Microindentation – a tool for measuring cortical bone stiffness?
A SYSTEMATIC REVIEW

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Objectives
Microindentation has the potential to measure the stiffness of an individual patient’s bone. Bone stiffness plays a crucial role in the press-fit stability of orthopaedic implants. Arm- ing surgeons with accurate bone stiffness information may reduce surgical complications including periprosthetic fractures. The question addressed with this systematic review is whether microindentation can accurately measure cortical bone stiffness.

Methods
A systematic review of all English language articles using a keyword search was undertaken using Medline, Embase, PubMed, Scopus and Cochrane databases. Studies that only used nanoindentation, cancellous bone or animal tissue were excluded.

Results
A total of 1094 abstracts were retrieved and 32 papers were included in the analysis, 20 of which used reference point indentation, and 12 of which used traditional depth-sensing indentation. There are several factors that must be considered when using microindentation, such as tip size, depth and method of analysis. Only two studies validated microindentation against traditional mechanical testing techniques. Both studies used reference point indentation (RPI), with one showing that RPI parameters correlate well with mechanical testing, but the other suggested that they do not.

Conclusion
Microindentation has been used in various studies to assess bone stiffness, but only two studies with conflicting results compared microindentation with traditional mechanical testing techniques. Further research, including more studies comparing microindentation with other mechanical testing methods, is needed before microindentation can be used reliably to calculate cortical bone stiffness.

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Article focus
To provide a comprehensive review on the use of microindentation for measuring cortical bone stiffness.
To assess whether microindentation can accurately measure cortical bone stiffness.

Key messages
Only two studies were found which directly compared microindentation with traditional mechanical testing methods.
These both used reference point indentation.

They showed contrasting results, however, and therefore it is currently unclear whether microindentation can accurately measure cortical bone stiffness.

Strengths and limitations
The study followed guidelines suggested by Cochrane and PRISMA organisations.
It reviews a mechanical testing technique which can further develop clinical practice.
Introduction
Knowledge of a patient’s bone stiffness would enable surgeons to determine the amount of force needed to be applied during impaction of an uncemented implant in joint replacement surgery. With the number of revision operations and periprosthetic fractures increasing each year, it is necessary to reduce any factor that may contribute to implant failure or fracture.

Stiffness is the ability of a structure to resist deformation, and is defined as the slope of the linear, reversible portion of the load-deformation plot. Stiffer bone undergoes less deformation than more compliant bone when subjected to a given load, and hence more force may be needed in order to seat an implant in a patient who has stiffer bone. However, when seated in stiffer bone, the implant will be less likely to loosen because the radial stresses effectively gripping the implant and known as the “elastic grip”, will be greater for a given amount of elastic deformation. Similarly, in less stiff bone, less force is required to seat the implant, but loosening is more likely as the radial stresses are weaker for the same amount of elastic deformation. In the elderly population, the structural stiffness and fracture resistance of bone is reduced due to increasing porosity. This causes a looser fit for a given amount of elastic deformation, and leads to difficulty in achieving a safe press fit without causing a fracture. The elastic interaction between the bone and implant is also critical in ensuring good bony ingrowth.

It is therefore important to understand the mechanical properties of bone so that implants with the appropriate mechanical properties can be chosen for each patient in order to improve implant longevity. Currently, mechanical compression testing is used experimentally to measure bone stiffness, but cannot feasibly be performed in vivo. Dual-energy radiograph absorptiometry (DEXA) has been used to assess stiffness, although it is not sensitive enough to be used as a clinical tool on its own. This is mainly due to cortical bone porosity being fundamental to bone stiffness and DEXA does not have the spatial resolution to detect these pores.

One method that does have the potential to measure bone stiffness is microindentation. Traditional indentation testing involves pressing a hard tip with a known force into a material, and measuring directly or indirectly the resulting hardness impression. Indentation tips may come in several shapes: spherical; three-sided (Berkovich); or four-sided pyramidal (Vickers) (Fig. 1). Macroindentation has been used since the mid-20th century, and recently micro- and nanoindentation methods have been developed. Nanoindentation measures the mechanical properties of bone at the level of trabeculae or osteons, and microindentation has the potential to measure bone properties at the millimetre-scale level. Microindentation of bone can broadly be divided into two categories: traditional depth-sensing indentation and the more recently developed reference point indentation (RPI). Traditional indentation, commonly used for macroindentation, involves pressing a sharp, hard tip into a material and measuring the residual hardness impression under a microscope. Depth-sensing microindentation was developed so that the hardness and modulus of a material could be calculated by indenting it, but without having to use a microscope to measure the resulting hardness impression.
carefully calibrated machine, which must be secured to a surface. From the load displacement data, the elastic modulus and hardness can be calculated with various techniques, including the Oliver-Pharr method.17

Conversely, RPI can be undertaken with a handheld device which uses a secondary probe as a reference point, as it is not fixed to a surface.18 RPI does not calculate stiffness or hardness values, but produces different outputs that are exclusive to the technique, and are described below.

