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■ BIOMATERIALS

Standardizing compression testing for measuring the stiffness of human bone

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Objectives

The ability to determine human bone stiffness is of clinical relevance in many fields, including bone quality assessment and orthopaedic prosthesis design. Stiffness can be measured using compression testing, an experimental technique commonly used to test bone specimens *in vitro*. This systematic review aims to determine how best to perform compression testing of human bone.

Methods

A keyword search of all English language articles up until December 2017 of compression testing of bone was undertaken in Medline, Embase, PubMed, and Scopus databases. Studies using bulk tissue, animal tissue, whole bone, or testing techniques other than compression testing were excluded.

Results

A total of 4712 abstracts were retrieved, with 177 papers included in the analysis; 20 studies directly analyzed the compression testing technique to improve the accuracy of testing. Several influencing factors should be considered when testing bone samples in compression. These include the method of data analysis, specimen storage, specimen preparation, testing configuration, and loading protocol.

Conclusion

Compression testing is a widely used technique for measuring the stiffness of bone but there is a great deal of inter-study variation in experimental techniques across the literature. Based on best evidence from the literature, suggestions for bone compression testing are made in this review, although further studies are needed to establish standardized bone testing techniques in order to increase the comparability and reliability of bone stiffness studies.

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Article focus

- To provide a comprehensive review on the experimental technique of compression testing of bone.
- To provide recommendations on how best to perform compression testing of bone in the future.

Key messages

- There is a great deal of inter-study variation in the experimental technique for compression testing of bone.
- Factors such as specimen preparation, specimen geometry, testing configuration, and strain rate can affect the measurement of bone stiffness.

- Further studies looking specifically at aspects of the compression testing technique are required in order to establish a standardized method for bone.

Strengths and limitations

- This review followed guidelines suggested by the Cochrane and Preferred Reporting Items for Systematic Reviews and Meta-Analyses organizations.
- This review of compression testing can help to develop a standardized experimental bone testing technique in the future.

Introduction

Stiffness can be defined as the resistance of a structure or material to deformation.¹ This property is of great importance for understanding the relationship between the structure and function of bone, and is clinically relevant in areas such as orthopaedic prosthesis design and characterization of bone properties across anatomical locations.²⁻⁵ Thus, the ability to determine bone stiffness in an accurate and efficient way is crucial for enabling clinicians to understand the effect of factors such as disease, age, and medical intervention on bone quality.

Compression testing is a widely used experimental technique for determining the mechanical properties of bulk tissue specimens excised from cortical or cancellous regions of bone.⁶⁻¹⁰ It is relatively straightforward to perform, and is capable of producing quick measurements of apparent elastic modulus and other properties, such as ultimate strength.¹¹ Other bulk tissue testing techniques for determining stiffness include three-point bending, tensile testing, and torsional testing.^{12,13} The apparent properties obtained from these tests are independent of the whole bone geometry, but include effects of porosity and anisotropy arising from osteon or trabecular orientation.¹⁴ Currently, there is wide variation in the literature about how compression testing of bone is performed, with no benchmark protocol. Differences between studies may be due to differing processes of extraction, machining, and preserving the bone samples.¹⁵ They may also be due to the method used to measure the strain in the bone as well as the strain rate used during testing.¹⁶

Currently, strict standards are universally established for the experimental testing of engineering materials. However, this is not the case for compression testing of bone specimens, where standardized material testing methods cannot always be applied due to restrictions related to using biological tissue. These include the heterogeneity and finite size of bone specimens, difficulties with gripping bone surfaces, as well as the relatively low loads that can be applied. Subsequently, there are variations in testing methodology and specimen preparation across the literature, and direct comparison of studies is difficult.¹⁵ Therefore, this paper aims to review systematically the literature surrounding compression testing of human bone and its reliability for measuring stiffness. Specifically, this systematic review aims to determine how best to perform compression testing of human bone in order to help develop a standardized testing technique for future studies.

Materials and Methods

A systematic review of published literature up until December 2017 relating to compression testing of human bone was undertaken using Medline, Embase, PubMed, and Scopus databases according to the

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.¹⁷ This was considered representative of the literature. A combination of the search terms, “compression test*” OR “compressive test*” OR “axial compression” AND “Bone*” OR “cortical bone*” OR “compact bone*” AND “stiffness” OR “rigidity” OR “elasticity” OR “elastic* modulus” OR “Young’s modulus” were used. Exclusion criteria included studies using non-human tissue, those that did not undergo compression testing (e.g. tensile testing or finite element modelling), non-bulk tissue testing (e.g. whole bone specimens), non-accessible or non-English papers, and those that did not measure stiffness directly from the compression test. Two authors (SZ and MA) were responsible for independent article extraction and inclusion. Any disagreements were resolved with discussion as recommended by Cochrane Collaboration guidelines.¹⁸

Results

Search results. Figure 1 shows the study extraction flow-chart, according to PRISMA guidelines. A total of 4712 abstracts were retrieved. Following duplicate removal and abstract screening, a total of 807 eligible full-text articles remained. After review of these manuscripts, 177 papers were deemed to fit the inclusion criteria for compression testing of human bone and were consequently included in this review.

Qualitative assessment. Overall, there was a great deal of variation across the literature in terms of testing protocol. Supplementary tables i, ii, and iii show details of the testing protocol and experimental setup used in each study, with articles separated according to the type of bone used (cancellous, cortical, or mixed specimens). If the method of strain measurement was not specifically reported in the article, it was assumed that it was measured via the machine crosshead displacement. A total of 20 studies directly analyzed aspects of the compression testing technique,^{7,16,19-36} and provided recommendations for improving the accuracy and precision of future testing. A brief overview of the studies retrieved is provided in the results section of this review. The analysis of individual studies is in the discussion section.

