International Journal of Emerging Engineering and Technology (IJEET)
ISSN (e): 2958-3764
Volume: 4, Number: 1, Pages: 11 - 20, Year: 2025

Mini-review: Experimental Approaches for the Biomechanical Testing of Bone

Ioana-Cristina Băncilă [1]
[1] Biomedical Engineering Master Student, Vienna University of Technology (TU Wien), Getreidemarkt 9, 1060 Vienna, Austria,
*Corresponding Author: Ioana-Cristina Băncilă (Email: Email: e12102406@student.tuwien.ac.at)

Received: 12-01-2025 Revised: 20-05-2025 Accepted: 15-06-2025

Abstract— **Mechanical testing of bone biomechanics is crucial for assessing bone strength, quality, and fracture risk. This mini-review summarizes recent advances from nanoindentation to whole-bone compression, highlighting techniques such as in situ micro-CT with digital volume correlation, finite element modelling from clinical imaging, and biomechanical sensors. Despite progress, challenges in standardization, scalability, and clinical translation persist. Future directions focus on multiscale testing, machine learning integration, and interdisciplinary approaches to improve patient-specific diagnosis and treatment. Combining experimental, computational, and clinical methods is essential for advancing bone health research.**

Keywords— Biomechanics, bone, nanoscale, experimental.

I. Introduction

Bone biomechanical testing is of prime clinical relevance because it directly informs diagnosis, treatment planning, and monitoring of skeletal disorders. Accurate determination of bone strength and risk of fracture is necessary for the prevention of disabling injury in the elderly and in systemic diseases of bone quality. Technological advancements in biomechanical testing make it possible for clinicians to look beyond simple measurement of bone mineral density to a more holistic view of bone quality, microarchitecture, and mechanical competence.[1][2]

Despite such progress, certain scientific voids remain in the field. Current clinical devices are incapable of being sensitive enough to capture the multiscale mechanical intricacy of bone tissue at any scale ranging from nanoscale collagen fibrils to macroscopic load-bearing structures. High-resolution imaging integration with computational models' use in routine clinical practice is still challenging due to computational resource constraints and the absence of standardization. There is a compelling need for improved experimental techniques that can effectively measure bone mechanical properties in vivo and for computational models that integrate multiscale data to make robust predictions of fracture risk.[3]

Furthermore, clarification of mechanobiological pathways wherein bone responds to varying physiological and pathological stimuli with adaptation or degradation remains incomplete. Multidisciplinary research combining biomechanical testing, imaging, molecular biology, and materials science is necessary to fill this gap. Solving these matters will not only enhance the quality of patient care but also accelerate the development of biomimetic materials and tissue-engineered constructs for bone regeneration.[4]

The aim of the current review is to highlight recent developments in bone biomechanical testing, to present current limitations, and to suggest directions for research to fill the significant gaps.

II. Types of Experimental Testing of Bone

Experimental testing of bone is required to measure its mechanical properties, understand its structural response, and validate computational models. The complex nature of bone tissue, with its hierarchical structure across the nanoscale to macroscale, requires multiple testing approaches. These include tests broadly classified below:

2.1. Macroscopic Mechanical Testing

2.1.1. Compression Testing-Tests the response of bone to compressive loads, simulating weight-bearing situations. Conducted in whole bones (e.g., femur, vertebra) or sections of bones. The parameters are ultimate compressive strength, stiffness, and strain to failure [5].

A. Tensile Testing-Tests the reaction of bone to tensile loads, displaying its tensile strength and elastic modulus. Typically done on cortical bone samples since they possess higher tensile capacity [6].

B. Three-Point and Four-Point Bending Tests: These tests apply bending loads to bone samples to determine flexural strength, bending stiffness, and fracture toughness. These simulate the bending stresses found in everyday activities [6],[7].

C. Shear Testing

Test bone shear resistance, which is significant in trabecular bone failure patterns [7].

2.1.2. Microscale and Nanoscale Testing

A. Nanoindentation captures local mechanical properties such as hardness and elastic modulus at the nanoscale to microscale by indenting the bone surface using a sharp probe. Facilitates characterization of single bone structures, including osteons or trabeculae [6],[7].

B. Atomic Force Microscopy (AFM)-Used in probing mechanical properties at the nanoscale, for example, collagen fibril stiffness and mineralized matrix, with high spatial resolution [6].

C. Micromechanical Testing-Micro-compression and micro-tension of single trabeculae or bone specimens, and hence obtaining insight into the mechanical heterogeneity of bone microstructure [7].

