Editorial

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# *Ex Vivo* Models: Transforming Standards in Preclinical Bone Regeneration Research

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With musculoskeletal disorders, such as osteoporosis, arthritis, fractures, and bone cancers affecting millions of people globally, research aimed at uncovering the molecular, cellular, and biomechanical aspects of bone is essential [1]. These conditions not only impair mobility and quality of life but also impose a substantial socioeconomic burden on healthcare systems. Advancing bone research is therefore crucial for uncovering the mechanisms that govern bone development, maintenance, degeneration, and repair, ultimately enabling the discovery of effective diagnostic tools, therapeutic strategies, and preventive measures.

Conventional bone research has relied heavily on two primary experimental approaches: *in vitro* cell culture systems and *in vivo* animal models. Two-dimensional cell culture, while offering high throughput and precise experimental control, fails to recapitulate the complex three-dimensional architecture, cellular heterogeneity, and biomechanical microenvironment characteristic of native bone tissue [2]. Cells cultured on plastic surfaces exhibit altered phenotypes, loss of tissue-specific functions, and responses that often poorly predict *in vivo* outcomes [3,4]. Experimental animal models, particularly those involving rodents and large animals, provide physiologically relevant environments but are associated with ethical concerns, high costs, lengthy experimental timelines, and species-specific differences that may limit their translational relevance [5]. The gap between *in vitro* simplicity and *in vivo* complexity has created a critical need for intermediate models that better mimic the native bone microenvironment while maintaining experimental accessibility.

## *Ex Vivo* Bone Models

*Ex vivo* bone models have emerged as a powerful platform that bridges the gap between traditional *in vitro* and *in vivo* approaches [6]. These systems employ intact bone tissue maintained under controlled culture conditions, thereby preserving native cellular diversity, extracellular matrix composition, three-dimensional architecture, and cell-cell interactions that are crucial for physiological bone function [6,7]. By maintaining bone tissue viability outside the body under controlled conditions, researchers can conduct detailed mechanistic studies, test therapeutic interventions, and evaluate biomaterial-tissue interactions in a more physiologically relevant context than conventional cell culture [7]. The development of perfusion technologies and culture optimization strategies has significantly enhanced the viability and functional maintenance of *ex vivo* bone cultures, extending culture periods from hours to weeks and enabling longitudinal studies of bone biology [8,9]. These advances have positioned *ex vivo* bone models as valuable tools for studying bone development, disease mechanisms, drug screening, and tissue engineering applications.

*Ex vivo* bone models offer an advance in preclinical bone research because they address many of the ethical, logistical, and scientific limitations inherent in traditional animal experimentation pathways [7]. One of their defining advantages is the significant reduction of systemic factors found in whole-animal studies, such as circulating hormones, immune system modulation, and organ-organ crosstalk, which can confound results and obscure the direct influence of mechanical and biological interventions on bone tissue itself [10,11]. By providing a controlled microenvironment, *ex vivo* systems enable researchers to independently manipulate variables such as oxygen tension, nutrient diffusion, perfusion rates, and mechanical loading [8,12]. This refined control leads to clearer attributions of cause and effect, facilitating the detailed study of cellular signaling, mechanotransduction, and biomaterial-bone interactions without interference from systemic physiology.

Replacement is a critical mandate of the 3Rs (Replacement, Reduction, and Refinement), and *ex vivo* models directly address this by substituting live animal testing with isolated, perfused, and mechanically stimulated bone tissues [13]. Perfused *ex vivo* bone explants emulate the dynamic conditions of living bone, maintaining cellular viability and function over extended culture periods, which enables studies that previously would have required large numbers of live animals [8]. Advanced bioreactor systems provide further refinement by replicating natural bone stimuli, including cyclic mechanical loading and pulsatile fluid shear, under fully sterile and controlled conditions [8,14]. Reduction, the second mandate of the 3Rs, is achieved when researchers proactively use bones from animals that have already been euthanized for other Institu-