Bone specimen preparation and storage. It was found that the most common method for storing and preserving bone specimens prior to testing was freezing and subsequent thawing before testing.^{5,6,8,10,16,20-22,24-31,33-139} The temperatures used varied across the literature and samples were commonly frozen in physiological (0.9%) saline solutions. Other solutions used for storage, or for thawing samples, include ethanol,^{5,22,32,70-72,140-145} Ringer’s solution,^{16,31,42,43,57,60,74,80,83,84,105,142,144,146} embalming fluid (commonly formalin-based solutions),^{31,102,118,141,147-152} and phosphate-buffered saline (PBS) solution.^{65,115,124,135,153} Some studies freeze-dried the samples.^{21,59,154-156}

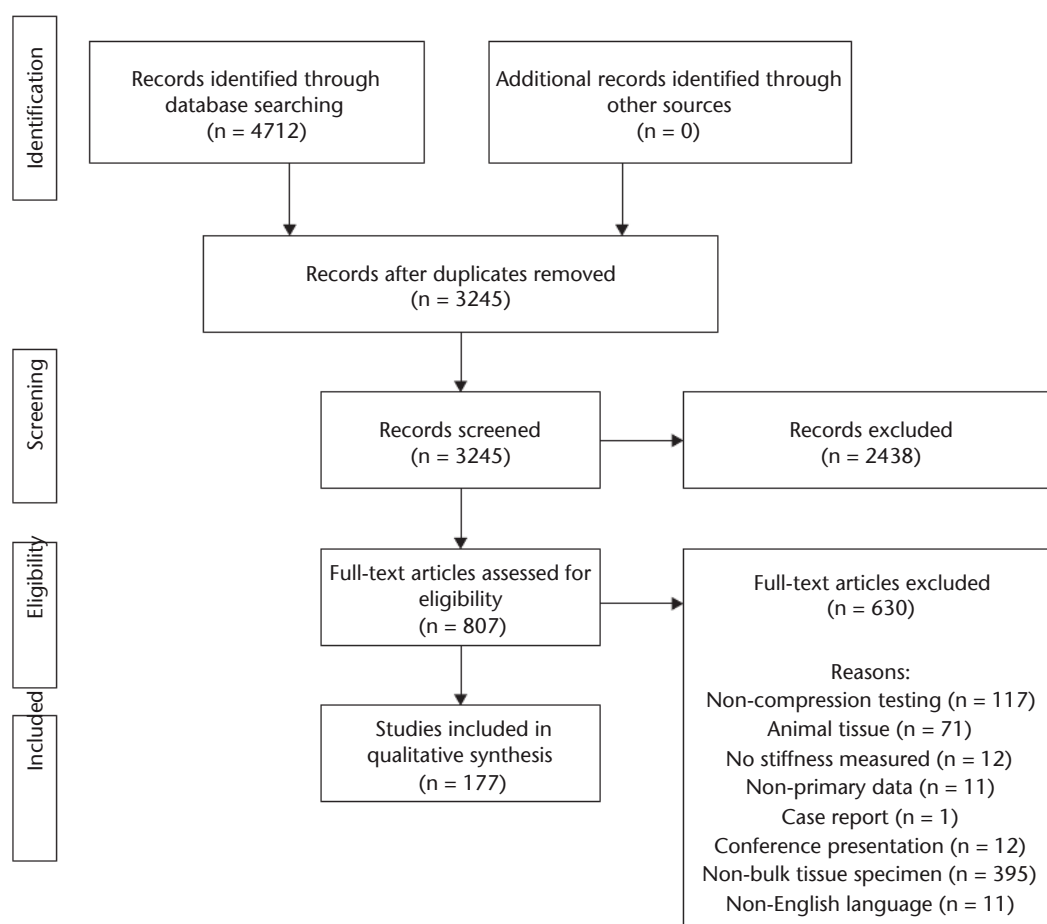


Fig. 1

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of study selection.

During the testing process, fresh wet specimens were most commonly used. Six studies used dried specimens in the compression test.^{33,51,135,138,157,158} The majority of studies tested bone specimens in unconfined conditions, but some tested in confined conditions using confinement chambers,^{22,98,159,160} a steel annulus,⁵² or polytetrafluoroethylene (PTFE) tape.¹³⁰

Testing setup. The standard compression test traditionally involves compressing the bone specimen between two fixed parallel stainless-steel platens or anvils (Fig. 2). Certain studies used a combination of a fixed platen paired with an adjustable, rotating platen.^{38,43-45,69,101,104,107,113,118,119,146,157,161-163} A few studies used variations of the standard machine setup such as use of grooved platens,¹⁵⁴ custom-built compression machines,^{82,122,132,164,165} and drop tower¹⁵⁹ or hand-loaded testing machines.¹⁴¹ Some studies tested bone specimens in water baths filled with saline solution.^{53,66,70,72,87,107,122,166,167} Other solutions in which samples were immersed during testing included Ringer's solution,¹⁶⁸ Hank's Balanced Salt Solution,¹⁶⁶ and PBS with protease inhibitors.¹³²

