lesions are a major source of morbidity and mortality. They typically persist without healing and prove difficult to treat effectively. Even after successful systemic therapy, bone lesions often remain

the final detectable sites of disease activity [5-7] when assessed by magnetic resonance imaging or positron emission tomography. In this shielded microenvironment, malignant cells appear capable of evading treatment and continue to prevent proper repair through sustained interference with bone turnover mechanisms. Although systemic myeloma therapies have progressed markedly with the advent of small-molecule agents and immuno-oncologic approaches developed by multiple research groups, including our own [8-13], and despite the achievement of deep responses with tumor burden reduced below detectable limits (minimal residual disease negativity) [14, 15], patients nevertheless experience disease relapse and eventual mortality.

Through a sustained 9-year multidisciplinary research program, the goal was to engineer bone-substitute materials that exhibit initial mechanical stability comparable to that of trabecular bone. These materials were designed to actively support regeneration by driving net bone formation, achieved through partial suppression of osteoclast activity without complete inhibition, alongside preservation or enhancement of osteoblast performance. To enable precise modulation of biological effects, the materials were engineered as versatile drug-delivery platforms. For use in myeloma, they were required to deliver localized tumor control, ideally through both inherent material properties and the action of incorporated therapeutic agents.

The mesoporous silica-collagen xerogels (sicXer) developed in this work are constructed on a mineralized collagen scaffold analogous to that found in natural bone. Xerogels are open-porous networks formed by the complete removal of swelling agents from a gel matrix. Familiar examples include gelatin and rubber. Here, silica gel was formed via hydrolysis of silicon alkoxy compounds. Mineralization with silicic acid was employed to bolster initial mechanical strength, drawing from the exceptional durability of marine glass sponge spicules. The silica-to-collagen ratio was systematically adjusted to achieve mechanical characteristics closely resembling those of trabecular bone [16, 17]. Further refinements to material composition allowed fine-tuning of degradation kinetics and modulation of bone remodeling processes by influencing osteoblast and osteoclast development and activity. This was accomplished through alterations in silica-collagen proportions, the specific form of silica used [18], and incorporation of (calcium) phosphate phases [19, 20]. Owing to the heterogeneous nature of silica-collagen

xerogels, which contain disordered binding sites, ions, and low-molecular-weight organic compounds, these structures readily adsorb various substances [21]. This adsorption capacity applies to soluble mediators of bone remodeling in the vicinity of the implant and to therapeutic payloads loaded into the material before use. Bortezomib was selected as the payload because of its documented ability to promote net bone formation [22, 23] and its established approval and efficacy in systemic myeloma treatment [24]. The drug-loaded versions are referred to as “boXer”.

This study reports the fabrication and comprehensive *in vitro* and *in vivo* assessment of sicXer and boXer, with particular emphasis on their ability to stimulate bone formation in models of both normal and myelomatous bone, their effectiveness in locally controlling myeloma cells, and their capacity to induce cell death in primary myeloma cells derived from patients resistant to bortezomib.

Materials and Methods

Generation and analysis of sicXer and boXer

Xerogel fabrication was performed according to previously established methods [17, 25]. Homogeneous suspensions of collagen at 30 mg/mL were prepared by dialysis (MWCO 12–14 kDa, Carl Roth) of bovine tropocollagen type I (GfN) against deionized water. This was followed by fibrillation within a 30 mM neutral sodium phosphate buffer, lyophilization using a Christ Alpha 1-4 laboratory freeze-dryer, and subsequent resuspension in 0.1 M TrisHCl at pH 7.4 (Roth) [17]. The lyophilized collagen used for sicXer and boXer was gamma-sterilized at 25 kGy. Silicic acid was produced by acid-catalyzed hydrolysis of tetraethoxysilane (TEOS, 99%, Sigma) at a molar ratio of TEOS/water = 1/4 in 0.01 M HCl. Vigorous vortexing of the silicic acid and collagen suspension yielded 800 μ L of hydrogels with a final composition of 30% collagen and 70% silica. Bortezomib (VELCADE®) was introduced into the collagen suspensions before xerogel formation to obtain final loadings of 100 μ g (boXer-100), 500 μ g (boXer-500), and 2500 μ g (boXer-2500) per 1 g of silica/collagen xerogel. Following gel formation, hydrogels were stabilized for three days and then dried at 37 °C to constant weight. The resulting monolithic silica/collagen xerogels (diameter: 5 mm, height: 3 mm) were pulverized and fractionated to achieve desired particle size ranges. Variants designated siXer-iaf and boXer-iaf (irradiation

after fabrication) received gamma sterilization at 25 kGy once manufacturing was complete.

Sample preparation for degradation studies

In the *in vitro* degradation experiments, monoliths of sicXer and sicXer-iaf were cultured in 1.2 mL of α -MEM medium (α -MEM) supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin (all obtained from Biochrom). The medium was fully exchanged three times each week on days 2, 5, and 7. The collected culture supernatants were stored at -20°C until subsequent analysis by inductively coupled plasma mass spectrometry (ICP-MS).

Quantification of collagen, silica, calcium, and phosphate

Collagen release into the supernatant was determined with an o-phthaldialdehyde-based assay. In short, 90 μ L aliquots of the cell culture supernatant were incubated overnight at 37°C with 100 μ L of 0.5 mg/mL collagenase from *Clostridium histolyticum* (Sigma-Aldrich). Afterward, 20 μ L of the resulting digest was combined with 200 μ L of fluoroaldehyde reagent (Thermo Scientific), and the fluorescence signal was recorded at excitation/emission wavelengths of 340/440 nm on an Infinite® M200Pro reader (Tecan). Standard curves were established using known concentrations of bovine collagen dissolved in α -MEM containing 10% FCS, 2 mM glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin. Silica, calcium, and phosphate levels in the supernatants were measured by ICP-MS (IRIS Intrepid II XUV, ThermoFisher Scientific). Before analysis, samples were diluted with water and HNO_3 (Carl Roth), and ion concentrations were calculated against a calibration curve prepared from certified element standards (High Purity).

Sample preparation for bortezomib, sicXer and sicXer-iaf release studies

Bortezomib elution from boXer-100 and boXer-500 was assessed across separate particle size fractions ($<125\ \mu\text{m}$, $125\text{--}250\ \mu\text{m}$, and $250\text{--}710\ \mu\text{m}$). For each test, 9 mg of material was incubated in 700 μ L of α -MEM containing 10% FCS, 2 mM glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin (all from Biochrom). Complete supernatant removal and replacement occurred at predetermined intervals: 1 h, 2 h, 4 h, 8 h, 24 h, 48 h, 72 h, 96 h, 168 h, 336 h, 504 h, and 672 h. All harvested

supernatants were stored at -20°C until processed by ultrahigh-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). Quantification of collagen, silica, calcium, and phosphate followed the procedures detailed earlier.

*Impact of sicXer and boXer on *in vitro* osteoblastogenesis and osteoclastogenesis*

Osteoblastogenesis

Human mesenchymal stromal cells (hMSC) were derived from bone marrow aspirates generously donated by Prof. Bornhäuser *et al.* from Medical Clinic I at Dresden University Hospital. Expansion of hMSC was performed in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, 2 mM glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin (Biochrom). The effects of sicXer and sicXer-iaf on osteoblastic differentiation were examined. Twenty-four-well polystyrene plates were inoculated with 1.7×10^4 hMSCs (passage 5) in 800 μ L of α -MEM containing 10% FCS, 2 mM glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin. Granules of sicXer and boXer (15 mg each) were loaded into ThinCerts (1 μm pore size) and pre-equilibrated overnight in 1 mL of α -MEM with 10% FCS. The following day, medium containing 50 μM ascorbate (Sigma-Aldrich) was added to the adherent hMSCs at the start of culture (day 0), with the xerogel granules in the ThinCerts inserts serving as the negative control. Osteogenic induction was initiated on day 3 by supplementing the medium with 50 μM ascorbate, 5 mM β -glycerophosphate, and 10 nM dexamethasone (all from Sigma-Aldrich). The culture medium was renewed three times per week. For later biochemical evaluation (described below), the cells were rinsed twice with phosphate-buffered saline (PBS) and cryopreserved at -80°C .

Osteoclastogenesis

Monocyte preparation

Peripheral blood mononuclear cells (PBMCs) were harvested from buffy coats obtained from five healthy individuals (German Red Cross). The buffy coats were first mixed with an equal volume of PBS containing 2 mM ethylenediaminetetraacetic acid (EDTA) and 0.5% bovine serum albumin (BSA; both from Sigma-Aldrich) (PBS E/B). The diluted preparations were then centrifuged for 20 min at 836 g across a 1.077 g/mL layer (Nycoprep™ 1.077, Progen, Germany) in Leucosep™ tubes (Greiner) with the brake disabled. Platelet depletion was achieved via subsequent density gradient

centrifugation at 1.063 g/mL (prepared by diluting NycoPrep™ 1.077 with PBS E/B) for 15 min at 353 g. Following this, cells were rinsed with PBS E/B and pelleted by centrifugation for 8 min at 301 g. Monocytes were purified from the PBMC fraction through negative magnetic bead selection employing the Monocyte Isolation Kit II (Miltenyi Biotec) as specified by the manufacturer. The isolated monocytes were suspended at 1×10^7 cells per mL and cryopreserved in α MEM supplemented with 10% dimethylsulfoxide (Sigma-Aldrich) and 40% heat-inactivated FCS for later use.

Osteoclastogenesis

Thawed monocytes were enumerated with a Scepter™ counter (Millipore, Germany). A total of 1.5×10^5 monocytes were plated in 800 μ L of α MEM containing 7.5% heat-inactivated FCS, 7.5% human A/B serum (ccpro), 2 mM glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin, further supplemented with 50 ng/mL macrophage colony-stimulating factor (M-CSF; R&D Systems) into twenty-four-well polystyrene plates. Granules of sicXer or sicXer-iaf (15 mg) were loaded into ThinCerts (1 μ m, Greiner) and allowed to equilibrate overnight in 1 mL of α MEM with 10% heat-inactivated FCS. Two PBS washes were performed the next day (day 0) to remove loosely adherent cells. Medium containing 25 ng/mL M-CSF and 25 ng/mL receptor activator of nuclear factor kappa B ligand (RANKL; R&D Systems) was then introduced to the adherent monocytes at the start of the culture period (day 0), with the xerogel granules maintained in the ThinCert system. Medium renewal occurred every third day. For biochemical assays, cells underwent two PBS washes before being frozen at -80°C .

Biochemical analysis

cells lysis was performed using 1% Triton X-100 (Sigma-Aldrich) in PBS for 60 min, followed by 10 min of sonication. In osteoblastogenesis experiments, lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities were assessed in the lysates. In osteoclastogenesis studies, tartrate-resistant acid phosphatase (TRAP) 5b activity was measured in lysates. LDH activity served as an indicator of hMSC proliferation. For this purpose, 50 μ L of lysate was combined with 50 μ L of the substrate solution from the LDH Cytotoxicity Detection Kit (Takara) and incubated in the dark. The reaction was terminated after 15 min through addition of 50 μ L 0.5 M HCl (Roth). Optical

density was determined at 492 nm on an Infinite® M200Pro reader (Tecan). A standard curve prepared from lysates of known cell counts was used to relate LDH activity to cell numbers.