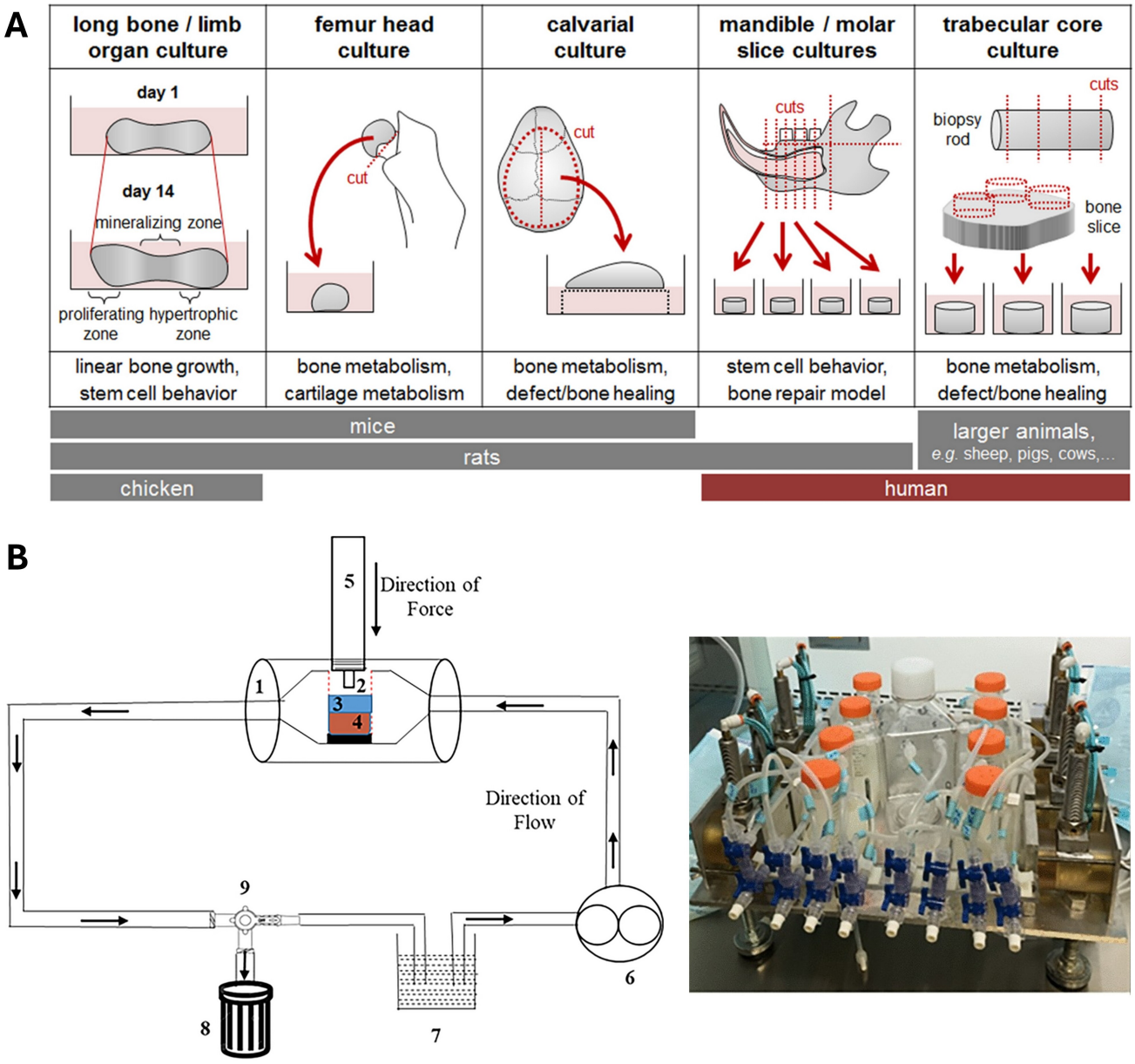


**Fig. 1. *Ex vivo* bone culture systems.** (A) Some of the commonly used *ex vivo* bone culture systems. Reproduced from [22] under the Creative Commons Attribution 4.0 International License (©Ehnert *et al*. 2020). (B) Schematic representation of a complete specimen chamber within the *ex vivo* bioreactor system. (1) Specimen chamber; (2) Polycarbonate holder; (3) Fiber mesh metallic scaffold; (4) Bone core; (5) Pneumatic actuator; (6) Peristaltic pump; (7) Media container; (8) Waste container; (9) Three-way valve. Reproduced from [12] under the Creative Commons Attribution 4.0 International License (©Dua *et al*. 2021).