Many studies used additional measures to improve accuracy of the testing technique, including the use of mineral oil to lubricate the specimen-platen interface^{22,24,26,34,61,88,103,127-129,133,147,148,162} and embedding the specimens in brass, aluminium alloy, or stainless-steel endcaps.^{4,8,19,28,29,34-36,39,40,49,73,76,77,90,99,113,118,119,131-133,142,144,153,159,164,167,169} Some studies used latex,^{33,38,69,132} Teflon plates,^{20,104} or poly(methyl methacrylate) (PMMA) cement^{22,27,32,166,170,171} on the specimen ends to help stabilize the severed trabecular free ends. Other studies glued specimens with cyanoacrylate or epoxy adhesive to the platen.^{51,55,116,172-174}

Strain measurement. The most common method for strain measurement was using the machine crosshead displacement. The following studies applied machine compliance correction when using this method of strain measurement.^{34,37,46,47,51,52,57,75,88,160,166,168} Only one study³⁷ gave specific details of the compliance correction algorithm. Other studies used extensometers^{3,8,16,22,24,26-32,35,40,47,49,50,53,60-62,65,68,76,77,81-84,90,97,103-105,111,112,119,127,129,131-133,135,136,142,144,147,148,168,170,171,173-181} and electric resistance wire strain gauges^{33,133,158,160} to measure strain. A

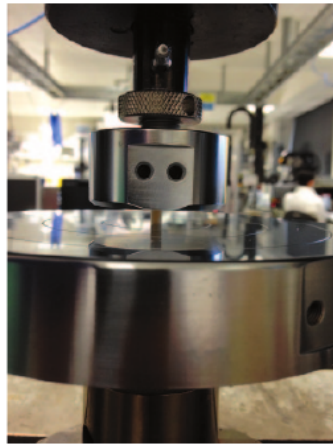


Fig. 2a

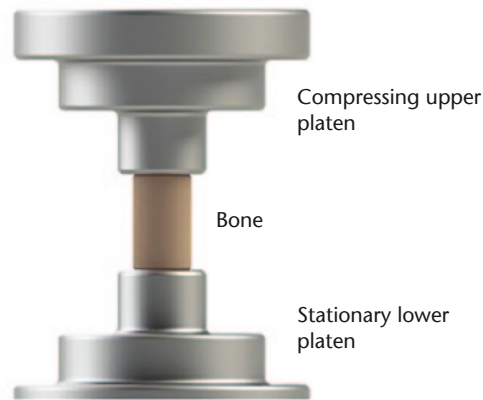


Fig. 2b

Images showing a) standard platens compression testing setup of a bone specimen, and b) a close-up schematic of a bone specimen placed between two polished platens.

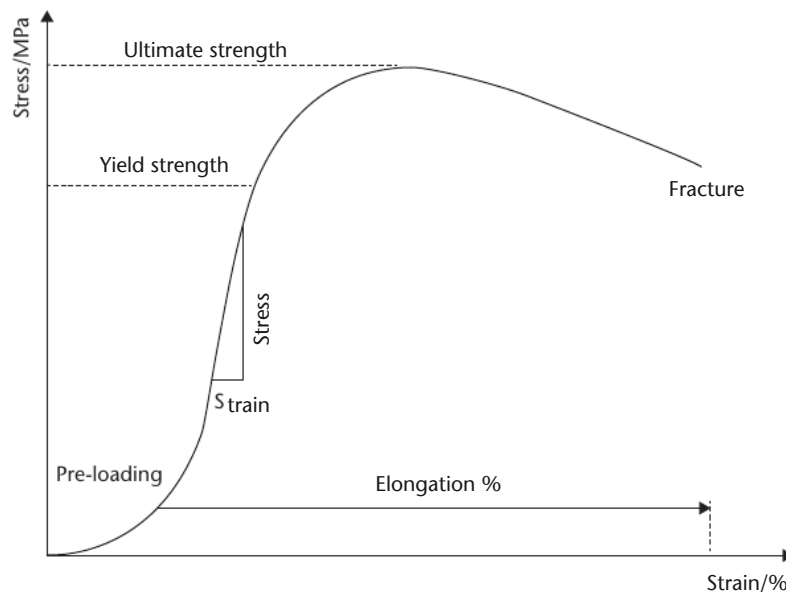


Fig. 3

Stress-strain graph of a typical compression test with corresponding mechanical parameters measured.

few studies used Digital Image Correlation (DIC), an optical, non-contact technique.^{30,67,86,120,125,140,145,182} It was found that the majority of studies used physiological¹⁸³ strain rates in the range of 0.005 s^{-1} to 0.08 s^{-1} , with a few testing at substantially higher strain rates.^{16,26,68,159,161}

Discussion

This systematic review set out to determine how best to perform compression testing of human bone, in order to suggest a standardized method for determining bone stiffness to be used in future studies. A total of 177 articles were retrieved which help to answer this question. The retrieved articles have been analyzed and, where possible, recommendations for compression testing have been made.

Determination of stiffness. The load-displacement curve and the resultant stress-strain curve can be divided into elastic and plastic deformation regions. Within the elastic region, the structure undergoes deformation, which returns to its original shape after the load is released.⁹ The pre-yield region of the stress-strain curve is usually linear and is considered, often incorrectly, to be elastic. The slope of the linear portion is taken by many researchers as the elastic modulus or Young's modulus (Fig. 3). However, bone is a complex, anisotropic, and heterogeneous material that does not behave as a purely elastic material. Thus, it has been shown to demonstrate non-linear behaviour, particularly at the lower portion of the pre-yield elastic region of the stress-strain curve, likely due to factors such as irregularities in the specimen

surface layers.^{25,29,34} Morgan et al²⁹ thus used a second-order polynomial fit for strain ranges of up to 0.2% when calculating the slope to minimize systematic errors. For strain ranges up to 0.25% and 0.3%, they used third and fourth order polynomial fits, respectively.