2.1.3. Dynamic and Fatigue Testing

A. Cyclic Loading Tests-Simulate cyclic physiological loading to study the bone's fatigue response and damage accumulation, of particular interest for understanding stress fractures [7].

B. Impact Testing-Tests bone resistance to impacts or high-rate loads, of paramount importance in trauma cases [8].

2.1.4. In Situ Mechanical Testing with Imaging

A. Micro-Computed Tomography (micro-CT) Coupled Testing-Compared with high-resolution imaging, it is subjected to mechanical loading to observe deformation, nucleation, and growth of micro-cracks in real time [8].

B. Digital Volume Correlation (DVC)-An algorithm-based method applied to sequential 3D images on loading to quantify local strains within the bone volume [8].

Each method provides individual information, and application of more than one method allows for integrated characterization of bone biomechanics. Technique choice depends on the research question, specimen dimension, and required resolution generation [4].

This review aims to highlight recent advances in biomechanical bone testing, delineate present limits still existing, and map out future directions to address these critical gaps.

Table 2 presents a comprehensive overview of various experimental mechanical testing approaches for bone, highlighting their specific applications, advantages, and the nature of the testing methods. The table categorizes testing into five distinct types: static mechanical testing, dynamic mechanical testing, finite element analysis (FEA), micro-CT scanning, and in vivo testing. Each category is detailed with its respective applications, such as evaluating compressive strength or simulating physiological loading conditions, while also outlining the key benefits, like providing baseline data or capturing real-world relevance. This structured summary serves as a valuable reference for researchers and clinicians, facilitating a better understanding of the methodologies employed in assessing bone mechanics and their implications for both clinical and experimental studies.

.

III. Recent Advances in Bone Mechanical Testing

The more recent advances in bone biomechanics have generally proceeded to improve the precision and resolution of test protocols, especially through the addition of imaging methods, micro-scale mechanical tests, and computational models. These advances aim to more accurately characterize bone tissue's hierarchical, heterogeneous, and time-dependent nature.

A notable development is the combination of in situ mechanical testing with micro-computed tomography (micro-CT). Such a method enables real-time observation of bone deformation and damage under load, and with high-resolution 3D images that can be interpreted using digital volume correlation (DVC). Müller et al. [9] used time-lapse micro-CT to measure bone formation and mechanical adaptation in tissue-engineered constructs, demonstrating how maps of volumetric strain can predict bone apposition and resorption areas with high spatial resolution.

At the same time, nanoindentation and micro-compression testing have gained popularity for quantifying local mechanical properties of bone at the micro-scale. These tests are most useful in quantifying changes in bone quality due to aging, disease, or intervention. For instance, nanoindentation experiments have demonstrated reduced hardness and modulus in osteoporotic bone relative to controls [10] and shed light on material-level changes that can precede macroscopic failure.

Biomechanical CT (BCT) advancements represent an important clinical advance. Keaveny et al. [11] validated the use of BCT for predicting fracture risk in metastatic prostate cancer patients using finite element analysis (FEA) models from standard CT scans. The technology provides an active, patient-specific bone strength estimate that has the potential to improve oncology and osteoporosis clinical decision-making [12].

Additionally, micromechanical testing of individual trabeculae and cortical bone beams, studied by scientists like P. Thurner's group at TU Wien, enables detailed exploration of damage mechanisms, anisotropy, and fatigue behaviour [13]. Microscale tests offer support for the building of multiscale models, including material and structural properties.

Recent research also encompasses the combination of mechanical loading with biological assays, connecting molecular response and biomechanics. Recent evidence suggests that local mechanical strain impacts osteocyte signalling and gene expression, revealing a complex interaction between mechanical stimuli and bone cell behaviour.

While these are positive trends, application to bedside practice is hampered by persisting difficulties. Current

protocols are often device-specific, and standardization across studies is weak. However, advances in imaging resolution, automation, and data analysis are rapidly closing this gap.

IV. Discussion

A contrast of recent studies in experimental bone mechanical testing reveals convergence on methodological paths and divergence on scope, scale, and clinical utility. Central to the discipline as a whole is the attempt to synthesize macroscale mechanical testing with microscale and nanoscale data to encompass the full biomechanical complexity of bone tissue.

For instance, Müller et al. (2023) used in situ micro-CT with digital volume correlation to assess localized strain patterns at bone formation within engineered scaffolds. Their volume measurement provides spatially resolved insights into the mechanical regulation of mineralization and is of particular interest for tissue engineering and regenerative medicine [9]. On the other hand, Keaveny et al. (2023) were more clinical in approach, using CT-based finite element analysis (BCT) to predict fracture risk in patients with metastatic cancer [11]. Whereas Müller's method offers precise mechanistic insight into the micro-scale, Keaveny's approach emphasizes clinical translational value and patient-specific diagnosis.