tional Animal Care and Use Committee (IACUC) approved studies [13,15]. Rather than discarding animal carcasses after primary experiments, implementing a well-coordinated program for harvesting these tissues promptly post-mortem can maximize the scientific value of each animal, supporting multiple experiments from a single experimental animal and reducing the overall number of animals needed annually for bone research.

In bone biomaterials research alone, where material and implant evaluation have historically required large numbers of animal surgeries and sacrificial endpoints for biomechanical, histological, and imaging analysis, this approach can positively impact welfare, cost, and throughput [2,16]. Current estimates suggest that hundreds to thousands of animals per year may be required at the institutional or national level for preclinical bone studies, particularly when multiple biomaterials and time points need to be evaluated [2,17]. By integrating *ex vivo* bone explant models into the research pipeline, especially those sourced from secondary-use tissues, the scientific community can minimize animal usage while increasing experimental control and reproducibility.

## Fundamental Principles of *Ex Vivo* Bone Culture

Successful *ex vivo* bone culture requires careful consideration of multiple factors that collectively maintain tissue viability and function [6]. Nutrient and oxygen supply are critical, as bone tissue contains a dense network of cells embedded within a mineralized matrix that limits diffusion distances [18]. Native bone receives nutrients through a sophisticated vascular network; in *ex vivo* systems, perfusion-based delivery is essential to overcome diffusion limitations and prevent the development of necrotic cores [8,19]. Mechanical stimulation plays a crucial role in maintaining the phenotype and function of bone cells [20]. Osteocytes, the most abundant cells in mature bone, function as mechanosensors that respond to mechanical loading by producing signaling molecules that regulate osteoblast and osteoclast activity [20,21]. *Ex vivo* systems that incorporate physiologically relevant mechanical stimuli better preserve the characteristics of bone tissue compared to static culture conditions. Some of the commonly used *ex vivo* bone culture systems are summarized in Fig. 1A (Ref. [22]).

Appropriate culture medium composition is also essential, typically including basal media supplemented with fetal bovine serum or alternatives, calcium and phosphate ions, vitamins, and hormones that support bone cell metabolism [6]. The oxygen tension of the culture environment significantly impacts bone cell behavior, as bone marrow naturally exists under hypoxic conditions, while the bone surface experiences higher oxygen levels [23]. Controlling oxygen tension to mimic physiological gradients can enhance the maintenance of stem cell populations and osteogenic differentiation [24].

## *Ex Vivo* Bone Culture Systems and Technologies

Bone explant culture represents the most direct approach to *ex vivo* bone modelling, utilizing intact fragments of bone tissue maintained under controlled culture conditions [10]. These models preserve the native cellular architecture, extracellular matrix composition, and three-dimensional organization of bone tissue. Calvarial bone explants, harvested from the flat bones of the skull, are widely used due to their accessibility and relatively uniform thickness, which facilitates the diffusion of nutrients [7,10]. These explants have proven particularly valuable for studying intramembranous bone formation, osteoblast differentiation, and responses to hormonal and pharmacological agents [6].