Strictly speaking, the slope of the linear portion of the stress-strain curve is not the Young's modulus of the bone specimen because this deformation is not purely elastic. Bones have complex hierarchical structures and contain many defects, including pores. Some degree of plastic deformation is present, even at a relatively low compressive stress or strain. An appropriate term used to describe the slope of the initial linear part is the apparent modulus, which is a useful stiffness parameter for comparison purposes.¹⁸⁴ However, this parameter simply represents the ratio between stress and strain, and should not be confused with the elastic or Young's modulus of the material. Many papers in the literature reported the apparent modulus as Young's modulus.^{3-5,7,10,16,19,20-25,27,29,30-33,37,40-42,45,47,48,50-53,55,57-71,73-84,86-100,102-124,126-137,139,140,142-148,150,151,153-159,161-164,166-168,176-182,185-195} This likely explains why many reported Young's modulus values are generally lower than expected, and with a wide range of variance.

The Young's modulus of bone specimens ideally should be obtained by an intermittent loading-unloading procedure. Specifically, the stress is increased at a low strain rate to a level somewhat below the yield point, i.e. before the stress-strain curve deviates from the linear portion. It is then released and immediately increased again, forming a loading-unloading cycle.¹⁹⁶ The unloading curve is steeper than the linear portion of the normal stress-strain curve, and its gradient can be used to generate a value for Young's modulus.⁶⁵ However, the complexity of bone material and variation between specimen and specimen may mean that this technique (which is commonly used in testing porous metals¹⁹⁶) is not feasible. Thus, we suggest for pragmatic purposes that the technique outlined by Keaveny et al³⁶ is used, whereby modulus is determined from a best fit line to the steepest portion of the stress-strain curve over a range of 0.2% strain. This is still technically measuring apparent modulus, but at a strain below 0.2% the amount of plastic deformation is likely to be small. Thus, this method may provide a value close to the true elastic modulus. However, care should be taken when interpreting the validity of data taken from studies using this method of calculation in the absence of endcaps or extensometers. This is because there is often a non-linear toe-in region present on the loading curve over this strain range if endcaps are not used.

All future studies should report the correct terminology, particularly where absolute values for modulus are concerned.

Other factors contributing to the variance in the literature regarding bone stiffness include patient demographic

or health status, sample location, orientation, and testing conditions. It can be misleading to provide an authoritative reference range for moduli, as in reality this range would differ between studies due to these numerous aforementioned compounding factors. But as a guide, for wet, cortical femoral bone (the most commonly tested), one can expect the stiffness to fall approximately in the range of 15 GPa to 20 GPa.^{8,65,112,142} This range should be interpreted accordingly with the test conditions and sample origin. Lower values should be anticipated when using cancellous samples,¹⁹⁷ when compressing in the transverse direction rather than the longitudinal,⁶⁵ when testing metaphyseal bone, rather than diaphyseal bone,¹⁹⁸ in a patient group of older age,⁶¹ or when extensometers and endcaps have not been used (i.e. structural end effects are not accounted for).³⁶ Higher values may be anticipated when testing dry samples^{135,158} or at higher strain rates.¹⁶ These factors will be discussed in more detail in the subsequent sections of this paper.

Stiffness of animal bone is outside the scope of this review, but may be of interest to researchers. For example, where bovine bone is commonly used as a test material. For further discussion regarding other mechanical testing techniques and non-human bone, we recommend the broad review by Novitskaya et al¹⁹⁷ as a good starting point.

Sample preparation. By far the most common method of specimen storage was shown to be freezing of wet bone specimens. Linde and Sørensen²² have shown that this method has minimal effect on stiffness. Therefore, freezing in physiological saline should be the standard method of storage as it is easily accessible and facilitates consistency and comparability between studies.

Although the majority of studies tested wet specimens, six studies tested dried specimens.^{33,51,135,138,157,158} Testing dry specimens may help facilitate coupling to the metal platens and hold the specimen in place. However, Carter et al⁵¹ found that specimens that had been dried then rewetted gave significantly greater moduli values than fresh specimens. This is consistent with studies by Bargren et al¹⁵⁸ and Samuel et al,¹³⁵ who found that bone specimens that had been dried had increased stiffness compared with the hydrated specimens. Therefore, studies using fresh specimens should not be compared with those using dry. When testing fresh specimens, care should be taken to ensure that the surface layers of the specimens do not dry out.

Bone can be considered a composite structure, with a solid phase (mineralized bone tissue) and a fluid phase (i.e. bone marrow, vessels, nerves, blood, and interstitial fluid).⁵² Thus, properties will change depending on whether or not specimens are tested with marrow *in situ* or *ex situ*,^{22,52} as well as depending on the hydration state of the tissue.^{135,199} Several studies have shown that defatting and removal of bone marrow from bone specimens prior to testing has significant effects on the mechanical

properties measured.^{3,22,33,52} The storage method before testing is therefore important. It is recommended to freeze samples in saline with marrow intact. It not only has a minimal effect on the mechanical properties, but also is a widely accessible and commonly used technique.