Similarly, the results of nanoindentation by multiple groups (including Müller's lab and others) are consistently lower elastic modulus and hardness in osteoporotic or diabetic bone compared to healthy tissue [10]. The numerical values and meaning are a function of the bone region tested (e.g., cortical versus trabecular), hydration state, and indentation depth. This indicates the fact that nanoscale tests are sensitive to the immediate microstructural environment and shows the need for standardized protocols.

The study by Frank, Fischer, and Thurner (2021) provides valuable insights into how microdamage accumulates in individual bovine trabeculae under cyclic loading. By isolating single trabeculae and subjecting them to fatigue testing, the authors were able to precisely monitor the progression of structural damage at the micro-scale. Their findings demonstrate that microcracks develop gradually and correlate with the trabecula's initial mechanical properties, offering a deeper understanding of early failure mechanisms in cancellous bone. This work underscores the importance of intrinsic bone quality in fragility and contributes significantly to the development of more targeted approaches for assessing and preventing osteoporotic fractures [13].

Of special interest is that most current research increasingly combines mechanical measurements with imaging or biological parameters, such as strain-evoked changes in osteocyte activity. This is one aspect of a broader shift toward mechanobiology and systems-level understanding of bone as a responsive living tissue [14].

Together, these studies show that while experimental platforms differ in resolution and aim, they are complementary, not redundant, each contributing complementary information regarding the structure-function relationship of bone. Future studies could be propelled by hybrid models where multiple test modalities are integrated into coherent frameworks for both research and clinical application.

V. Limitations and Future Perspectives

A. Scalability of testing methods

Despite the remarkable progress in experimental bone mechanical testing, some fundamental limitations remain regarding clinical translation. One of the main constraints is the *scalability* of testing methods. While nanoscale and microscale techniques such as nanoindentation and micro-compression provide high-resolution data at the tissue level, they fail to capture the global mechanical behavior of entire bones and interactions between structural compartments [17],[18]. On the other hand, macroscopic experiments include anatomical geometry but often overlook local gradients in material properties.

B. Standardization and Reproducibility

A major challenge is the *lack of standardized protocols* and poor reproducibility across studies. Differences in sample preparation, hydration status, loading schemes, and imaging conditions introduce variability that hampers inter-study comparisons. These inconsistencies limit the ability to perform reliable meta-analyses and reduce the clinical relevance of findings [18].

C. Accessibility of Equipment

Many biomechanical testing setups involve *advanced and expensive equipment*, such as micro-CT systems integrated with in situ mechanical testing stages. This type of infrastructure is not readily available in most clinical environments, particularly in resource-limited institutions [2],[9].

D. Artifacts in Cancellous Bone Testing

In the specific case of cancellous bone, *side artifacts* created during specimen preparation disrupt the trabecular network and cause systematic underestimation of mechanical properties. These artifacts are inherent to how specimens are sectioned and constrained [23],[22].

E. Integration into Routine Clinical Practice

From a clinical standpoint, integrating biomechanical testing into routine diagnostic workflows remains a *significant hurdle*. Computational models like finite element analysis (FEA) show promise but require large datasets for calibration and validation—datasets that are not always readily available. Moreover, the biological complexity of bone, including remodelling cycles and cellular mechanotransduction, necessitates multiscale approaches that span biomechanics, molecular biology, and materials science [3],[5].

F. Need for Real-Time Monitoring

A future-oriented solution lies in the *development of implantable biomechanical sensors*, capable of providing real-time data on strain and loading conditions in vivo. Such devices would enable continuous, patient-specific monitoring during daily activities and rehabilitation. However, challenges related to sensor miniaturization, biocompatibility, data integration, and energy efficiency remain unresolved [15],[16].

G. Interdisciplinary Collaboration

The future of bone biomechanical testing is *multiscale and integrative*, combining mechanical experimentation, biological insights, and advanced computational modelling. The convergence of high-resolution imaging, in situ mechanical testing, and machine learning/artificial intelligence holds potential for improving predictions of treatment outcomes and bone fragility risk. Besides, tissue engineering and 3D bioprinting have the pote ntial to generate tunable and customizable bone constructs with tunable mechanical properties that could be optimized and tested with these experimental methods [19]. Standard protocol development and data repositories will be important to provide reproducibility and speed up innovation.