ALP activity was measured to evaluate the extent of osteogenic differentiation and osteoblast functionality. Lysates (25 μ L) were incubated with 100 μ L of substrate solution containing 5.4 mM 4-nitrophenylphosphate disodium salt in a buffer of 100 mM diethanolamine, 1 mM magnesium chloride, and 0.1% Triton X-100 (all Sigma-Aldrich) at pH 9.8. After 30 min at 37°C , the enzymatic reaction was halted by adding 50 μ L of 0.5 M NaOH (Carl Roth). Absorbance was read at 405 nm (Infinite® M200Pro, Tecan). Calibration was performed using varying concentrations of p-nitrophenol (Sigma-Aldrich) in substrate buffer. The resulting ALP values were adjusted based on cell numbers derived from LDH measurements.

TRAP 5b activity, a marker specific for osteoclasts, was determined with naphthol-ASBI phosphate substrate. Lysates (10 μ L) were mixed with 50 μ L of substrate buffer containing 2.5 mM naphthol ASBI phosphate (Sigma-Aldrich) in 100 mM sodium acetate (Carl Roth), 50 mM sodium tartrate, 2% Nonidet™ NP 40, and 1% ethylene glycol monomethyl ether (all Fluka) at pH 6.1. Following 30 min incubation at 37°C , the reaction was quenched with 125 μ L of 0.1 M NaOH (Roth). Fluorescence was quantified at excitation/emission wavelengths of 335/405 nm (Infinite® M200Pro, Tecan). Calibration was performed using graded concentrations of TRAP (BoneTRAP).

Scanning electron microscopy

Prepared specimens were mounted onto aluminum stubs and coated with a carbon layer. Images were acquired using an ESEM XL 30 scanning electron microscope (Philips) at an accelerating voltage of 3 kV in secondary electron mode.

Impact of sicXer and boXer on primary myeloma cells and myeloma cell lines

Patients and samples

Eight consecutive patients diagnosed with either newly diagnosed, treatment-requiring multiple myeloma (based on myeloma-defining CRAB features [26]) or relapsed disease were recruited into this study. The research protocol received approval from the ethics committee of the Medical Faculty of the University of Heidelberg (#S-

152/2010), and all participants provided written informed consent.

Myeloma cells were isolated from bone marrow aspirates through magnetic separation with anti-CD138 microbeads on an AutoMACS Pro Separator (Miltenyi Biotec). Purity and quality were verified by flow cytometry [9, 27, 28]. The human myeloma cell lines AMO-1, KARPAS-620, KMS-11, KMS-12-BM, L363, OPM-2, RPMI-8226, and U266 were sourced from the German Collection of Microorganisms and Cell Cultures, the American Type Culture Collection, or the Japan Health Science Research Resources Bank. Additionally, the HG1 and HG9 lines were developed in-house at the Myeloma Research Laboratory in Heidelberg, Germany. Authentication of cell lines was confirmed by DNA fingerprinting; PCR verified the absence of mycoplasma; and EBV status was assessed using standard clinical PCR diagnostics.

Killing of myeloma cell lines

Myeloma cell lines were first incubated with pre-incubated xerogels for 24 h. They were then treated with sicXer-iaf, boXer-20-iaf, boXer-100-iaf, or boXer-500-iaf and incubated for an additional 72 h. Viability was quantified with a WST-1 colorimetric assay (Roche) and normalized against untreated medium controls. All experimental conditions were performed in triplicate.

Survival of primary myeloma cells

Primary myeloma cells obtained from eight patients were maintained in co-culture with their autologous bone marrow microenvironment and treated with either sicXer-iaf or boXer-500-iaf. Following a six-day incubation period, viability was determined by dual staining with CD138-FITC (IQ products, clone B-A38) and propidium iodide (PI; Pharmingen). Results were expressed relative to medium controls according to established protocols [9, 27, 28]. Viable myeloma cells were defined as CD138⁺/PI⁻, whereas viable bone marrow microenvironment cells were identified as CD138⁻/PI⁻. Experiments were conducted in duplicate, and flow cytometric data were analyzed using FACSDiva software (BD Biosciences).

Rat model (drill hole defect) bone healing

sicXer-iaf and boXer-100/500/2500-iaf particles (size range: 250–710 µm) were inserted into a standardized drill hole defect in the left femur of healthy 4-month-old female Sprague-Dawley rats ([Crl:CD(SD)]; Charles

River). All animal care and experimental interventions adhered strictly to institutional guidelines and German animal welfare legislation, with prior approval granted by the responsible local animal welfare committee (reference number: V54-19 c20/15-F 31/38). Under general anesthesia, an anterolateral approach exposed the distal femur, where a metaphyseal drill hole defect measuring 2.5 mm in diameter and 4 mm in depth was created. Postoperatively, rats were housed individually with ad libitum access to food and water. After four weeks, the animals were euthanized under anesthesia via CO₂ inhalation. Both the operated femur and the contralateral femur were collected from each animal for further processing. To evaluate local bortezomib release, tissue samples were obtained from the defect site, adjacent muscle, and bone regions including the femoral condyle, distal shaft, and proximal shaft.

Histology and histomorphometry

Assessment of new bone formation

The harvested rat femora were fixed using phosphate-buffered 4% paraformaldehyde solution and maintained at 4 °C before further preparation. Subsequently, the specimens were embedded in Technovit® 9100 NEU resin according to the supplier's recommended procedure (Heraeus Kulzer). After polymerization, the resin blocks were cut into 5 µm sections with the support of Kawamoto's film (Section-Lab) to ensure retention of all biomaterial components. Sectioning was standardized to include the plane that displayed both femoral condyles in every experimental group.

Histomorphometric analyses

Qualitative and quantitative evaluations were performed on undecalcified bone sections stained with Movat pentachrome and toluidine blue. Representative images were captured at 10× magnification on a light microscope (Axioplan 2 imaging system with Axiophot 2 photomodule, Carl Zeiss) equipped with a Leica DC500 camera. Images were recorded using Leica IM1000 software and edited in Adobe Photoshop version CS6. For the histomorphometric assessment of newly formed bone, expressed as bone volume relative to trabecular volume (BV/TV), analysis was conducted in Adobe Photoshop CS6. A defined region of interest (ROI) corresponding to the original drill hole defect was selected. This area incorporated both the regenerated bone within the defect and the adjacent biomaterial-tissue boundary. The ROI dimensions remained uniform for all

study groups. Quantitative measurements included the areas of the ROI, bone tissue, implanted material, osteoid, and unoccupied spaces to evaluate the degree of new bone formation.

On toluidine blue-stained sections, osteoblasts were identified as clusters of cuboidal cells exhibiting blue staining along bone surfaces. The parameter osteoblast surface per bone surface (Ob.S/BS) was quantified through direct tracing of these cells. All assessments were carried out by an observer blinded to the experimental groups.

Enzyme histochemical analysis

Osteoclast activity was examined through tartrate-resistant acid phosphatase (TRAP) staining. Following deplastification, sections were incubated in sodium acetate buffer containing Naphthol-AS-TR phosphate dissolved in N,N-dimethylformamide (Sigma-Aldrich), together with sodium tartrate (Merck) and Fast Red TR salt (Sigma-Aldrich) at 37 °C for 1 hour.

Counterstaining was performed with hematoxylin (Shandon). TRAP-positive cells, representing osteoclasts, were enumerated within the standardized ROI to calculate the number of osteoclasts per trabecular area (Oc./Tb. Ar).

Immunohistochemistry and immune histomorphometry

Immunostaining was conducted with the following primary antibodies: rabbit anti-BMP-2 polyclonal antibody (AP20597PU-N; Acris), rabbit anti-osteoprotegerin (OPG) polyclonal antibody (250,800; Abbiotec, USA), rabbit anti-CD254/RANKL polyclonal antibody (AP30826PU-N; Acris), rabbit anti-CD31 antibody (250,590; Abbiotec), and mouse anti-rat monocytes/macrophages monoclonal antibody ED1 (MAB1435; Chemicon).

A goat anti-rabbit secondary antibody (BA-1000, Vector) was applied for the detection of BMP-2, OPG, and RANKL, followed by signal amplification with the Vectastain ABC kit (Elite PK-6100, Vector Laboratories). Chromogenic development was performed with Nova Red (SK4800, Vector Laboratories), and hematoxylin (Shandon) was used as the counterstain. For ED1 detection, the Dako Envision+ System-HRP (DAB) protocol specific for mouse primary antibodies was utilized (Dako, K4006).

All images were obtained via the Axioplan 2 Imaging system (Carl Zeiss) with a Leica DC500 camera,

recorded using Leica IM1000 software, and processed in Adobe Photoshop CS6.

The OPG/RANKL ratio [%] was calculated through manual counting of cells positively stained for each respective marker. Similarly, the number of ED1-positive cells was counted manually within the defined ROI.

5T33-mouse model—bone healing and control of myeloma cell proliferation

5T33-mouse model

All animal care and experimental work followed the regulations set by the Institutional Animal Care and Use Committee of the Vrije Universiteit Brussels (license number LA1230281), with full approval obtained from the Ethics Committee for Animal Experiments (#15–281-6).

sicXer particles (n = 8) and boXer-500/–2500 particles (n = 10 per group), each with sizes below 125 µm, were placed into a 1 mm diameter and 1 mm deep drill hole defect created in the left femur of mice bearing the 5T33 myeloma model [29, 30]. Anesthesia was achieved through intraperitoneal injection of ketamine at 100 mg/kg combined with xylazine at 10 mg/kg. Postoperative pain management involved subcutaneous administration of meloxicam at 1 mg/kg. Three weeks following the surgical procedure, the mice were euthanized. The femora were harvested and fixed in 4% paraformaldehyde; within each group, one femur was preserved in yellow fixative while another was immediately frozen on dry ice for additional testing.

Histology and immunohistochemistry

Femoral condyles were fixed in 4% paraformaldehyde (Carl Roth) and subsequently embedded in Technovit 9100 (Heraeus Kulzer) resin according to the manufacturer's specified protocol for histological and immunohistochemical evaluation.

Sagittal sections measuring 4 µm in thickness were prepared from all condyles on a rotary microtome (RM 2265, Leica Microsystems).

Masson-Goldner staining was performed exactly as outlined in the manufacturer's protocol (Carl Roth).

Immunohistochemical detection of CD138 involved de-embedding of sections in 2-methoxyethyl acetate (Merck) for 15 min, followed by rehydration in a descending alcohol series. Antigen retrieval was performed by heating the sections in 0.1 M citrate buffer at 90 °C for 20 min. Endogenous peroxidase activity was blocked with 3% H₂O₂ for 5 min. Primary antibody

incubation occurred overnight at 4 °C using a polyclonal anti-CD138 antibody reactive against human, rat, mouse, and hamster epitopes (Acris, PAB 9567) at a 1:1000 dilution in antibody diluent (Dako, DK, S3022). Detection was performed using SuperVision 2 Single Species HRP-Polymer Rabbit (DCS, PD 000POL-K) for 2 20-min incubations, followed by chromogenic development with DAB (DCS, DC 137 C 100) for 10 min. Sections were then mounted using DePeX (Serva).

Histomorphometry

Sections were imaged at 5× objective magnification with a Leica DFC 320 digital camera attached to a Zeiss Axiophot microscope. Masson-Goldner-stained slides were used to outline the defect region and assess osteoid deposition in the immediate vicinity of the defect. Using the Lasso tool in Adobe Photoshop CS6 Extended, an area of interest was delineated that included the defect zone plus a surrounding margin of 100 µm.

Osteoid areas stained red were selectively highlighted and quantified in mm² via the Magic Wand Tool in Adobe Photoshop CS6 Extended.