Orientation and anatomical location. When compression testing anisotropic materials such as bone,²⁰⁰ care must be taken to control and specify orientation and axis of loading of the specimen. Not only is this important in studies wishing to replicate *in vivo* conditions, but it also helps ensure comparability between studies. Care must be taken to avoid misalignment of the principal material axes with the anatomical axes during the specimen machining process. This is because off-axis angulation can lead to errors in the mechanical properties measured, as demonstrated experimentally by Öhman et al.³² Schwiedrzik et al¹⁷³ used bone specimens that were extracted perpendicular to the main trabecular orientation rather than parallel, which explained the lower yield stress values obtained in their study. Morgan and Keaveny⁹⁹ quantified the degree of misalignment in their study to enable them to calculate the average estimated percentage error in their modulus measurements. They used microCT scanning, as did Perilli et al,¹⁴⁴ to determine any off-axis angulation of the specimens. Bourgnon et al⁴⁵ used a microscopy camera during their experiment to ensure correct alignment before testing. Although microCT and microscopy cameras can offer more accurate alignment information, they are not always available and can be time-consuming to use. Researchers should use cameras or visual inspection for checking orientation.

The orientation considerations can cause constraint in the sample extraction procedure. For example, in two studies by Wachter et al,^{127,129} the thin cortical shell in the donor patient's femur only allowed for small cortical specimens to be extracted in one direction. If resources allow, it would be beneficial to take radiographs of specimens to ascertain the trabecular direction, and subsequently ensure correct orientation prior to testing.^{32,133,179,201} It is recommended that the misalignment angle is measured and considered when analyzing the outcomes.

Due to the nature of human tissue collection, it may be difficult to determine the orientation and location of biopsied specimens relative to the donor's host bone. The most commonly sampled locations throughout the relevant literature were the femur, followed by the vertebrae and tibia. Variation in results due to anatomical variation¹² can be minimized by sampling from the same precise anatomical site. Lv et al¹⁵⁶ designed a personalized mould for each femoral head used in their study to ensure that biopsies were extracted from precisely the same anatomical location in each patient. While this method confers a higher level of precision and ensures standardized sample extraction, it is both time- and

resource-consuming. It is hardly practical when a large number of samples are involved, and may not be as reproducible in other locations as the femoral head. In most cases, the use of anatomical landmarks to determine specimen extraction location would be sufficient.

Specimen geometry. In compression testing, the specimens are typically cylindrical or parallelepiped. A few studies used cross-sectional slices ranging from 8 mm to 20 mm in height.^{39,44,59,94} This is not recommended because the height-to-width ratio is too small, and it is also difficult to give an accurate measurement of the cross-sectional area. Thus, stiffness derived from studies using non-uniform specimen geometries should be interpreted with caution. Studies have found that modulus decreases with decreasing specimen cross-sectional area and length.^{24,34} Linde et al²⁴ suggest a 6.5 mm cube or cylinder of 7.5 mm diameter and 6.5 mm length, and Keaveny et al²⁰² recommended the use of cylindrical specimens over cubic in order to minimize errors related to Poisson's ratio. However, this error is likely to be small. As long as an appropriate aspect ratio is maintained, geometry of the cross section, either cylindrical or cubic, should not matter much.

It is appreciated that it would be difficult to set a standardized specimen size for testing as this ultimately depends on the machine setup and volume of tissue available. However, given the literature and with guidance from current testing standards for similar engineering materials, the following aspects should be considered when selecting specimen geometry:

- The aspect ratio (i.e. the height to width ratio of the specimen). This should be between 1 and 2 to avoid buckling of the specimen during the compression test and thus maintain axial load application.^{201,203}
- Cylindrical cross-section specimens should be used where possible. A 2:1 cylinder of minimum 5 mm diameter, as suggested by Keaveny et al,²⁰² would be sufficient to satisfy the continuum assumption.
- The method of strain measurement. In general, larger specimens should be used if using machine crosshead motion for strain measurement to minimize the effect of the structurally compromised free surface layers. Zhu et al³⁴ recommended a minimum height of 10 mm and cross-sectional area of 100 mm². If this cannot be achieved, regional strain measurement should be carried out.

Machining. It is important to maintain a standardized process for machining the top, bottom, and side surfaces of the bone specimen. This may be one of the most time-consuming steps of sample preparation but is imperative for ensuring a maximally ideal stress field and state

during compression testing. Both ends of the specimen should be parallel to each other, with these surfaces also lying perfectly perpendicular to the long axis of the specimen.¹⁴¹

Irrigated, low-speed, low-force cutting devices should be used to prevent overheating of the specimens, and thus reduce thermal and mechanical damage.^{9,204} Diamond is a material that has been shown to be resistant to wear²⁰⁵ and is biocompatible.²⁰⁶ Thus, where possible, this should be used for tool materials. If cylindrical specimens are used, these are commonly cut using coring tools, by milling, or by lathing.⁹ Keaveny et al²⁰⁷ found that lathing their cylindrical specimens produced clean cut sides, and no additional damage compared with coring the specimens. Some studies recommend machining the samples while frozen to prevent damage,^{42,68} although there is little supporting evidence for this method. No matter how careful, any process of machining a heterogeneous tissue may lead to potential damage. We want to highlight a key step in the specimen preparation process, which is to inspect visually the specimen surfaces for any damage or unacceptable irregularities before proceeding to the compression test phase.

Testing configuration. It is possible to test bone specimens in confined or unconfined conditions. The latter allows the escape of bone marrow during testing, which reduces the influence of viscoelasticity. Confined testing not only keeps bone marrow in place but also restricts lateral deformation during testing. A study by Linde and Hvid²⁷ looking at the effect of side constraint found that bone specimens tested in confined conditions produce increased values for stiffness compared with results obtained from the same specimen tested in unconfined conditions. Although, arguably, it is more representative to test specimens in constrained conditions, it is clearly difficult during testing to replicate the degree of constraint conferred by neighbouring tissue *in vivo*. It is thus recommended that, for measuring properties of the bone alone, unconfined testing remain the standard at least to facilitate comparability. This study highlights one of the potential limitations of *ex situ* techniques such as compression testing, where the results may have limited use clinically.