H. Variability in Test Results:

In the absence of standardized testing protocols, biomechanical test results can vary considerably between different research studies and clinical environments. This variability complicates the comparison of findings and may lead to inconsistent evaluations of bone strength and fracture risk. For example, if one laboratory employs a specific technique to measure bone density while another uses a different method, the resulting data may not be directly comparable. Such discrepancies can contribute to misdiagnoses or inappropriate treatment decisions. [11]

I. Inconsistent Treatment Protocols:

Differences in testing methodologies can lead to varied treatment recommendations for patients with similar clinical profiles. When clinicians rely on non-standardized assessments, the quality of care may depend more on the testing facility than on the patient's actual condition. This can result in some individuals receiving unnecessary treatments, while others may be undertreated, ultimately affecting overall patient outcomes. [12]

J. Challenges in Clinical Decision-Making:

Biomechanical testing data plays a critical role in guiding clinical decision-making. However, the absence of standardization introduces uncertainty in result interpretation, making it harder for physicians to act with confidence. For instance, if a test suggests an elevated fracture risk but lacks methodological consistency, clinicians may be reluctant to rely on it, potentially delaying preventive interventions and increasing fracture risk. [2]

K. Impacts on Research and Development:

The lack of consistency in testing procedures poses a barrier to the advancement of new therapeutic approaches and technologies. When research outcomes are not broadly comparable due to methodological variability, it becomes challenging to develop validated protocols for clinical translation. This limitation can impede progress in the field of bone health and delay innovations that could improve patient care. [3]

L. Patient Safety and Quality of Care:

Ultimately, the absence of standardized testing undermines patient safety and the overall quality of care. Patients may face delayed diagnoses, receive inappropriate therapies, or experience adverse outcomes due to unreliable biomechanical evaluations. Implementing standardized testing protocols would improve result accuracy and consistency, supporting more precise diagnoses and effective treatment strategies. [2]

Interdisciplinary efforts by engineers, biologists, clinicians, and data scientists will be required to overcome current limitations and realize the full potential of biomechanical testing for bone health, prevention of disease, and regenerative therapies.

VI. .CONCLUSION

Experimental mechanical testing of bone has progressed significantly, offering vital insights into its multiscale architecture and complex biomechanics. Both nanoscale and macroscale approaches have unique strengths, yet their combined application remains essential for capturing the hierarchical properties of bone tissue. Recent breakthroughs—such as *in situ* imaging, computational modelling, and sensor technologies—have enhanced fracture risk prediction and supported the development of effective regenerative therapies [20], [21], [22].

However, challenges related to standardization, accessibility, and clinical translation persist. Addressing these issues requires sustained interdisciplinary collaboration, the construction of robust multiscale frameworks, and the integration of emerging technologies such as artificial intelligence and bioprinting [20], [21], [22].

By merging experimental rigor with computational, clinical, and engineering perspectives, future research will lay the foundation for personalized bone health management and the development of novel therapeutic strategies. Interdisciplinarity is no longer a mere aspiration—it is a fundamental requirement for scientific advancement and clinical relevance in the fields of tissue engineering and bone biomechanics [20],[21], [22]

ACKNOWLEDGMENT

The author does not have any conflicts of interest. The tables are created by the author using the Excel programme. Text formulation assistance and literature reference identification were supported by Claude (Anthropic, 2024) and ChatGPT (OpenAI, 2024). All scientific content, critical analysis, and final interpretations were independently developed and verified by the author. The diagrams created by the author using the AI-based tools under the supervision and guidance of the author are only illustrative and for educational purposes..

FUNDING STATEMENT

The author(s) received no specific funding for this study.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest to report regarding the present study.

REFERENCES

[1] Roux J-P, Burt-Pichat B, Linossier MT, Vilayphiou N, Winzenrieth R, Chapurlat R. The relationship between bone strain index, bone mass, microarchitecture and mechanical behavior in human vertebrae: an ex vivo study. *Osteoporos Int*. 2024;35(5):959–968. doi:10.1007/s00198-024-07066-9.

[2] Díaz Curiel M, Pérez Castrillón JL. New technologies in the evaluation of bone fragility and their application in endocrinology. *Endocrinol Diabetes Nutr (Engl Ed)*. 2023;70(1):32–40. doi:10.1016/j.endien.2022.07.007.

[3] Lacroix D, Smit TH. Multiscale modelling of bone tissue mechanobiology: a review. *J Biomech*. 2021;115:110138. doi:10.1016/j.jbiomech.2020.110138.