To quantify CD138-positive cells in the bone marrow, a separate ROI was created using the Lasso tool in Adobe Photoshop CS6 Extended. This region encompassed the bone marrow of the distal epiphysis near the defect site and extended an additional 2 mm into the distal diaphysis. Membrane-bound CD138 staining within this ROI was selected using the Magic Wand Tool, and the percentage of the area occupied by CD138-positive signal was determined.

UPLC-MS/MS analysis

Quantification of bortezomib was performed by UPLC-MS/MS analysis following liquid-liquid extraction, as previously described [31]. Tissue samples explanted from the rat model were homogenized in 500 µL of 0.1 M hydrochloric acid with an ULTRA-TURRAX device (IKA) and centrifuged at 400 × g for 5 min at 4 °C. An internal standard (25 µL) was introduced to each homogenate, followed by brief vortexing (10 s) and sonication (3 min). Methyl tert-butyl ether (5 mL) was then added, and the mixtures were agitated overhead for 15 min before centrifugation at 3000 × g for 10 min. The resulting organic layer was dried under a nitrogen stream and redissolved in 250 µL of ACN/H₂O (30/70, v/v) supplemented with 0.01% formic acid.

A 20 µL portion of each reconstituted extract was injected onto the UPLC-MS/MS platform, which

comprised an Acquity UPLC System (Waters Sample Manager and Binary Solvent Manager) linked to a Waters Xevo TQ-S triple quadrupole mass spectrometer. Separation occurred on a Waters Acquity BEH C18 column (1.7 µm, 2.1 × 50 mm) equipped with an integrated filter. The mobile phase, consisting of acetonitrile and H₂O acidified with 0.01% formic acid, was delivered at a flow rate of 0.8 mL/min. In positive electrospray ionization mode, precursor ions [M + H]⁺ were selected at m/z 367.1 for bortezomib and m/z 375.2 for the D8-bortezomib internal standard in Q1. Collision-induced fragmentation occurred in argon within Q2, and specific product ions were monitored in Q3 at m/z 225.9 (bortezomib) and m/z 233.8 (D8-bortezomib).

Calibration standards and quality control samples were generated by spiking blank tissue with 25 µL of appropriate solutions, producing 10 calibration points ranging from 0.5 to 2500 pg per sample, and three QC levels (1.65, 472, and 708 pg per sample) were analyzed in duplicate. Peak area ratios were plotted against concentration using weighted (1/x) linear least-squares regression. Calibration curves consistently showed correlation coefficients exceeding 0.998, enabling a lower limit of quantification of 0.5 pg per sample. Both intra-batch and inter-batch accuracy for QC samples remained within the ±15% limits stipulated by FDA and EMA guidelines [32, 33]. All data acquisition, integration, and quantitative processing were managed with Waters TargetLynx V4.1 software (Waters).

Micro-CT analysis of xerogel-bearing mice femora

Samples embedded in Technovit were scanned using the SkyScan 1173 micro-computed tomography system (Bruker MicroCT). Acquisition of raw projection data, image reconstruction, and all post-processing procedures were performed in accordance with the recommended standards for bone microstructure evaluation [34]. Scan parameters were configured as follows: tube current 200 µA, tube voltage 40 kVp, rotation increment 0.24°, and four-frame averaging to suppress noise. A 0.5 mm aluminum filter was applied for beam filtration. Reconstruction of cross-sectional images with an isotropic voxel size of 5.7 µm was performed using NRecon (Bruker MicroCT) with a Gaussian filter.

Quantification of newly formed bone involved selection of a 0.5 mm thick slab in the mid-sagittal orientation, positioned perpendicular to the axis of the drill hole defect. Inside this defined volume, the extent of implant resorption, intra-defect bone regeneration, and

surrounding bone accrual were measured according to established density thresholds. The analytical strategy was derived from earlier published methodologies with appropriate modifications [35, 36].

Morphometric parameters were extracted after binarization of the grayscale μ CT datasets through a locally adaptive thresholding procedure implemented in CTAn software (Bruker MicroCT).

Consistent with the guidelines proposed by Bouxsein *et al.* [34], modifications in bone structure resulting from the interventions were evaluated by determining relative bone volume, mean trabecular thickness, and trabecular number.

ToF-SIMS analysis

Time-of-flight secondary ion mass spectrometry (ToF-SIMS) provides high-resolution, simultaneous mapping of both organic and inorganic surface constituents, with lateral resolution down to 100 nm. The process begins with bombardment of the specimen surface by a focused primary ion beam, which causes the ejection of secondary ions. These ions are accelerated into a time-of-flight mass analyzer and separated based on their mass-to-charge ratios. Rastering the primary beam across the surface yields spatially resolved chemical distributions, enabling generation of detailed molecular maps. In this investigation, ToF-SIMS was used to characterize bone tissue quality, resorption of the implanted xerogels, and localized bortezomib release from the constructs. Further technical information and examples of its application in skeletal research can be found in Kern *et al.* [37].

Analyses were conducted on a ToF-SIMS 5–100 instrument (ION-TOF Company) fitted with a 25 keV Bi-cluster primary ion gun and an Ar gas cluster sputter source. Bi_3^+ ions were selected as the primary beam. The gun operated in high-mass-resolution spectrometry mode, delivering a lateral resolution of approximately 10 μm . All spectral and image data were processed with Surface Lab 6.3 software (ION-TOF Company). Resin overlying the sample surface was removed beforehand by repeated scanning with a 10 keV Ar_{1500}^+ cluster beam at 0.010–0.025 mm/s for two to three passes, adjusted according to the resin layer thickness.

Large-area imaging of rat femoral cross-sections was accomplished by stitching multiple individual $500 \times 500 \mu\text{m}^2$ fields into composite “stage scans” covering several square millimeters. For bortezomib mapping in the boXer-treated specimens, stage scans were recorded at 100 pixels/mm, with a 50 μs cycle time and 500 shots per

pixel, across 5 patches with three repeated scans, resulting in 7500 total shots per pixel. Empty defect controls and sicXer samples were imaged using the same settings, with only a single scan per patch to reduce total acquisition duration; the measured ion counts were subsequently multiplied by 3 to enable direct comparison between groups.

Statistical analysis

Statistical calculations were executed in R version 3.1.1 (<http://www.r-project.org/>) supported by Bioconductor release 2.14 [38]. Results were regarded as statistically significant when the corresponding P value was less than 5%. Data are reported as mean $\pm$ standard deviation unless noted otherwise. Significance testing for cell culture and drug release experiments was performed using two-way analysis of variance (ANOVA) with Bonferroni adjustment for multiple comparisons in GraphPad Prism; immunohistochemistry outcomes were evaluated using IBM SPSS Statistics version 28.0. Because the data did not meet normality assumptions, nonparametric approaches were adopted. Inter-group differences were initially screened with the Kruskal–Wallis H test, and subsequent pairwise comparisons between treatment conditions were carried out using the Mann–Whitney U test when overall significance was attained.

Results and Discussion

Development and synthesis of (bortezomib-releasing) mesoporous sol–gel silicafibrillar collagen xerogels

The preparation of sicXer and boXer materials relies on a sol–gel process. Combining a buffered suspension of bovine collagen fibrils with pre-hydrolyzed silica sol results in gelation upon pH elevation. Subsequent drying of the hydrogel yields a solid, monolithic, nanoporous silica-collagen composite (**Figures 1A and 1B**). To modulate in vivo resorption rates, the monolithic xerogels (**Figure 1c1**) were milled into granules of varying dimensions. Bortezomib was integrated into the silica-collagen matrix during the sol–gel phase, before gelation. The pharmacological activity of bortezomib remained intact throughout the sol–gel reaction, xerogel formation, and subsequent grinding steps (data not shown). Two distinct gamma-irradiation protocols were applied: sterilization of the finished product (denoted by the suffix -iaf, e.g., sicXer-iaf), (**Figure 1C2**) or pretreatment of the collagen component (sicXer),

(Figure 1C3). The latter approach was implemented to enhance material breakdown and minimize potential radiation-induced interactions with bortezomib.

Bortezomib content is indicated by a numerical suffix representing $\mu\text{g/g}$ of material (e.g., boXer-100 denotes 100 $\mu\text{g/g}$ bortezomib loading).

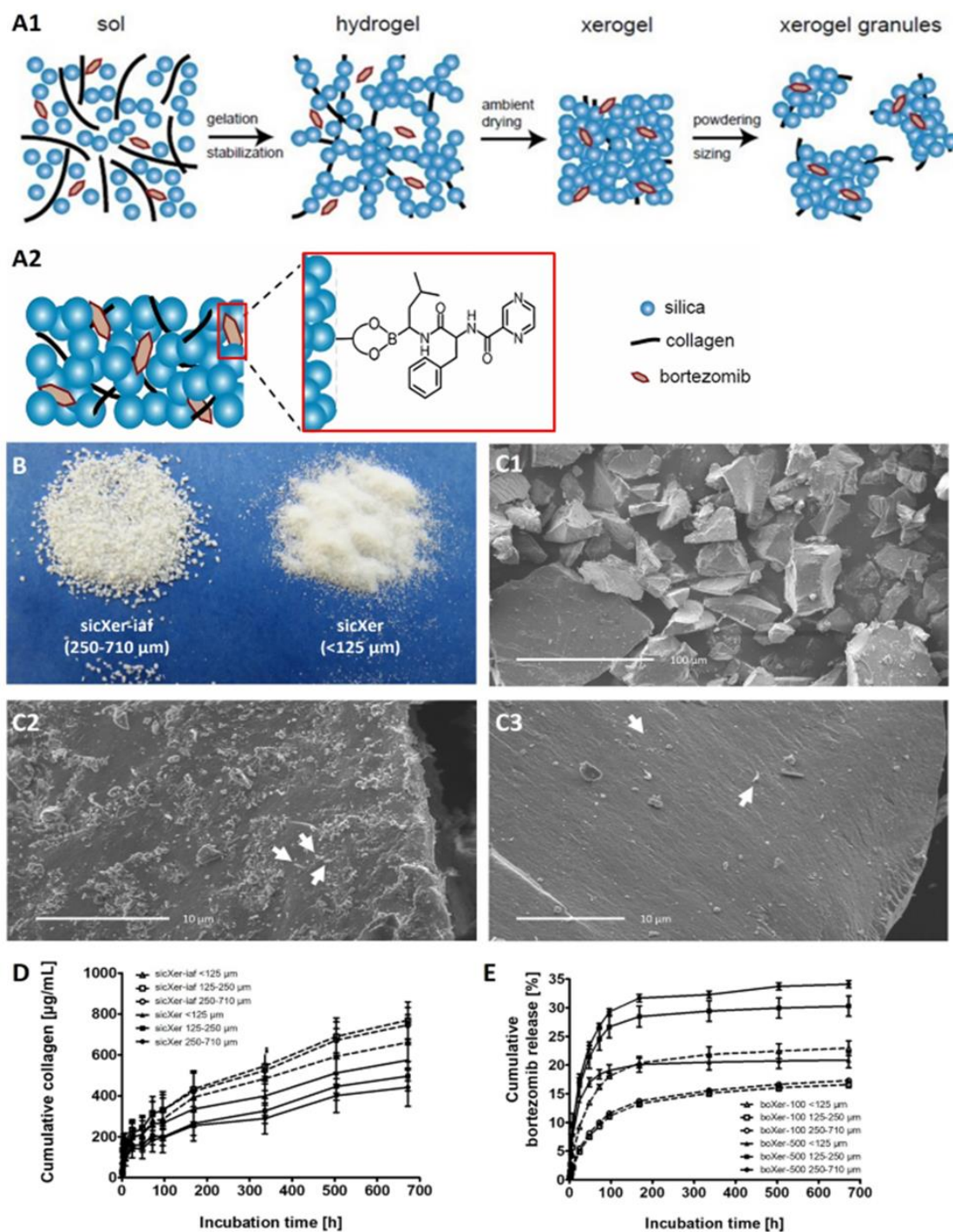


Figure 1. Synthesis of silica-collagen xerogels (sicXer) and bortezomib-loaded silica-collagen xerogels (boXer) together with their collagen and bortezomib release profiles