Platen setup. With traditional platens compression testing, the specimen can be placed between two fixed parallel platens, or a combination of a fixed platen paired with an adjustable platen. This adjustable platen, usually pivoted on a ball bearing, is often used to reduce the effects of specimen non-parallelism and misalignment. This would enable full engagement between the platen surface and specimen ends, and encourages evenly distributed loading during compression.^{69,77} Overall, with regard to the testing apparatus, the type of machine is unlikely to be a critical factor in relation to the testing results so long as the effects of specimen

misalignment, friction, and slippage can be avoided or minimized.

Precautional measures (to improve accuracy). The surface of the compression platens can be polished and/or lubricated with mineral oil to minimize friction between the platen surface and the specimen surface. This can help reduce the influence of “end effects” and allow for free transverse expansion of the specimen (Poisson’s effect).^{15,34,36,103} The disadvantage of this, however, is highlighted in preliminary studies by Linde et al²⁶ where the presence of an oil film on the platen resulted in a significant load signal before the specimen had any contact with the compression column. Similarly, the use of latex, Teflon, PMMA cement, or glue to secure the specimens directly to the platen should generally be avoided, if possible, as it is unclear whether these additions have an effect on the mechanical properties measured.²⁰⁸

Endcaps may be made of brass, aluminium alloy, or stainless steel, and specimens are commonly secured within them using PMMA cement, cyanoacrylate glue or latex rubber. These may help to eliminate the effects of specimen slippage, while also providing a suitable homogeneous surface for the attachment of extensometers. This confers the advantage of increased precision.³⁶ Furthermore, the process of embedding the specimens into the endcaps can introduce off-axis tilting or uncontrolled preloading.

Keaveny et al³⁶ published a paper describing several systematic and random errors present when compression testing trabecular bone samples. They reported a systematic underestimation error in the range of 20% to 40% for compression testing of trabecular specimens. They suggested a testing technique aimed at minimizing end effects, which involves embedding the bone specimen in endcaps and using an extensometer to sample in four directions around the specimen. This study shows the beneficial effect of using an external method of strain measurement, and future studies should use this endcap-extensometer technique where possible.

One such case where it may not be possible to use extensometers is with small specimens. Speirs et al¹¹⁶ describe this issue in their study of the effect of sterilization techniques on ear ossicles. The naturally small size of these bones did not allow for use of endcaps or extensometers. They used data obtained from pre-testing on synthetic bone specimens of the same size and under similar conditions to help estimate the reproducibility of their testing technique. However, we suggest that, in these cases, alternative methods of strain measurement such as strain gauges or linear variable differential transformers (LVDTs) can be used to similar effect.

Temperature and environment. The temperature and testing environment can be controlled through use of temperature-controlled baths filled with solution (most commonly saline). These baths are commonly used to

regulate the testing temperature to 37°C to emulate *in vivo* testing conditions. For most studies, the use of saline baths is not necessary, provided that the specimen is not left to dry out on the platen, and the test is carried out within a reasonable time frame.

It is appreciated that the machine setup and environmental condition depend not only on the objectives of the study but also on the resources available to the researcher. Thus, standardization is impractical in this aspect. We suggest that, where possible, any testing method used should be validated against an appropriate material standard with known properties (e.g. rigid plastic) in order to identify and quantify any systematic error present.¹⁴ This will enable adjustment to the testing technique to assure accuracy prior to testing.

Testing procedure. Preconditioning or preloading protocols are cyclic loading tests applied before the intended test is carried out. These are used to achieve a steady viscoelastic state and ensure that zero-strain during testing is reproducible by defining the zero-strain at a set preload.⁷ Preloading and preconditioning also help to ensure that the entire end surface of the bone specimen is uniformly in contact with the platen before testing. These multiple compression cycles may also be used to check for any substantial plastic damage to the specimens by identifying reductions in modulus between the cycles, provided that these cycles are within the elastic range.³⁶

Preconditioning should be carried out if measures have not been taken to eliminate end effects.²⁰⁷ A cyclic, displacement-controlled preconditioning protocol of low strain levels in the range of 0.3% to 0.5%^{7,207} can be used to minimize irreversible damage to the specimens. Note that this threshold level of preload will differ between specimens and so, for more precision, a protocol where the specimen is cyclically loaded and unloaded until linearity may be used. This can be used to identify the zero-point, i.e. the effective starting point of zero-strain, where the specimen can then be subsequently reloaded for the test.³⁶

Strain measurement. There are a wide variety of techniques for measuring strain, including machine crosshead displacement, strain extensometers, and strain gauges. With the traditional platens testing technique, strain can be easily measured via the motion of the test machine crosshead. This method works on the assumption that the displacement of the platen is identical to the deformation of the specimen. When measuring the relatively small deformations of bone specimens, this assumption introduces error due to deflection of the entire load frame of the machine when under stress. This effect is related to the stiffness of the test machine apparatus and is known as “machine compliance”.²⁰⁹ Thus, studies that use this method of strain measurement should correct for the machine compliance to ensure that they are measuring the strain of the specimen alone. The machine compliance may be determined directly by loading the system

without a specimen or with a standard uniform material specimen such as steel with known properties. Augat et al³ reported systematic measurement errors in their study due to the measurement of strain across the machine platens, and not the specimen itself.