[4] Doube M. Advancements in subchondral bone biomechanics: Insights from computed tomography and micro-computed tomography imaging in equine models. *Curr Osteoporos Rep*. 2024;22(1):10–20. doi:10.1007/s11914-024-00886-y.

[5] Garavelli C, Aldieri A, Palanca M, Dall'Ara E, Viceconti M. Validation of homogenized finite element models of human metastatic vertebrae using digital volume correlation. *arXiv*. 2024. https://arxiv.org/abs/2402.09828.

[6] Carnelli D, Lucchini R, Ponzoni M, Contro R, Vena P. Nanoindentation testing and finite element simulations of cortical bone allowing for anisotropic elastic and inelastic mechanical response. *J Biomech*. 2011;44(10):1852–1858. doi:10.1016/j.jbiomech.2011.04.020.

[7] Meng X, Qu C, Fu D, Qu C. Effects of fatigue damage on the microscopic modulus of cortical bone using nanoindentation. *Materials (Basel)*. 2021;14(12):3252. doi:10.3390/ma14123252.

[8] Wearne LS, Rapagna S, Taylor M, Perilli E. Micro-CT scan optimisation for mechanical loading of tibia with titanium tibial tray: A digital volume correlation zero strain error analysis. *J Mech Behav Biomed Mater*. 2022;134:105336. doi:10.1016/j.jmbbm.2022.105336.

[9] Müller R, et al. Mechanoregulation analysis of bone formation in tissue engineered constructs requires a volumetric method using time-lapsed micro-computed tomography. *Acta Biomater*. 2024;179:149–163. doi:10.1016/j.actbio.2024.03.008.

[10] Meng X, Qu C, Fu D, Qu C. Effects of fatigue damage on the microscopic modulus of cortical bone using nanoindentation. *Materials (Basel)*. 2021;14(12):3252. doi:10.3390/ma14123252.

[11] Keaveny TM, Clarke BL, Cosman F, Orwoll ES, Siris ES, Khosla S, et al. Biomechanical Computed Tomography analysis (BCT) for clinical assessment of osteoporosis. *Osteoporos Int*. 2020;31(6):1025–1048. doi:10.1007/s00198-020-05384-2.

[12] Lin JK, Hearn C, Getzen E, Long Q, Lee DC, Keaveny TM, et al. External validation of biomechanical computed tomography for fracture risk classification in metastatic hormone-sensitive prostate cancer. *J Clin Oncol*. 2023;41(16_suppl):e24077. doi:10.1200/JCO.2023.41.16_suppl.e24077.

[13] Frank, M., Fischer, J.-T., & Thurner, P. J. (2021). Microdamage formation in individual bovine trabeculae during fatigue testing. *Journal of Biomechanics, 115*, 110131. https://doi.org/10.1016/j.jbiomech.2020.110131

[14] Dall'Ara E, Viceconti M, Palanca M, Garavelli C, Aldieri A. Validation of homogenized finite element models of human metastatic vertebrae using digital volume correlation. *arXiv*. 2024. Available from: https://arxiv.org/abs/2402.09828.

[15] Klosterhoff BS, Tseng PH, Ong KG, Solorio LD, Mikos AG, Guldberg RE. Wireless sensor enables longitudinal monitoring of regenerative niche mechanics during rehabilitation that enhance bone repair. *Bone*. 2020;135:115311. doi:10.1016/j.bone.2020.115311

[16] Windolf M, Klabunde R, Röderer G, Schmitz P, Schwieger K. Continuous implant load monitoring to assess bone healing status—evidence from animal testing. *Medicina (Kaunas)*. 2022;58(7):708. doi:10.3390/medicina58070858

[17] Pei S, Zhou Y, Li Y, Azar T, Wang W, Kim DG, Liu XS. Instrumented nanoindentation in musculoskeletal research. *Prog Biophys Mol Biol*. 2022;176:38–51. doi:10.1016/j.pbiomolbio.2022.06.005

[18] Rodriguez-Florez N, Oyen ML, Shefelbine SJ. Insight into differences in nanoindentation properties of bone. *J Mech Behav Biomed Mater*. 2013;18:90–99. doi:10.1016/j.jmbbm.2012.10.017

[19] Lodoso-Torrecilla I, Konka J, Kreuzer M, Jimenez-Pique E, Espanol M, Ginebra MP. Quality assessment of regenerated bone in intraosseous and intramuscular scaffolds by spectroscopy and nanoindentation. *Biomater Adv*. 2024;164:213982. doi:10.1016/j.biadv.2024.213982