(A) Schematic illustration. (A1) Mixtures containing bovine collagen suspensions (with or without bortezomib) and silica precursors undergo sol–gel transition to form hydrogels. Drying produces mesoporous xerogels. (A2) Bortezomib incorporation occurs within the xerogel network during the sol–gel manufacturing stage, before complete gelation. (B) Macroscopic appearance of sicXer-iaf granules (left panel; 250–710 μm) and sicXer granules (right panel; $< 125 \mu\text{m}$). Addition of bortezomib produced no visible changes (data not shown). C. Scanning electron micrographs showing (C1) monolithic xerogels ground into granules of different sizes, (C2) irradiation after gel formation (sicXer-iaf), and (C3) irradiation before gel formation (sicXer). These treatments influence the arrangement and morphology of collagen fibrils (indicated by white arrows) within the silica framework. (D) In vitro degradation behavior of xerogel granules. Collagen release from sicXer-iaf and sicXer particles of varying sizes into culture medium. Both granule size and the timing of irradiation (before or after gelation) govern the release kinetics of collagen from the silica-collagen composites. (E) In vitro bortezomib release. Cumulative bortezomib liberation from boXer-100 and boXer-500 formulations, quantified by UPLC-MS/MS and expressed as a percentage of the initially incorporated drug load.

Tailoring material properties

Mechanical properties

sicXer and boXer demonstrated initial mechanical stability comparable to that of native trabecular bone [10, 11].

In vitro degradation

Degradation kinetics could be modulated by adjusting granule size and fabrication approach (sicXer versus sicXer-iaf). This was evidenced by the liberation of silica and collagen from monolithic specimens into the surrounding culture medium (**Figure 1D**). Collagen elution profiles from sicXer and sicXer-iaf (**Figure 1D**) revealed greater matrix stability for sicXer compared with sicXer-iaf. Release rates diminished with increasing

particle diameter ($< 125 \mu\text{m}$, 125–250 μm , and 250–710 μm) for both variants, in accordance with predictions. Following the initial rapid phase, component release continued at a relatively steady pace for the remainder of the incubation (**Figure 1D**). Silica release from monolithic sicXer rose progressively during the first 16 days upon repeated medium renewal, attaining 0.8 mM, after which levels stabilized between 0.6 and 1 mM. Parallel to material breakdown, the culture medium experienced adsorption-driven reductions in calcium and phosphate concentrations. Calcium levels initially dropped to 0.7 mM before recovering and plateauing at 1.3–1.4 mM from day 7 onward. Phosphate concentrations rose initially to approximately four times the control medium value, then declined, reaching roughly half the peak concentration by day 5 (with medium exchanges on days 2, 5, and 7).

Bortezomib elution from boXer-100 and boXer-500 into culture medium (**Figure 1E**) was quantified by UPLC-MS/MS. As expected, release decreased with increasing granule size ($< 125 \mu\text{m}$, 125–250 μm , 250–710 μm). All formulations exhibited an early burst phase followed by prolonged sustained release. By day 28, the cumulative fraction of incorporated bortezomib liberated was 23.9%, 16.6%, and 17.3% from boXer-100 and 20.9%, 30.3%, and 34.1% from boXer-500, respectively, for the three size ranges examined.

In vitro and ex vivo activity of sicXer and boXer

Bone forming and bone resorbing activity

The effects of sicXer and sicXer-iaf granules ($< 125 \mu\text{m}$) on hMSC proliferation, osteogenic differentiation, and osteoclast formation were examined.

Osteoblastogenesis was studied by culturing hMSCs under osteogenic conditions either without additives (control) or together with sicXer or sicXer-iaf granules ($< 125 \mu\text{m}$). Cell counts rose significantly with time in all groups (**Figure 2A1**), (Jonckheere-Terpstra test, $P < 0.001$). Osteoblastic activity, evaluated through alkaline phosphatase (ALP) measurement, also increased markedly over the culture duration across all conditions (**Figure 2A2**), (Jonckheere-Terpstra test, $P < 0.001$).

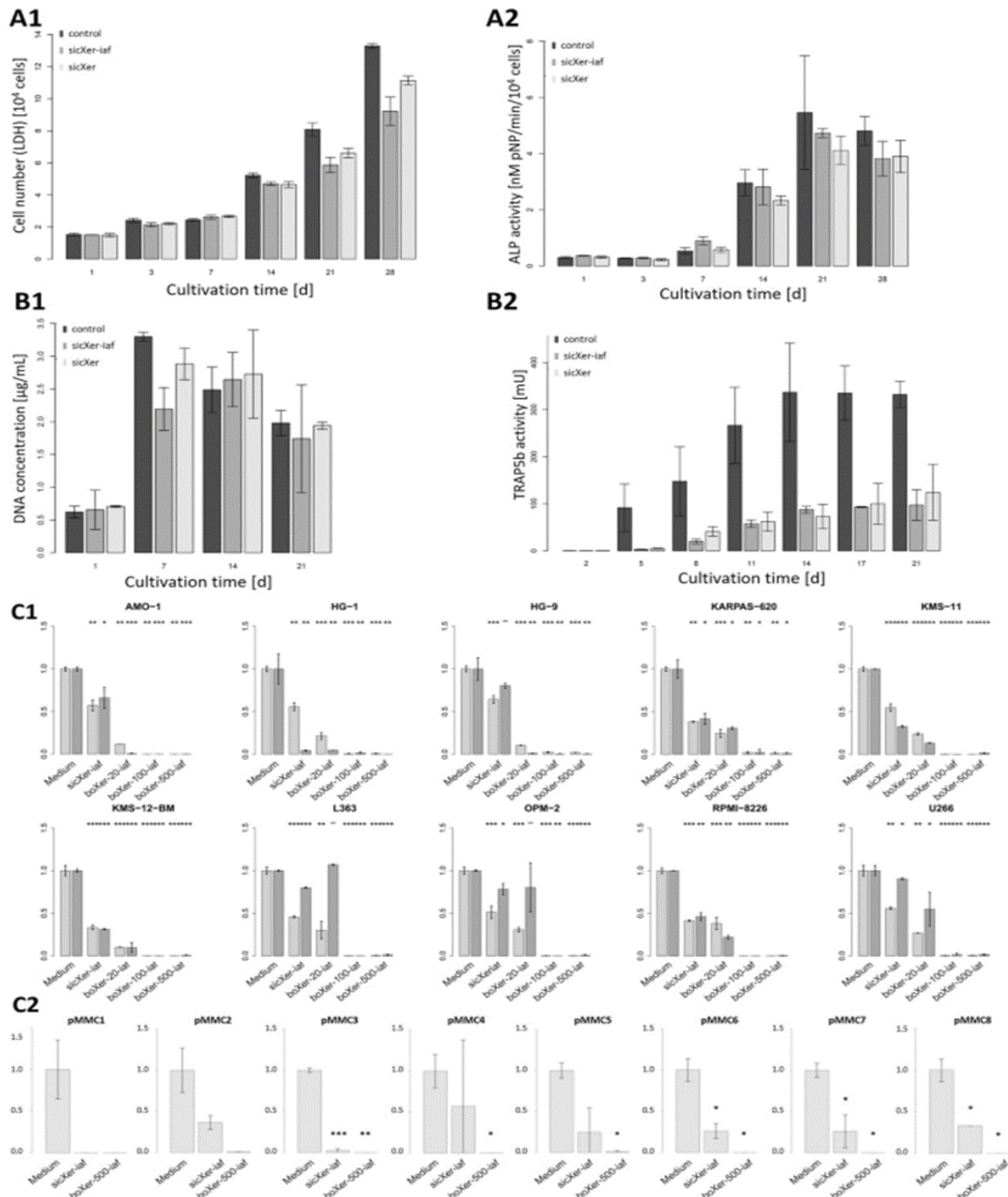


Figure 2. In vitro functional assessment. Exposure to sicXer and sicXer-iaf promoted progressive expansion and activation of osteoblasts while strongly attenuating osteoclast formation and function compared with untreated controls, resulting in favorable net bone formation while preserving core remodeling processes

(A1) Osteoblast formation. hMSCs were maintained under osteogenic conditions without (control) or with sicXer or sicXer-iaf granules ($< 125 \mu\text{m}$). Cell numbers increased significantly throughout the observation period (Jonckheere-Terpstra test, $P < 0.001$). (A2) Osteoblast activity, as indicated by alkaline phosphatase (ALP)

levels, rose significantly over time in every experimental group (Jonckheere-Terpstra test, $P < 0.001$). (B1) Osteoclast number and (B2) osteoclast activity. Monocytes were differentiated into osteoclasts in vitro in the absence or presence of sicXer or sicXer-iaf granules ($< 125 \mu\text{m}$). Osteoclast activity, measured by

intracellular TRAP5b, increased significantly with time in all conditions (Jonckheere-Terpstra test, $P < 0.001$), yet remained considerably lower when either xerogel material was present. (C1) Ten human myeloma cell lines were incubated with sicXer-iaf, boXer-20-iaf, boXer-100-iaf, or boXer-500-iaf. Clear cytotoxic effects on myeloma cells were already apparent in sicXer-treated wells. Untreated medium served as control. Light-grey and dark-grey bars correspond to separate experiments, each run in triplicate. (C2) Primary myeloma cells (pMMC, $n = 8$) maintained together with their bone marrow microenvironment were treated with sicXer-iaf or boXer-500-iaf and compared against medium controls. Primary myeloma cells from four patients (pMMC3, 4, 6 and 8) were resistant to systemic bortezomib treatment.

Osteoclast formation and function were investigated by *in vitro* differentiation of monocytes into osteoclasts with or without sicXer or sicXer-iaf granules ($< 125 \mu\text{m}$). Osteoclast numbers rose initially, reaching a maximum around day 7 in all conditions, followed by a gradual decline until day 21 (**Figure 2B1**). Osteoclast activity, quantified by TRAP5b, increased significantly over time across all groups (Jonckheere-Terpstra test, $P < 0.001$); (**Figure 2B2**), but was notably suppressed in the presence of either bone substitute material relative to controls.

Collectively, the materials exerted only minor influence on osteoblast numbers and activity while producing pronounced reductions in osteoclast function compared with untreated controls. This pattern was expected to generate net bone formation with retention of physiological remodeling, a hypothesis subsequently verified *in vivo*.

Anti-myeloma activity

Treatment with sicXer-iaf resulted in effective myeloma cell elimination across all 10 human myeloma cell lines examined. Incorporation of bortezomib further augmented cytotoxicity: boXer-20-iaf produced enhanced killing, while boXer-100-iaf and boXer-500-iaf led to virtually total eradication of the remaining myeloma cells (**Figure 2C1**). In primary myeloma cells derived from bone marrow samples obtained during standard diagnostic assessments [9, 39], sicXer-iaf triggered marked cell death. Application of boXer-500-iaf achieved over 95% myeloma cell reduction in all 8 patient samples tested. Myeloma cells from four patients (pMMC3, 4, 6, 8) proved resistant to conventional bortezomib treatment (**Figure 2C2**). Impact on non-myeloma cells of the bone marrow microenvironment proved minimal. These anti-myeloma properties observed *in vitro* were successfully corroborated in subsequent *in vivo* experiments (described below).