Bioreactor technology has revolutionized *ex vivo* bone culture by providing controlled perfusion of culture medium through bone tissue, mimicking the nutrient and oxygen delivery provided by the vascular system *in vivo* [9,14]. Perfusion bioreactors overcome the diffusion limitations inherent in static culture, enabling extended culture periods and maintaining cellular viability in larger tissue constructs. Direct perfusion bioreactors force culture medium through the natural porosity of bone tissue, utilizing the interconnected canalicular network that normally houses osteocytes [25]. This approach provides efficient nutrient delivery directly to cells embedded within the mineralized matrix. Studies have shown that direct perfusion maintains bone viability for over four weeks, which is significantly longer than in static culture conditions [9,25]. Indirect perfusion systems circulate medium around bone explants without forcing flow through the tissue, relying on enhanced convection and reduced boundary layer effects to improve mass transport [25]. While less efficient than direct perfusion, these systems are simpler to implement and reduce the risk of mechanical damage to delicate tissue structures. Dynamic mechanical loading bioreactors combine perfusion with cyclic mechanical compression or fluid shear stress, recapitulating the mechanical environment experienced by bone tissue *in vivo* [14]. These systems have demonstrated superior maintenance of bone cell phenotype and enhanced osteogenic responses compared to perfusion alone. A schematic representation of the *ex vivo* bioreactor system and its components is shown in Fig. 1B (Ref. [12]).

## Key Considerations and Challenges

The oxygen microenvironment has a significant influence on bone cell behavior, with different regions of bone tissue experiencing distinct oxygen tensions *in vivo*. Replicating these physiological oxygen gradients in *ex vivo* systems presents both challenges and opportunities for enhancing the relevance of the model. Nutrient diffusion limitations represent another fundamental challenge in *ex vivo* bone culture, particularly for thicker tissue constructs [26]. The dense, mineralized nature of bone matrix restricts diffusion distances, necessitating active perfusion to maintain cellular viability in deep tissues [8,25]. Computational modelling approaches can be employed to predict oxygen and nutrient gradients within cultured bone tissue, informing bioreactor design and optimization strategies [27].

Despite significant advances, *ex vivo* bone culture systems face numerous technical challenges that limit their widespread adoption and long-term viability. Maintaining sterility during extended culture periods is critical but challenging, particularly for perfusion systems with multiple fluid connections and mechanical components. Contamination can lead to experimental failure and presents safety concerns when using tissue samples. Tissue degradation over time represents a fundamental limitation, as even optimal culture conditions cannot perfectly replicate the *in vivo* environment [28]. The balance between tissue maintenance and experimental duration must be carefully considered [29]. Complexity and cost of sophisticated biore-

actor systems may limit accessibility, particularly for laboratories without specialized equipment or expertise. The need for custom-designed bioreactors, precise environmental control, and continuous monitoring adds significant expense compared to conventional culture approaches. However, these costs must be weighed against the expense and ethical concerns of *in vivo* studies.

## Summary

While traditional *in vitro* cell culture systems have provided valuable insights, they suffer from limitations in recapitulating the complex three-dimensional architecture and physiological microenvironment of native bone tissue. *Ex vivo* bone models represent a powerful and increasingly sophisticated platform for bone tissue engineering research, bridging the gap between simplified *in vitro* systems and complex *in vivo* animal models. These systems preserve native tissue architecture, cellular diversity, and extracellular matrix composition while enabling precise experimental control and detailed mechanistic investigations. The applications of *ex vivo* bone models span a broad range of research areas, including biomaterial evaluation, cell-based therapy development, growth factor delivery optimization, mechanobiology studies, drug screening, and disease modelling. These systems have provided valuable insights into osteocyte mechanotransduction, osteoblast-osteoclast coupling, vascularization strategies, immune-inflammatory modulation, and the complex interactions between bone cells and their microenvironment. Despite significant progress, challenges persist in maintaining long-term tissue viability, achieving standardization across laboratories, translating findings into clinical applications, and meeting regulatory requirements for tissue-engineered products. As standardization efforts progress and regulatory frameworks mature, *ex vivo* bone models are expected to play an increasingly important role in the clinical translation of bone tissue engineering innovations.

## Availability of Data and Materials

Not applicable.

## Author Contributions

KS, SAB, TEK and CPP conceived this study. KS, SAB, TEK and CPP were involved in the drafting and critical revision of the manuscript. All authors have read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

## Ethics Approval and Consent to Participate

Not applicable.

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## Conflict of Interest

The authors declare no conflict of interest.

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