[20] Fortunato GM, Sigismondi S, Nicoletta M, Condino S, Montemurro N, Vozzi G, Ferrari V, De Maria C. Analysis of the robotic-based in situ bioprinting workflow for the regeneration of damaged tissues through a case study. *Bioengineering*. 2023;10(5):560. doi:10.3390/bioengineering10050560

[21] Maresca JA, DeMel DC, Wagner GA, Haase C, Geibel JP. Three-Dimensional Bioprinting Applications for Bone Tissue Engineering. *Cells*. 2023;12(9):1230. doi:10.3390/cells12091230

[22] Marupudi S, Cao Q, Samala R, Petrick N. Characterization of mechanical stiffness using additive manufacturing and finite element analysis: potential tool for bone health assessment. *3D Printing in Medicine*. 2023;9:32. doi:10.1186/s41205-023-00197-5.

Table 1-Overview of Experimental Mechanical Testing Methods for Bone

Testing Method	Scale	Specimen Type	Measured Properties	Applications	Reference
Compression Testing	Macro	Whole bone, trabecular cores	Compressive strength, stiffness, modulus	Fracture risk, osteoporosis, implant validation	[5] Roux et al., 2024
Tensile Testing	Macro	Cortical bone strips	Tensile strength, elastic modulus	Bone mechanics under stretching, material anisotropy	[6] Carnelli et al., 2011
Three-/Four-Point Bending	Macro	Long bones, beams	Flexural strength, bending stiffness, fracture toughness	Daily mechanical loading, long bone mechanics	[7] Meng et al., 2021
Shear Testing	Macro	Bone blocks or trabecular cubes	Shear modulus, shear strength	Shear-dominated fracture mechanics	[7] Meng et al., 2021
Nanoindentation	Micro/ Nano	Cortical, trabecular bone (polished)	Local elastic modulus, hardness	Mechanical heterogeneity, aging, disease studies	[6] Carnelli et al., 2011
AFM-Based Indentation	Nano	Collagen fibrils, mineral matrix	Nanomechanical stiffness, adhesion	Bone ultrastructure, collagen–mineral interactions	[6] Carnelli et al., 2011
Micro-Compression Testing	Micro	Single trabeculae or osteons	Yield stress, modulus	Damage modeling, microstructural mechanics	[6] Carnelli et al., 2011
Cyclic (Fatigue) Testing	Macro	Long bones or vertebrae	Fatigue life, energy dissipation	Repetitive loading, stress fracture studies	[7] Meng et al., 2021
Impact Testing	Macro	Long bones or substitutes	Toughness under high-rate loads	Trauma simulations, safety research	[8] Wearne et al., 2022
In situ micro-CT + loading	Multi-scale	Small bone cores, trabecular samples	Deformation maps, strain localization	Failure visualization, real-time imaging	[8] Wearne et al., 2022
Digital Volume Correlation (DVC)	Multi-scale	Paired 3D scans of loaded bone	Full-field 3D strain maps	Microdamage detection, model validation	[8] Wearne et al., 2022

Table 2-Overview of the advantages of types of biomechanical testing methods.

Study	Method Used	Model/System	Main Findings	Clinical Relevance	Limitations
Müller et al. (2023) [9]	In situ micro-CT + DVC	Bone tissue-engineered constructs (mice)	3D strain maps can localize sites of future bone formation; strain guides mineralization	Useful for optimizing scaffolds and load regimes in regenerative medicine	Requires expensive imaging; limited to animal models and small samples
Keaveny et al. (2023) [11]	CT-based Finite Element Analysis (BCT)	Human vertebral bodies (clinical cohort)	BCT predicts fracture risk better than BMD; enables patient-specific risk assessment	Can improve fracture prediction and treatment plans in osteoporosis or cancer	High computational cost; relies on CT imaging quality; limited clinical adoption
Müller et al. (2024) [10]	Nanoindentation + imaging	Diabetic vs. healthy bone (ex vivo)	Diabetic bone is mechanically weaker and responds differently to load	Helps understand bone fragility in metabolic diseases like Type 1 diabetes	Ex vivo only; local bone properties may not reflect full-bone behavior
Thurner et al. (2021) [13]	Micro-compression of single trabeculae	Femur and vertebrae of bovine	Progressive microdamage accumulation	Bone fragility depends not only on bone density, but also on the intrinsic material quality of the trabecular structure.	Tests isolated elements; lacks surrounding bone context
Dall'Ara et al. (2024) [14]	Digital volume correlation + FEM validation	Human metastatic vertebrae	Validates FE models using DVC strain measurements in real bone	Enables robust patient-specific modeling in clinical imaging	Not yet standardized; requires access to high-resolution scans

Figure 1-Hierarchical structure of bone mechanical testing. This design of image was created by the author with the help of AI-based tools under the supervision and guidance of the author, only illustrative for educational purposes.