Although this method may be adequate, other methods of strain measurement with higher accuracy are necessary to obtain test values within an acceptable limit. Strain is often greater at boundary regions close to the platen than in the middle of cut specimens due to the severed struts of trabeculae on the outer surface. This can lead to overestimation of the average strain across the specimen, and thus a consequent underestimation of modulus.^{15,30,34} To minimize these end effects, strain is best measured at the middle region of the specimen. This can be achieved using an extensometer, which may be of a contact or non-contact type. Keaveny et al³⁶ recommended an extensometer technique that sampled deformation data from all around the specimen, thus accounting for any potential architectural heterogeneity within the bone specimen. However, there are limitations to measuring strain using extensometers in such bone studies. First, the use of extensometers requires large specimen sizes due to technical difficulties with securing the arms of the extensometer on the surface of small or irregularly shaped specimens. The typically slippery and smooth surfaces of fresh bone specimens further exacerbate these fixation difficulties. Several studies reported errors stemming from slippage of the extensometer, particularly when dealing with wet bone specimens.^{77,142} Cotton et al¹⁶⁸ reported that nearly 20% of their samples tested had unreliable extensometer readings, apparently due to slippage. There may have also been damage to specimens through transverse preload as a result of attaching contact-type extensometers.²⁹

When strain gauges are suitably attached to the test material, the deformation of the strain gauge is assumed to be identical to the deformation of the material. The deformation of the strain gauge leads to changes in electrical resistance that allow digital calculation of strain.²¹⁰ These can be used singularly for measuring strain in one direction or in the form of rosette strain gauges to measure strain multi-directionally. However, installation of these devices can be difficult when dealing with bone material in terms of specimen surface preparation and choice of adhesive. A common source of error lies in the bonding of the gauge to the test specimen and insufficient specimen surface preparation.²¹⁰ By technical standards, the specimen surface should be chemically clean and degreased, appropriately rough, and of appropriate pH.²¹¹ As imagined, this is difficult or inappropriate when testing organic material such as fresh bone.

An innovative technique for strain measurement is digital image correlation (DIC), an optical, non-contact technique for measuring displacement. The specimen

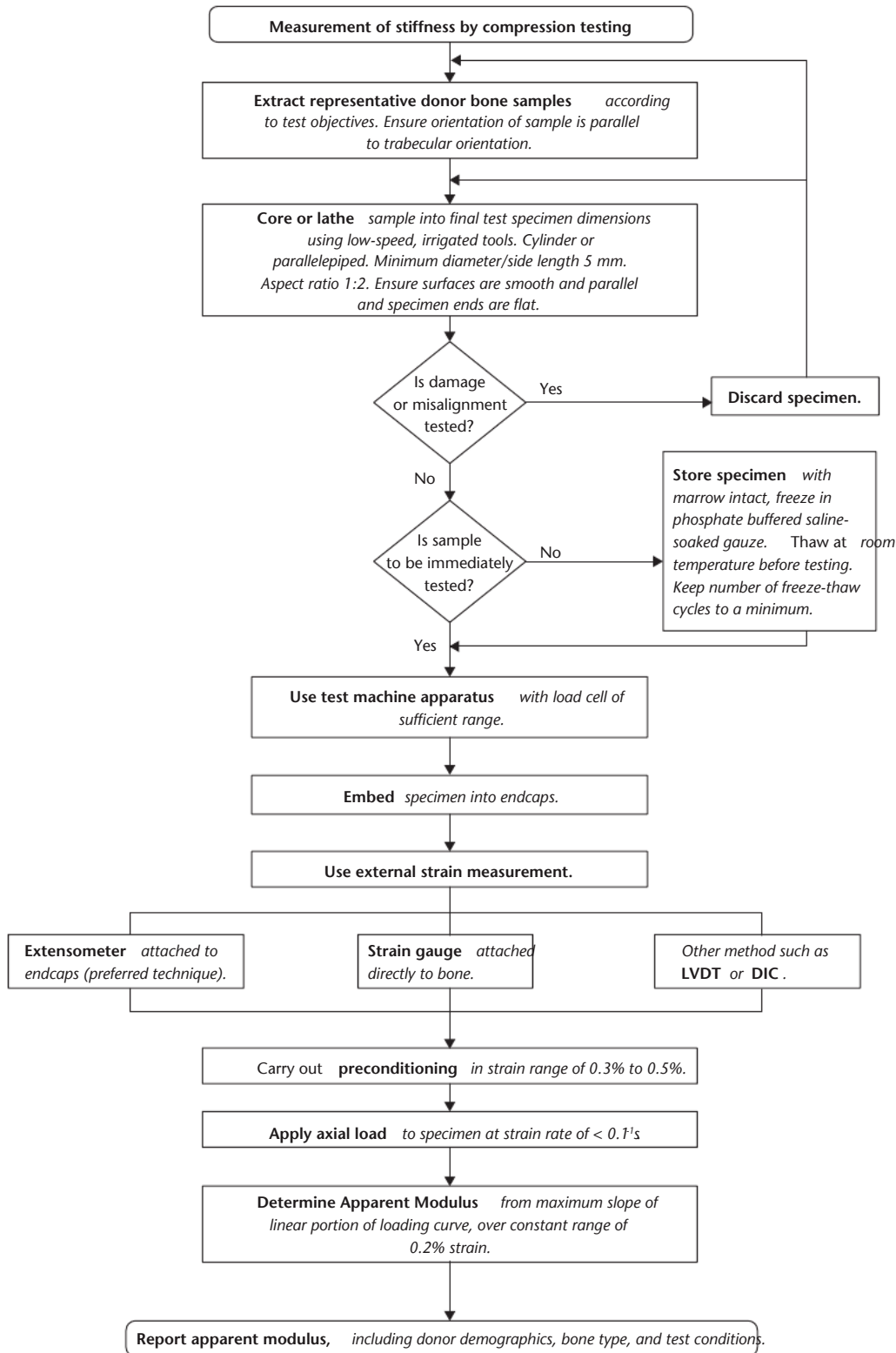


Fig. 4

Recommended testing protocol for measurement of bone modulus. DIC, Digital Image Correlation; LVDT, Linear Variable Differential Transformer.