In vivo activity

Drill hole defect in rat femora

Representative Movat pentachrome-stained histological sections from empty control defects and those filled with sicXer-iaf, boXer-100-iaf, boXer-500-iaf, or boXer-2500-iaf illustrated improved bone regeneration. The boXer-100-iaf group in particular displayed superior integration of newly deposited osteoid and restoration of continuous trabecular networks, closely resembling the architecture of intact bone (**Figure 3A1**). Complementary ALP staining confirmed heightened osteoblast function in boXer-100-iaf samples, reflecting vigorous bone-forming activity (**Figure 3A2**). In contrast, TRAP staining revealed a notable reduction in osteoclast numbers and activity, particularly in boXer-2500-iaf specimens where TRAP-positive cells were significantly fewer ($P = 0.002$), indicating attenuated resorptive processes (**Figure 3A3**).

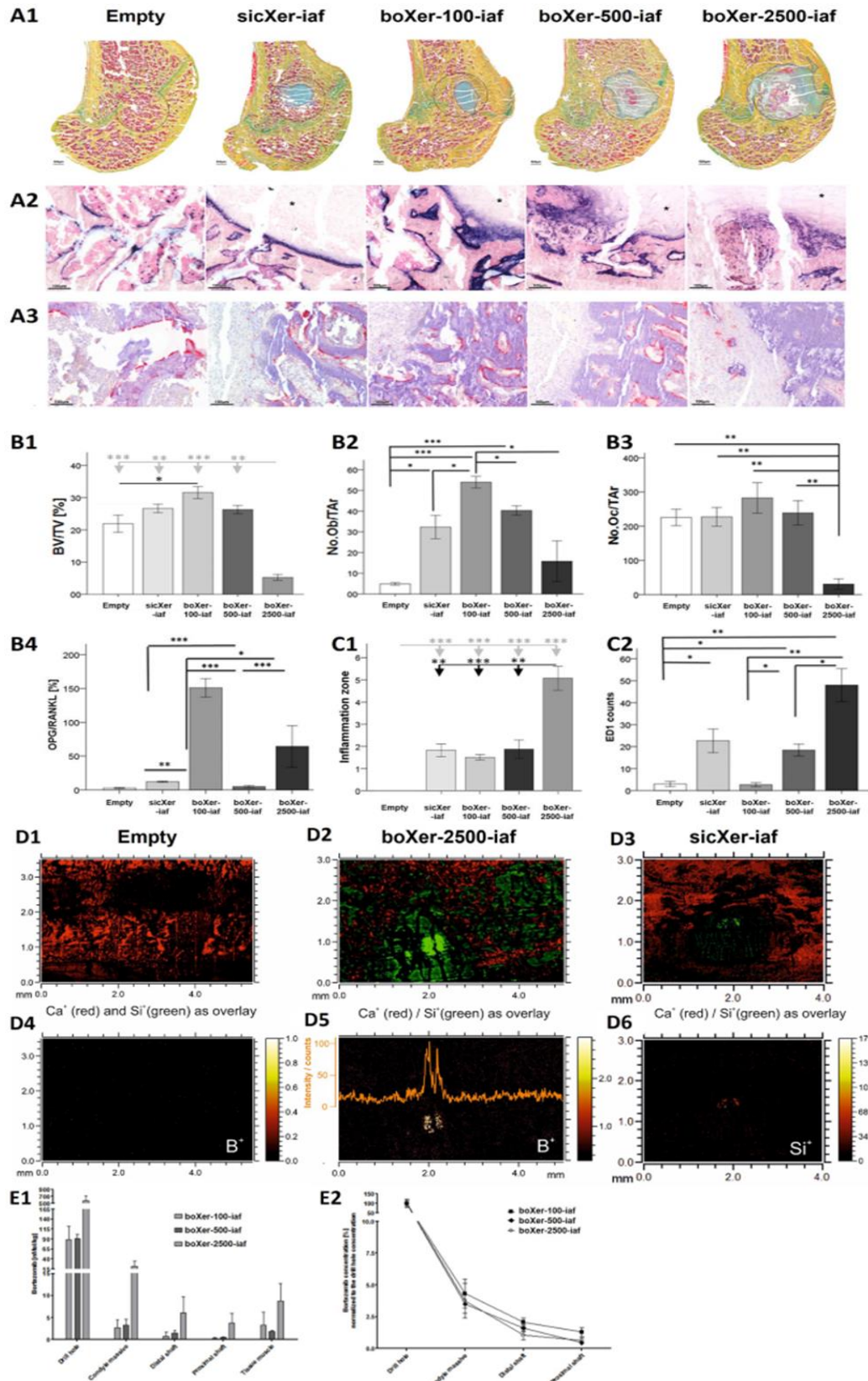


Figure 3. In vivo performance of sicXer-iaf and various boXer-iaf formulations implanted into 2.5 mm drill-hole defects in rat femora, evaluated four weeks after surgery

(A1) Low-magnification views of Movat pentachrome-stained sections arranged from left to right as empty defect, sicXer-iaf, boXer-100-iaf, boXer-500-iaf, and boXer-2500-iaf. All treated groups exhibited increased bone deposition, with boXer-100-iaf achieving the most advanced osteoid incorporation and trabecular reconnection, indicative of advanced structural restoration comparable to native bone. (A2) Alkaline phosphatase (ALP) staining highlighted elevated osteoblastic activity in the boXer-100-iaf group, consistent with active matrix production. (A3) Tartrate-resistant acid phosphatase (TRAP) staining showed a statistically significant reduction in positive cells within boXer-2500-iaf samples ($P = 0.002$), reflecting decreased osteoclast-mediated resorption. (B1) Quantitative histomorphometry of new bone formation expressed as bone volume (BV) relative to tissue volume (TV) within the original defect site. boXer-100-iaf treatment produced significantly greater mineralized tissue than empty defects ($P = 0.038$). Conversely, boXer-2500-iaf yielded markedly lower bone volume compared with empty defect ($P \leq 0.001$), sicXer-iaf ($P = 0.002$), boXer-100-iaf ($P \leq 0.001$), and boXer-500-iaf ($P = 0.002$). Mann-Whitney test; * $P < 0.05$, ** $P < 0.01$, *** $P \leq 0.001$. Grey significance bars mark comparisons of boXer-2500-iaf versus other groups; black bars indicate comparisons versus empty control. (B2) Histomorphometric quantification of osteoblast numbers (No. Ob) per trabecular area (TA_r) based on ALP staining and (B3) osteoclast numbers (No. Oc) per TA_r based on TRAP staining in the defect region. ALP staining intensity was highest in boXer-100-iaf samples. TRAP-positive cell counts were significantly reduced in boXer-2500-iaf specimens ($P = 0.002$). (B4) Immunohistomorphometric evaluation demonstrated a highly significant elevation of the OPG/RANKL ratio exclusively in the boXer-100 group relative to all other conditions ($P \leq 0.001$). (C1) The inflammatory zone expanded considerably in all implanted groups compared with empty defects, which displayed no inflammation (all $P \leq 0.001$). Inflammation reached the highest level in boXer-2500-iaf, significantly exceeding sicXer ($P = 0.002$), boXer-100-iaf ($P \leq 0.001$), and boXer-500-iaf ($P = 0.004$). Grey bars represent boXer-2500-iaf comparisons; black bars compare to empty controls. (C2) ED1-positive macrophage counts were concurrently lowered in the boXer-100-iaf group. Mann-Whitney test; * $P < 0.05$, ** $P < 0.01$, *** $P \leq 0.001$. (D) ToF-SIMS mass spectrometric images of bone cross-sections. (D1–

D3) Composite overlays depicting Ca^+ (red) and Si^+ (green) distributions in empty defect controls, sicXer-iaf, and boXer-2500-iaf samples. (D4) and (D5) Images of B^+ ion distribution, where pixel intensity reflects ion count rate. The orange profile line in (D5) displays the vertical summation of B^+ counts. (D6) Si^+ distribution map for sicXer-iaf. (E) Local bortezomib concentrations determined in vivo: (E1) levels measured inside the drill hole and in surrounding bone and soft tissue; (E2) relative concentration gradients expressed as percentages from the drill hole site (100%) declining to the condylar region ($< 5\%$), distal shaft ($< 2.5\%$), and proximal shaft (approximately 1%).

Bone formation

Compared with empty defects, sicXer-iaf treatment resulted in a significant elevation of osteoblast numbers (**Figure 3B2**), ($P \leq 0.001$) while osteoclast counts remained unchanged (**Figure 3B3**). This was associated with a substantially higher OPG/RANKL ratio ($P = 0.002$); (**Figure 3B4**), indicating a shift toward net bone accrual. Although BV/TV values exceeded those of controls, the increase did not attain statistical significance (**Figure 3B1**).

boXer-100-iaf produced a pronounced rise in osteoblast numbers relative to both empty defects and sicXer-iaf ($P \leq 0.001$ and $P < 0.01$, respectively), with osteoclast numbers staying stable (**Figure 3B3**). This translated into significantly greater bone formation (BV/TV) than in empty controls ($P = 0.038$); (**Figure 3B1**). The OPG/RANKL ratio was markedly elevated compared with empty defects and sicXer-iaf (both $P \leq 0.001$); (**Figure 3B4**). Increasing bortezomib dosage reduced these beneficial effects. boXer-500-iaf still significantly boosted osteoblast counts versus controls ($P \leq 0.001$) without changing osteoclast numbers. Yet, it did not improve the OPG/RANKL balance or produce significantly higher bone volume (BV/TV) than empty defects. boXer-2500-iaf failed to increase osteoblast numbers significantly (**Figure 3B2**) but led to a clear reduction in osteoclasts ($P < 0.01$ versus all other groups); (**Figure 3B3**). Bone formation in this group was significantly lower than in empty defects ($P \leq 0.001$), sicXer-iaf ($P = 0.002$), boXer-100-iaf ($P \leq 0.001$), and boXer-500-iaf ($P = 0.002$) (**Figure 3B1**).

Tolerability

Immunohistochemical quantification demonstrated expansion of the inflammatory zone in all implanted

groups (sicXer, boXer-100-iaf, boXer-500-iaf, and boXer-2500-iaf) compared with empty defects, which displayed no inflammation (all $P \leq 0.001$) (**Figure 3C1**). Inflammation was most extensive with boXer-2500-iaf, significantly surpassing sicXer ($P = 0.002$), boXer-100-iaf ($P \leq 0.001$), and boXer-500-iaf ($P = 0.004$). Foreign body giant cells expressing the macrophage marker ED1 (CD68) (**Figure 3C2**) showed no elevation in the boXer-100-iaf group relative to empty defects, whereas significantly higher counts were recorded for boXer-500-iaf ($P = 0.003$) and boXer-2500-iaf ($P \leq 0.001$).