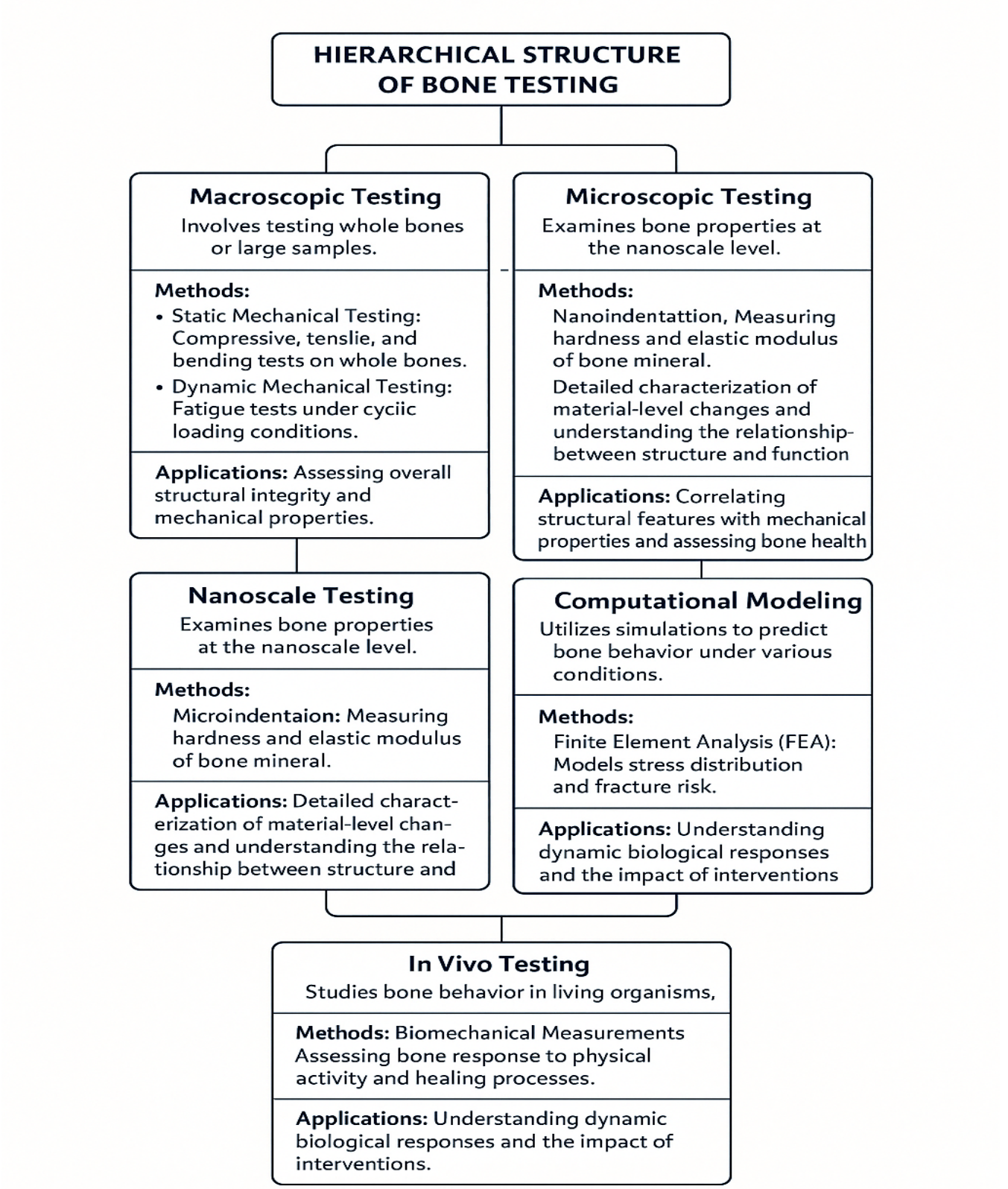
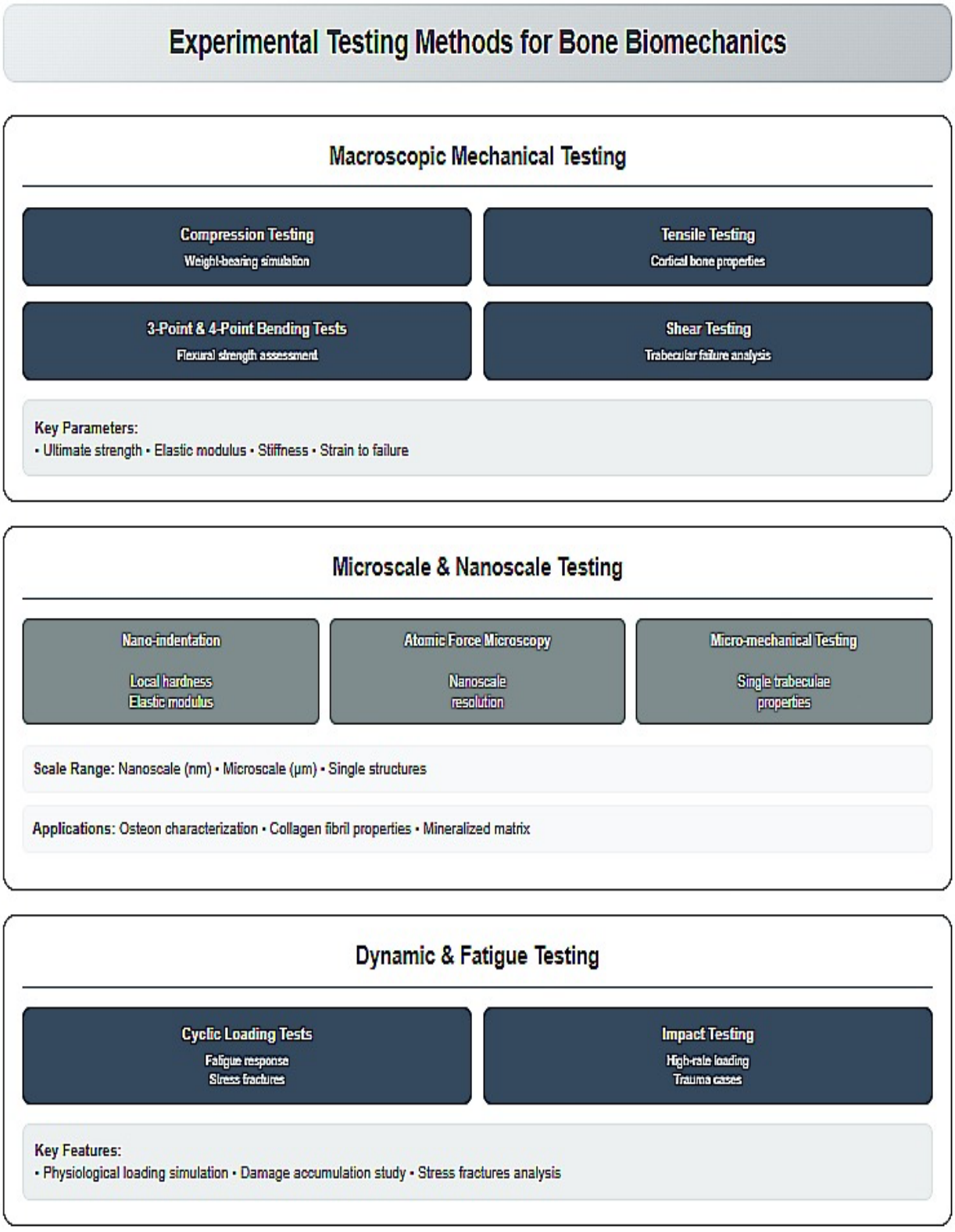


Figure 2-Types of experimental testing of bone- the image was created by the author using the AI-based tools for the design under the guidance and supervision of the author, only illustrative for educational purposes.



In Situ Testing with Imaging

Micro-CT Coupled Testing

Real-time deformation
Micro-crack observation

Digital Volume Correlation (DVC)

3D strain quantification
Sequential imaging

Advantages:
• Real-time observation of bone behavior • Non-destructive analysis

Hierarchical Scale Integration

Integrated characterization across multiple scales enables comprehensive understanding

Key Considerations for Method Selection

Research Question

- Mechanical properties?
- Failure mechanisms?
- Material validation?
- Clinical relevance?

Specimen Considerations

- Sample size availability
- Bone type (cortical/trabecular)
- Age and pathology
- Preservation method

Resolution Requirements

- Spatial resolution needed
- Temporal resolution
- Measurement precision
- Statistical requirements

Technical Limitations

- Equipment availability
- Sample preparation
- Testing environment
- Data analysis complexity