surface is usually painted or sprayed to produce a high-contrast speckle pattern. DIC works by tracking the pixels of serial digital photographs taken of the painted surface

at different stages of deformation. Here, the DIC system is capable of taking the influences of end effects into account and has the potential for full-field strain

measurement.²¹⁰ However, measurements are limited to the accuracy and resolution of the DIC system and authors highlighted limitations with using acrylic paint for surface preparation.¹⁴⁰ For example, paint that had penetrated into the pores could have been carrying applied loads or obstructing marrow flow, and thus could affect mechanical behaviour.¹⁴⁰

Each method of strain measurement has its advantages and disadvantages and, ultimately, the method used should depend on the objective of the study. Where relative values are sought, traditional crosshead displacement measurement of strain is sufficient and is quick and simple to implement. It also places fewest restrictions on the specimen, with no need for large-sized specimens or surface preparation to facilitate attachment of extensometers or strain gauges. Although the effect of machine compliance may be negligible when testing relatively compliant orthopaedic samples, it is strongly recommended that investigators still correct for this error. If absolute values are of interest in the study, then extensometers should be used to improve the accuracy of strain measurement as discussed above. If localized strain changes are of interest to the researcher, strain gauges can be used. Digital image correlation is a method that would be most useful in studies interested in full-field strain measurements (e.g. whole bone fracture analysis).²¹² Further studies using DIC on *ex vivo* bone samples are required before reliability of this method can be assessed.

Strain rate. Strain rate is strain change (deformation) per unit of time. Testing strain rate is an important factor to consider when measuring the mechanical properties of biological materials. The strain rate used may vary depending on the nature of the experiment, i.e. within normal physiological range or higher strain rates for simulating trauma and impact.¹⁶ Physiological strain rates are considered to be within the range of 0.005 s^{-1} and 0.08 s^{-1} , and the majority of studies use rates that fall within this range.¹⁸³ The studies which tested at substantially higher strain rates^{16,26,68,159,161} were studying high-impact situations.

Wet bone exhibits “viscoelastic” or strain rate-dependent behaviour due to the complex, multi-phase, porous structure of bone where fluid present in the bone matrix effectively acts as a “shock absorber”.^{135,213} Internal friction between the fluid phase, i.e. bone marrow, and the solid phase, i.e. mineral matrix, leads to losses of elastic energy.^{14,52} This viscoelastic effect has been shown to have the greatest influence at higher strain rates (greater than 10 s^{-1}) in confined boundary conditions where the marrow cannot move freely.^{26,52} Studies by Wells and Rawlings¹³⁰ and Linde et al²⁶ showed that the stiffness of trabecular bone increased as the testing strain rate increased. Hansen et al¹⁶ have experimentally demonstrated a similar effect when testing cortical bone. They

tested at moderate to high strain rates, as this was more representative of the strain rates encountered during traumatic events that resulted in bone fractures.

Due to viscoelasticity, the most accurate modulus values are obtained at very low strain rates, i.e. rates that are considered “quasistatic”.²⁵ It is suggested that a critical strain rate exists at which moduli values increase, although there is no definitive consensus in the literature.^{16,161} Clearly, compressing specimens at infinitely low strain rates would be impractical and inappropriate. At excessively slow loading durations over long periods of time, materials will suffer from creep deformation, a phenomenon which will also affect the measured modulus. Thus, we recommend a testing strain rate range of between 0.001 s^{-1} and 0.1 s^{-1} to be used where strain rate is not the studied factor. A strain rate within this range should be sufficient to minimize creep whilst still low enough to be considered quasistatic.²¹⁴ Again, researchers should bear in mind their objectives as testing at these low strain rates may not be representative of the dynamic or physiological strains present *in vivo*,¹⁸³ and many of the studies in the literature purposefully test at moderate or high strain rates. All studies should report the strain rate used to facilitate comparability.

In conclusion, variations in methodology for compression testing of bone across the literature due to lack of standardization in testing technique have made comparability and interpretation of current studies difficult.¹⁵ This paper aimed to review the literature systematically in order to determine how best to perform compression testing of human bone to help develop a standardized testing technique for future studies. The American Society for Testing Materials (ASTM) designations for compressive testing provide a current source of guidance for mechanical testing technique, but adaptations for use with bone tissue are necessary.²⁰³

We recommend that the testing protocol shown in Figure 4 should be used as a guide in conducting compression tests of human bone to obtain stiffness values. The following key factors in compression testing should be noted:

- Orientation during extraction of the specimen must be carefully considered to ensure correct anatomical alignment.
- Specimen geometry is important, and cylindrical bone cores with aspect ratios less than two are preferred.
- Use of fresh, wet, and unconfined specimens stored in physiological saline is recommended.
- The method of strain measurement should be carefully considered, taking into account the size and quality of the specimens.
- The strain range and fit used to determine the apparent modulus must be carefully considered due to the non-linear and plastic nature of bone.