Assessment of material degradation and bortezomib distribution

Tissue distribution of calcium, silicon, and boron was mapped using imaging mass spectrometry (ToF-SIMS) (**Figure 3D**). Calcium ion maps (**Figures 3D1–3**); (red) faithfully reproduced the anatomical features of the rat distal femur, including the defect area. Silicon signals (green), which are absent in healthy bone, enabled monitoring of material breakdown and spread. After four weeks, most of the implanted sicXer-iaf and boXer-iaf had been resorbed (**Figures 3D2 and 3D3**), with remaining fragments largely restricted to the original defect zone. Liberated silicon had diffused into surrounding tissues and accumulated particularly at the marrow-trabecular boundaries (**Figures 3D2 and 3D3**). Boron ions (B^+), representing bortezomib, could be detected only in the boXer-2500-iaf group at this time point due to differences in concentration and ionization. Bortezomib remained primarily localized within the defect, with a uniform, low-level distribution across the bone section, without a distinct concentration gradient into neighboring areas (**Figure 3D5**).

Bortezomib concentrations in the operated femora were measured four weeks post-surgery by UPLC-MS/MS. Detectable amounts depended on both the initial drug

loading and the distance from the implantation site (**Figure 3E1**) [absolute concentrations] and (**Figure 3E2**) [relative gradients] normalized to levels in the drill hole). Highest concentrations occurred at the defect in the distal metaphysis (69–700 nM), followed by the adjacent femoral condyle bone marrow (1.2–37 nM). Concentrations declined further with distance in the distal shaft (0.1–9.5 nM), proximal shaft (0.1–5.7 nM), and surrounding muscle/soft tissue (0.8–13.3 nM). In every region examined, the greatest bortezomib levels corresponded to the highest initial loading doses.

Drill hole defects in the 5T33-myeloma mouse model

Load-bearing and fracture stabilizing properties

Implantation of any of the three tested materials prevented fractures. The implants therefore successfully counteracted myeloma-induced bone weakening and provided the desired mechanical support to the defect sites.

Local control of myeloma cell infiltration

Following sicXer implantation (**Figure 4A1**), myeloma cells exhibited diffuse infiltration throughout the distal epiphysis and commonly extended directly into the drill hole. Despite this, sicXer treatment still permitted effective bone regeneration within three weeks even in the presence of nearby myeloma cells (**Figure 4B2**). In comparison, boXer implants (**Figures 4A2 and A3**) generated clear zones devoid of myeloma cells, with the size of these zones increasing in proportion to bortezomib content. Consequently, boXer-500 and boXer-2500 achieved strong local containment of myeloma cells, whether evaluated across the full bone marrow region examined (**Figure 4B1**) or restricted to a 100 μm zone immediately surrounding the implanted granules (**Figure 4B2**).

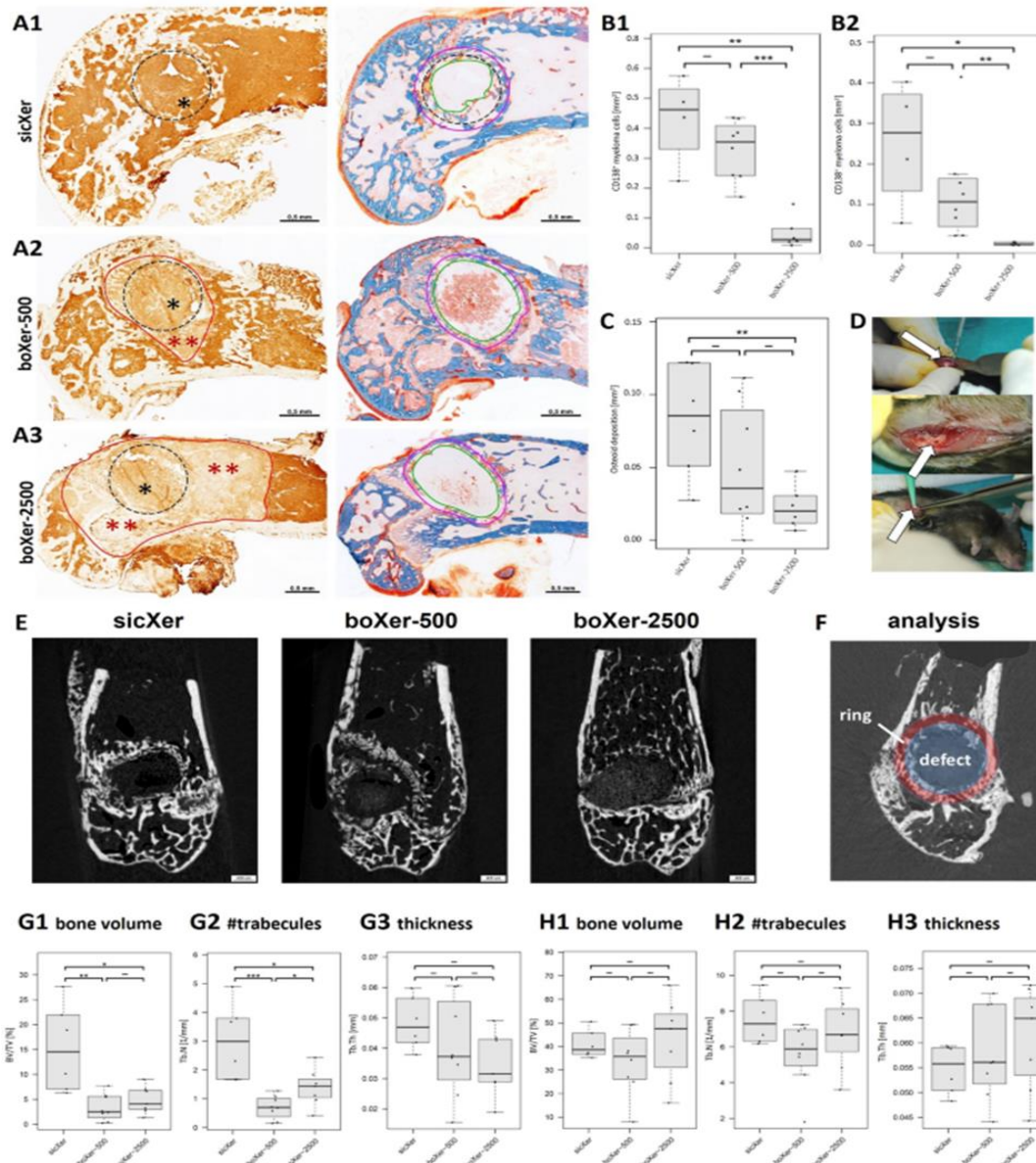


Figure 4. In vivo anti-myeloma effects and stimulation of bone formation by sicXer and boXer in the 5T33-myeloma mouse model

(A) Dose-dependent local suppression of myeloma cell accumulation visualized by anti-CD138 immunohistochemistry (left panels) and assessment of bone-forming potential by Masson–Goldner staining (right panels) for (A1) sicXer, (A2) boXer-500, and (A3) boXer-2500. sicXer implantation resulted in extensive CD138 positivity across the entire distal epiphysis due to dense myeloma cell infiltration that also penetrated the defect area. boXer materials produced bortezomib-dependent inhibition of myeloma cells, creating a protective zone around boXer-500 and a broader effect

extending into the diaphysis with boXer-2500. The dashed line delineates the original drill hole contour. The red line denotes the outer boundary of bortezomib's local anti-myeloma influence. Osteoid production was most extensive with sicXer. Masson–Goldner staining three weeks post-implantation showed woven bone advancing into the defect from the margins (dashed black line) in sicXer samples, whereas boXer-treated defects displayed larger unoccupied areas. Green lines indicate regions lacking bone ingrowth; magenta lines define the 100 μ m annular zone around the implant used for osteoid

quantification, (B) Boxplots summarizing quantitative results from panel A concerning myeloma cell infiltration reduction (B1) in the bone marrow compartment and (B2) within the 100 μm zone adjacent to the material, (C) Boxplot summarizing osteoid formation data from panel (A), (D) Placement of material into the drill hole defect. White arrows point to the defect location. (E) μCT evaluation of tissue sections. Coronal multiplanar reformations illustrate extensive resorption of sicXer (left panel) with robust new bone deposition both within and beyond the original drill hole boundaries. boXer-500 displayed incomplete resorption accompanied by moderate bone regeneration (middle panel). boXer-2500 exhibited very limited resorption and negligible new bone formation (right panel). (F) Diagram illustrating the regions used for quantitative μCT analysis. The defect zone corresponds to the original 1 mm diameter bone defect (quantified in I1–I3), while the “ring” represents the 1 mm wide surrounding peri-defect area (quantified in J1–J3). (G1) Bone percentage in the original defect zone, (G2) trabecular number [1/mm], (G3) trabecular thickness [mm]. All materials supported new bone formation inside the defect, with the strongest response observed for sicXer. (H1) Bone percentage in the peri-defect zone, (H2) trabecular number [1/mm], and (H3) trabecular thickness [mm]. Bortezomib release did not adversely affect the surrounding peri-defect region.

Induction of net bone formation and bone healing

sicXer implantation resulted in the ingrowth of newly formed woven bone from the drill hole margins into the defect area (**Figure 4A**). Histological measurements indicated that the average residual defect area decreased from the initial size of 0.79 mm^2 to 0.40 (± 0.17) mm^2 , representing approximately 49% coverage by bone. In contrast, boXer-treated defects showed less clearly defined margins, with mean defect areas expanding to 0.86 (± 0.17) mm^2 (mean increase of 9%) for boXer-500 and 0.91 (± 0.14) mm^2 (mean increase of 15%) for boXer-2500. Along the adjacent host bone surfaces, active bone apposition was visible as prominent red osteoid seams deposited on trabecular edges. Within the defined ring-shaped region of interest, the mean proportion of osteoid was 8.2 (± 3.8)% for sicXer, 4.9 (± 3.9)% for boXer-500, and 2.2 (± 1.3)% for boXer-2500 (**Figure 4C**). Due to ethical considerations and the high fracture risk in this model, empty defect controls were not feasible.

μCT evaluation of harvested specimens confirmed extensive degradation of sicXer associated with robust bone regeneration both inside the original defect and in surrounding areas. boXer-500 displayed incomplete resorption with some new bone development, while boXer-2500 showed very limited material breakdown and negligible bone formation (**Figures 4E and 4F**). Within the initial defect zone, average BV/TV reached $15.33 \pm 8.77\%$ for sicXer, $3.35 \pm 2.94\%$ for boXer-500, and $4.86 \pm 2.83\%$ for boXer-2500 (**Figure 4G1**). Trabecular thickness (**Figure 4G2**) was 0.048 ± 0.009 μm , 0.040 ± 0.016 μm , and 0.035 ± 0.011 μm for sicXer, boXer-500, and boXer-2500, respectively. The number of new trabeculae (**Figure 4G3**) averaged 3.00 ± 1.32 μm^{-1} , 0.70 ± 0.46 μm^{-1} , and 1.39 ± 0.50 μm^{-1} . In the surrounding peri-defect area, mineralization was similarly observed (**Figures 4H1–3**), aligning with histological osteoid findings (**Figures 4A and 4C**). Mean BV/TV in this region was $52.9 \pm 5.7\%$ for sicXer, $52.7 \pm 9.1\%$ for boXer-500, and $54.5 \pm 19.2\%$ for boXer-2500 (**Figure 4H1**). Trabecular number and thickness showed no significant inter-group differences in the peri-defect zone. The overall experimental setup for the 5T33-mouse model is illustrated in **Figure 4D**.

Collectively, the materials supported bone repair and successfully prevented fractures in the 5T33-myeloma mouse model. They also provided effective local inhibition of myeloma cell infiltration, with boXer variants extending this control into the adjacent tissue. The present study introduces silica-collagen xerogels, designated sicXer and boXer, that exhibit mechanical properties similar to trabecular bone and actively promote bone regeneration. These materials represent a promising new approach for the localized management of both non-malignant and malignant disorders associated with compromised bone repair. During the development of these silica-collagen xerogels, their mechanical integrity was thoroughly evaluated. Investigations on monolithic specimens confirmed that the materials can endure the physical forces associated with surgical insertion into bone. In the hydrated condition, their elastic modulus, along with their compressive and tensile strength values, fell within the ranges reported for human cancellous and cortical bone [16, 17]. The current work demonstrates that both formulations effectively stabilized the created defects in rats and mice, with no fractures observed in any animal. Several mechanisms likely mediate the biological effects of the materials. First, the presence and gradual release

of silicon ions, which have been shown to enhance osteoblast differentiation *in vitro* using calcium phosphate cements [40] and to stimulate osteogenesis *in vivo* [41], as well as in silicon-containing bioceramic scaffolds [42, 43]. Second, silica-based xerogels exhibit adsorptive properties that can sequester signaling molecules involved in local bone remodeling [44]. Earlier research has established that bortezomib promotes bone formation by enhancing osteoblast proliferation and activity at low nanomolar concentrations (approximately 5 nM) [22, 23] while simultaneously inhibiting bone resorption and triggering osteoclast apoptosis [22, 23, 45–48] ($IC_{50} \approx 92$ nM) [49]. These findings align closely with the bortezomib levels detected near the defect site in the rat model (approximately 37 nM after four weeks), which may account for the superior bone healing observed with boXer compared with sicXer. In the rat model, both materials favored bone formation by modulating the OPG/RANKL axis—raising OPG expression while lowering RANKL—alongside increased osteoblast numbers and overall bone mass. This anabolic influence was most evident with lower bortezomib doses, particularly boXer-100-iaf. Higher bortezomib release from boXer-500-iaf reduced these benefits, while the highest dose in boXer-2500-iaf triggered a pronounced inflammatory response. In the 5T33-mouse model, bone formation manifested as osteoid deposition and mineralization primarily in the peri-defect area (especially with boXer-500), although limited bone regeneration occurred inside the defect itself. The shorter experimental duration in this model—necessitated by systemic disease progression requiring euthanasia—provides an earlier view of the healing process compared with the rat model. The observed stimulation of bone repair is consistent with a previous investigation employing bortezomib-loaded porous nano-hydroxyapatite/alginate scaffolds in a rabbit femoral defect model [50]. Unlike bortezomib-releasing polymethylmethacrylate bone cement [51], sicXer and boXer permit physiological remodeling and replacement of the defect with native bone.

In multiple myeloma, the proliferation of malignant plasma cells disrupts normal bone homeostasis. Although both resorption and formation initially increase, bone formation eventually fails to compensate, resulting in net bone loss and osteolytic lesions [52]. Myeloma cells rely on autocrine and paracrine growth factors, such as insulin-like growth factor 1 [28, 53–55],

which are liberated from the bone marrow niche and degraded bone matrix, thereby perpetuating a vicious cycle. Removal of myeloma cells interrupts this cycle, enabling bone regeneration to resume.

Notably, sicXer alone exhibited clear anti-myeloma effects against both established human myeloma cell lines and primary patient-derived myeloma cells. Given that both normal and malignant plasma cells depend on external growth and survival signals [53], we propose that the strong adsorptive capacity of silica-collagen xerogels [21, 44] deprives myeloma cells of these essential factors, thereby contributing to cell death. The addition of bortezomib in boXer formulations markedly amplifies this cytotoxic action. Local tissue concentrations of bortezomib exceeded the IC_{50} values reported for myeloma cell lines (1.6–8.8 nM) [56], including in cells rendered resistant through exposure (showing up to 20-fold higher IC_{50}) [57]. This explains the efficacy of boXer even in primary myeloma cells from patients resistant to systemic bortezomib. In the 5T33-mouse model, myeloma cell clearance extended into the surrounding tissue.

sicXer and boXer offer a potential therapeutic option for managing fractured or fracture-prone osteolytic lesions (**Figure 5**). These materials stabilize the affected area while exerting localized control over malignant plasma cells, including those resistant to systemically attainable bortezomib levels. Compared with percutaneous radiotherapy, these locally applied biomaterials provide immediate biomechanical reinforcement, actively promote bone repair, and spare neighboring hematopoietic tissue.

In patients with asymptomatic (smoldering) myeloma, where the emergence of osteolytic bone lesions often represents the primary or sole indication for initiating systemic chemotherapy in approximately one third of cases [4], or in individuals with relapsed disease manifesting predominantly as progressive lytic lesions despite prior systemic therapy, such local fracture-preventive intervention could serve to postpone or bridge the need for further systemic treatment (**Figure 5**). Since osteolytic lesions frequently constitute the final sanctuary sites harboring residual myeloma cells after systemic therapy [5, 6], targeted elimination of these persistent foci may form an important component of integrated systemic and local therapeutic strategies in future clinical trials. For non-malignant bone conditions, both materials appear suitable, with boXer offering advantages through its enhanced bone-anabolic effects

and restricted action beyond the immediate implantation site.

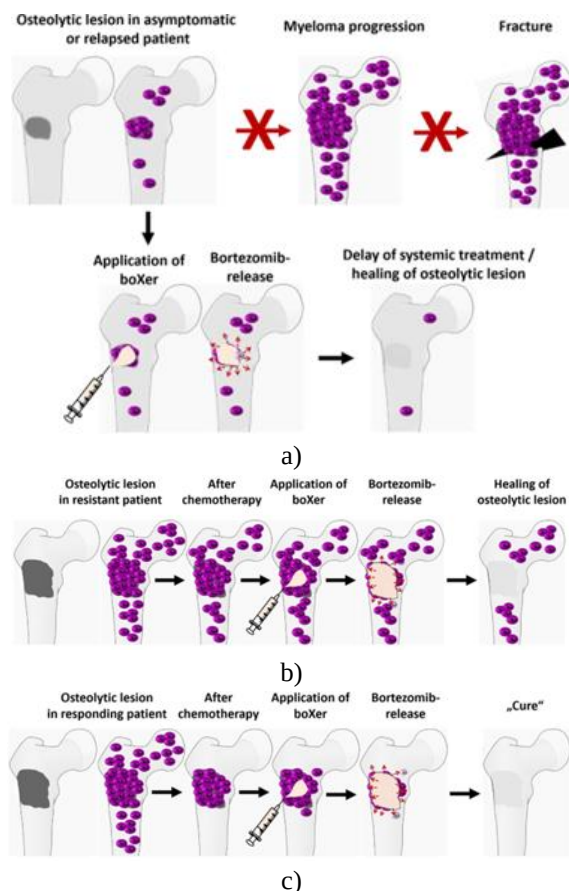


Figure 5. Potential clinical uses of sicXer and boXer in the management of multiple myeloma

(a) In patients with asymptomatic or relapsed myeloma, advancing osteolytic lesions frequently necessitate intervention to prevent pathological fractures and extensive skeletal damage. (b) A particularly challenging scenario arises in individuals who have become refractory to all available therapies or who can no longer tolerate additional systemic chemotherapy or radiotherapy due to cumulative toxicity. In such cases, local delivery of high bortezomib doses (for example, via boXer-500) may overcome resistance mechanisms and achieve local disease control while simultaneously providing mechanical stabilization. (c) When minimal residual disease is undetectable elsewhere, osteolytic lesions may function as protected niches containing surviving myeloma cells. Their targeted eradication could extend progression-free survival and, in optimistic scenarios, contribute toward achieving a cure for myeloma.

Although the highest bortezomib concentration tested in this study (boXer-2500) was primarily included to explore potential dose-limiting effects and identify an optimal therapeutic window rather than for direct clinical translation, the presented materials—sicXer, boXer-100 (low-dose bortezomib), and boXer-500 (high-dose bortezomib)—enable tailored application depending on the predominant clinical need, sicXer or boXer-100 would be appropriate when mechanical stabilization of fracture-prone lesions is the priority. In contrast, higher bortezomib release may be preferred for controlling residual myeloma cells. Naturally, clinical implementation of this approach requires evaluation in appropriately designed clinical trials.

Conclusion

In conclusion, sicXer and boXer represent an innovative therapeutic strategy. Their combined capacity to stabilize fracture-prone lesions, stimulate bone regeneration, and exert direct anti-tumor effects supports their advancement into clinical testing as part of multimodal systemic and local treatment regimens for multiple myeloma as well as for selected non-malignant bone disorders.

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References

1. Kyle RA, Rajkumar SV. Multiple myeloma. *Blood*. 2008;111(6):2962-72.
2. Kyle RA, Gertz MA, Witzig TE, Lust JA, Lacy MQ, Dispenzieri A, et al. Review of 1027 patients with newly diagnosed multiple myeloma. *Mayo Clin Proc*. 2003;78(1):21-33.
3. Melton LJ 3rd, Kyle RA, Achenbach SJ, Oberg AL, Rajkumar SV. Fracture risk with multiple myeloma: a population-based study. *J Bone Miner Res*. 2005;20(3):487-93.
4. Neben K, Jauch A, Hielscher T, Hillengass J, Lehnert N, Seckinger A, et al. Progression in smoldering myeloma is independently determined by the chromosomal abnormalities del(17p), t(4;14),

- gain 1q, hyperdiploidy, and tumor load. *J Clin Oncol*. 2013;31(34):4325-32.
5. Zamagni E, Nanni C, Mancuso K, Tacchetti P, Pezzi A, Pantani L, et al. PET/CT improves the definition of complete response and allows to detect otherwise unidentifiable skeletal progression in multiple myeloma. *Clin Cancer Res*. 2015;21(19):4384-90.
 6. Moreau P, Attal M, Caillot D, Macro M, Karlin L, Garderet L, et al. Prospective evaluation of magnetic resonance imaging and [(18)F]fluorodeoxyglucose positron emission tomography-computed tomography at diagnosis and before maintenance therapy in symptomatic patients with multiple myeloma included in the IFM/DFCI 2009 trial: results of the IMAJEM study. *J Clin Oncol*. 2017;35(25):2911-8.
 7. Bannas P, Hentschel HB, Bley TA, Stork A, Adam G, Weber C, et al. Diagnostic performance of whole-body MRI for the detection of persistent or relapsing disease in multiple myeloma after stem cell transplantation. *Eur Radiol*. 2012;22(9):2007-12.
 8. Emde-Rajaratnam M, Beck S, Benes V, Bohn M, Kotecha R, Raab MS, et al. RNA-sequencing based first choice of treatment and determination of risk in multiple myeloma. *Front Immunol*. 2023;14:1286700.
 9. Seckinger A, Delgado JA, Moser S, Moreno L, Neuber B, Grab A, et al. Target expression, generation, preclinical activity, and pharmacokinetics of the BCMA-T cell bispecific antibody EM801 for multiple myeloma treatment. *Cancer Cell*. 2017;31(3):396-410.
 10. Seckinger A, Hillengass J, Emde M, Beck S, Knauth K, Zojer N, et al. CD38 as Immunotherapeutic target in light chain amyloidosis and multiple myeloma—association with molecular entities, risk, survival, and mechanisms of upfront resistance. *Front Immunol*. 2018;9(1676).
 11. Goldschmidt H, Mai EK, Bertsch U, Fenk R, Nievergall E, Tichy D, et al. Addition of isatuximab to lenalidomide, bortezomib, and dexamethasone as induction therapy for newly diagnosed, transplantation-eligible patients with multiple myeloma (GMMG-HD7): part 1 of an open-label, multicentre, randomised, active-controlled, phase 3 trial. *Lancet Haematol*. 2022;9(11):e810-21.
 12. Barlogie B, Mitchell A, van Rhee F, Epstein J, Morgan GJ, Crowley J. Curing myeloma at last: defining criteria and providing the evidence. *Blood*. 2014;124(20):3043-51.
 13. Durie BGM, Hoering A, Abidi MH, Rajkumar SV, Epstein J, Kahanic SP, et al. Bortezomib with lenalidomide and dexamethasone versus lenalidomide and dexamethasone alone in patients with newly diagnosed myeloma without intent for immediate autologous stem-cell transplant (SWOG S0777): a randomised, open-label, phase 3 trial. *Lancet*. 2017;389(10068):519-27.
 14. Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol*. 2016;17(8):e328-46.
 15. Munshi NC, Avet-Loiseau H, Anderson KC, Neri P, Paiva B, Samur M, et al. A large meta-analysis establishes the role of MRD negativity in long-term survival outcomes in patients with multiple myeloma. *Blood Adv*. 2020;4(23):5988-99.
 16. Heinemann S, Heinemann C, Bernhardt R, Reinstorf A, Nies B, Meyer M, et al. Bioactive silica-collagen composite xerogels modified by calcium phosphate phases with adjustable mechanical properties for bone replacement. *Acta Biomater*. 2009;5(6):1979-90.
 17. Heinemann S, Coradin T, Worch H, Wiesmann HP, Hanke T. Possibilities and limitations of preparing silica/collagen/hydroxyapatite composite xerogels as load-bearing biomaterials. *Compos Sci Technol*. 2011;71.
 18. Weitzmann MN, Ha SW, Vikulina T, Roser-Page S, Lee JK, Beck GR Jr. Bioactive silica nanoparticles reverse age-associated bone loss in mice. *Nanomedicine*. 2015;11(4):959-67.
 19. Casarrubios L, Matesanz MC, Sánchez-Salcedo S, Arcos D, Vallet-Regí M, Portolés MT. Nanocrystallinity effects on osteoblast and osteoclast response to silicon substituted hydroxyapatite. *J Colloid Interface Sci*. 2016;482:112-20.
 20. Heinemann S, Heinemann C, Wenisch S, Alt V, Worch H, Hanke T. Calcium phosphate phases integrated in silica/collagen nanocomposite xerogels enhance the bioactivity and ultimately manipulate the osteoblast/osteoclast ratio in a human co-culture model. *Acta Biomater*. 2013;9(1):4878-88.

21. Song X, Ding T, Yao L, Lin M, Li Y, Wu C, et al. On the origin and underappreciated effects of ion doping in silica. *Small*. 2015;11(34):4351-65.
22. Giuliani N, Morandi F, Tagliaferri S, Lazzaretti M, Bonomini S, Crugnola M, et al. The proteasome inhibitor bortezomib affects osteoblast differentiation in vitro and in vivo in multiple myeloma patients. *Blood*. 2007;110(1):334-8.
23. Qiang YW, Hu B, Chen Y, Zhong Y, Shi B, Barlogie B, et al. Bortezomib induces osteoblast differentiation via Wnt-independent activation of beta-catenin/TCF signaling. *Blood*. 2009;113(18):4319-30.
24. Sonneveld P, Goldschmidt H, Rosinol L, Bladé J, Lahuerta JJ, Cavo M, et al. Bortezomib-based versus nonbortezomib-based induction treatment before autologous stem-cell transplantation in patients with previously untreated multiple myeloma: a meta-analysis of phase III randomized, controlled trials. *J Clin Oncol*. 2013;31(26):3279-87.
25. Kruppke B, Hose D, Schnettler R, Heinemann S, Ray S, Hanke T, et al. Drug release as a function of bioactivity, incubation regime, liquid, and initial load: release of bortezomib from calcium phosphate-containing silica/collagen xerogels. *J Biomed Mater Res B Appl Biomater*. 2018;106(3):1165-73.
26. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*. 2014;15(12):e538-48.
27. Hose D, Rème T, Meissner T, Moreaux J, Seckinger A, Kuehl A, et al. Inhibition of aurora kinases for tailored risk-adapted treatment of multiple myeloma. *Blood*. 2009;113(18):4331-40.
28. Seckinger A, Meissner T, Moreaux J, Goldschmidt H, Fuhler GM, Benner A, et al. Bone morphogenic protein 6: a member of a novel class of prognostic factors expressed by normal and malignant plasma cells inhibiting proliferation and angiogenesis. *Oncogene*. 2009;28(44):3866-79.
29. Radl J, De Groot E, Schuit HR, Zurcher C. Idiopathic paraproteinemia II. Transplantation of the paraprotein-producing clone from old to young C57BL/KaLwRij mice. *J Immunol*. 1979;122(2):609-13.
30. Vanderkerken K, De Raeve H, Goes E, Van Meirvenne S, Radl J, Van Riet I, et al. Organ involvement and phenotypic adhesion profile of 5T2 and 5T33 myeloma cells in the C57BL/KaLwRij mouse. *Br J Cancer*. 1997;76(4):451-60.
31. Clemens J, Longo M, Seckinger A, Weisel KC, Driessen C, Schmidt EM, et al. Stability of the proteasome inhibitor bortezomib in cell based assays determined by ultra-high performance liquid chromatography coupled to tandem mass spectrometry. *J Chromatogr A*. 2014;1345:128-38.
32. US Food and Drug Administration (FDA). Bioanalytical Method Validation Guidance for Industry [Internet]. [cited 2026 Aug 14]. Available from: <https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf>
33. European Medicines Agency (EMA), Committee for Medicinal Products for Human Use. Guideline on Bioanalytical Method Validation [Internet]. [cited 2026 Aug 14]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-bioanalytical-method-validation_en.pdf
34. Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R. Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *J Bone Miner Res*. 2010;25(7):1468-86.
35. Morgan EF, Mason ZD, Chien KB, Pfeiffer AJ, Barnes GL, Einhorn TA, et al. Micro-computed tomography assessment of fracture healing: relationships among callus structure, composition, and mechanical function. *Bone*. 2009;44(2):335-44.
36. Nyman JS, Munoz S, Jadhav S, Shepherd A, Bose A, Majumdar S, et al. Quantitative measures of femoral fracture repair in rats derived by micro-computed tomography. *J Biomech*. 2009;42(7):891-7.
37. Kern C, Kern S, Henss A, Rohnke M. Secondary ion mass spectrometry for bone research. *Biointerphases*. 2023;18(4).
38. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol*. 2004;5(10):R80.
39. Hose D, Beck S, Salwender H, Dürig J, Weisel K, Scheid C, et al. Prospective target assessment and multimodal prediction of survival for personalized and risk-adapted treatment strategies in multiple myeloma in the GMMG-MM5 multicenter trial. *J Hematol Oncol*. 2019;12(1):65.

40. Mestres G, Le Van C, Ginebra MP. Silicon-stabilized α -tricalcium phosphate and its use in a calcium phosphate cement: characterization and cell response. *Acta Biomater.* 2012;8(3):1169-79.
41. Ke D, Tarafder S, Vahabzadeh S, Bose S. Effects of MgO, ZnO, SrO, and SiO₂ in tricalcium phosphate scaffolds on in vitro gene expression and in vivo osteogenesis. *Mater Sci Eng C Mater Biol Appl.* 2019;96:10-9.
42. Bunpetch V, Zhang X, Li T, Lin J, Kuo YE, Wu H, et al. Silicate-based bioceramic scaffolds for dual-lineage regeneration of osteochondral defect. *Biomaterials.* 2019;192:323-33.
43. Wu C, Chang J. Multifunctional mesoporous bioactive glasses for effective delivery of therapeutic ions and drug/growth factors. *J Control Release.* 2014;193:282-95.
44. Wang Y, Zhao Q, Han N, Bai L, Li J, Liu J, et al. Mesoporous silica nanoparticles in drug delivery and biomedical applications. *Nanomedicine.* 2015;11(2):313-27.
45. Pennisi A, Li X, Ling W, Khan S, Zangari M, Yaccoby S. The proteasome inhibitor, bortezomib suppresses primary myeloma and stimulates bone formation in myelomatous and nonmyelomatous bones in vivo. *Am J Hematol.* 2009;84(1):6-14.
46. Oyajobi BO, Garrett IR, Gupta A, Flores A, Suva LJ, Barlogie B, et al. Stimulation of new bone formation by the proteasome inhibitor, bortezomib: implications for myeloma bone disease. *Br J Haematol.* 2007;139(3):434-8.
47. Zangari M, Terpos E, Zhan F, Tricot G. Impact of bortezomib on bone health in myeloma: a review of current evidence. *Cancer Treat Rev.* 2012;38(8):968-80.
48. Lund T, Soe K, Abildgaard N, Bødker JS, Møller MB, Abildgaard N, et al. First-line treatment with bortezomib rapidly stimulates both osteoblast activity and bone matrix deposition in patients with multiple myeloma, and stimulates osteoblast proliferation and differentiation in vitro. *Eur J Haematol.* 2010;85(4):290-9.
49. von Metzler I, Krebbel H, Hecht M, Manz RA, Fleissner C, Zustin J, et al. Bortezomib inhibits human osteoclastogenesis. *Leukemia.* 2007;21(9):2025-34.
50. Chen J, Wen J, Fu Y, Li S, Zhang Y, Wang S, et al. A bifunctional bortezomib-loaded porous nano-hydroxyapatite/alginate scaffold for simultaneous tumor inhibition and bone regeneration. *J Nanobiotechnol.* 2023;21(1):174.
51. Ren E, Ren W, Collins AC, Robinson A, Vaidya R. Chemotherapy release from bortezomib-impregnated polymethylmethacrylate-coated intramedullary nails: a novel in vitro study for a local chemotherapy delivery device. *Cureus.* 2024;16(7):e64181.
52. Andersen TL, Sondergaard TE, Skorzynska KE, Dagnaes-Hansen F, Plesner T, Hauge EM, et al. A physical mechanism for coupling bone resorption and formation in adult human bone. *Am J Pathol.* 2009;174(1):239-47.
53. Klein B, Seckinger A, Moehler T, Hose D. Molecular pathogenesis of multiple myeloma: chromosomal aberrations, changes in gene expression, cytokine networks, and the bone marrow microenvironment. *Recent Results Cancer Res.* 2011;183:39-86.
54. Hose D, Moreaux J, Meissner T, Seckinger A, Goldschmidt H, Benner A, et al. Induction of angiogenesis by normal and malignant plasma cells. *Blood.* 2009;114(1):128-43.
55. Moreaux J, Hose D, Kassambara A, Jourdan M, Shaughnessy JD Jr, Barlogie B, et al. Osteoclast-gene expression profiling reveals osteoclast-derived CCR2-chemokines promoting myeloma cell migration. *Blood.* 2011;117:1280-90.
56. Clemens J, Seckinger A, Hose D, Kunz C, Witzens-Harig M, Pschowski M, et al. Cellular uptake kinetics of bortezomib in relation to efficacy in myeloma cells and the influence of drug transporters. *Cancer Chemother Pharmacol.* 2015;75(2):281-91.
57. Ri M, Iida S, Nakashima T, Miyazaki K, Mori A, Ito A, et al. Bortezomib-resistant myeloma cell lines: a role for mutated PSMB5 in preventing the accumulation of unfolded proteins and fatal ER stress. *Leukemia.* 2010;24(8):1506-12.