The stiffness of cortical bone is important in orthopaedic surgery, and a reliable method of assessing cortical bone stiffness in patients is needed. Hence, a systematic review of the literature has been conducted to answer the following question: can microindentation accurately measure the stiffness of human cortical bone?

**Materials and Methods**
A systematic review of published literature relating to microindentation of cortical bone was undertaken using Medline, Embase, Cochrane, PubMed and Scopus databases up to November 2016. A combination of the search terms ‘microindentation’, ‘reference point indentation’, ‘indentation (micro)’, ‘bone’, ‘compact bone’, ‘cortical bone’, ‘elastic modulus’, ‘Young’s modulus’, ‘elasticity’, ‘rigidity’ and ‘stiffness’ was used. Exclusion criteria were as follows: studies only using nanoindentation or cancellous bone; studies on animal tissue only; foreign language papers; and papers where the full text could not be accessed. The first two authors selected articles for review, and any disagreements as to whether a paper should be included were resolved via discussion as recommended by the Cochrane Collaboration’s guidelines.19

**Results**
**Search results.** Figure 2 shows the systematic review flowchart, according to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guidelines.20 A total of 1076 abstracts were retrieved, as well as 18 from other sources, amounting to 1094 abstracts to be reviewed. There were 132 abstracts remaining, which after screening and removal of duplicates, left 90 eligible full-text papers. Following review of these 90 papers, 32 papers were deemed to fit the inclusion criteria for testing cortical bone using microindentation. A total of 20 of the studies used RPI (BioDent = 13 and Osteoprobe = 7, both produced by Active Life Scientific, Santa Barbara,
### Table I. Papers included in qualitative synthesis

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<td>20 cycles with peak force of 10 N</td>
<td>Elastic modulus (112 and 49 MPa after NaF)</td>
</tr>
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</table>

BMSi, bone material strength index; CID, creep indentation distance; ED, energy dissipation; IDI, indentation distance increase; LS, loading slope; TID, total indentation distance; US, mean unloading slope; US 1st, first cycle unloading slope; BMD, bone mineral density; MPa, Megapascal; NaF, Sodium fluoride
California) and 12 involved conventional depth-sensing microindentation.

**Qualitative assessment.** Only two papers directly compared microindentation measurements with traditional bulk tissue compression testing and both these studies used RPI. Papers were also retrieved which compared nanoindentation measures of bone mechanical properties with bulk tissue testing measures. As this review is focused on microindentation rather than nanoindentation, these latter papers will not be discussed further. Table 1 displays details of each included study. When extracting results, values were only given for parameters relevant to stiffness of bone in order to comply with PRISMA guidelines.

**Discussion**

Microindentation, which has two main categories: depth-sensing microindentation; and RPI, has been used to evaluate cortical bone stiffness. However, in this systematic review we found only two studies that have validated this technique by comparing microindentation of human bone with other mechanical testing methods, both of which used RPI but had contrasting results.

Currently, there are two instruments using RPI, the BioDent and the OsteoProbe. The BioDent produces parameters such as indentation distance increase (IDI: the difference in depth between the first and last indentation), total indentation distance (TID), and creep indentation distance (CID: progressive indentation distance during the stable force phase of the first indentation cycle at the maximum 10 N force). In contrast, the OsteoProbe has an output, referred to as bone material strength index (BMSi). The BioDent uses one of three 700 mm diameter reference probes which differ in their tip morphology: a tri-bevelled surface (BP1); a bevelled surface with a blunted end (BP2); or a flat concentric surface (BP3). Based on the manufacturer’s recommendations, BP1 probes are ideal for samples with intact soft tissue because the probe can be used to scrape the soft tissue away from the test site, which is important for in vivo studies. BP2 probes are used for ex vivo work on large bones, and BP3 probes are for small animal work. Within each of the three reference probes is a similar test probe (375 mm in diameter, 90° cono-spherical, 2.5 mm tip radius).

Interpretation of RPI values has proven to be problematic. Although no direct comparative studies have been undertaken, it is generally accepted that a higher BMSi value from the OsteoProbe indicates better bone mechanical properties. However, there is disagreement regarding the BioDent, with only two studies directly comparing the BioDent with traditional mechanical testing. Granke et al suggested that bone toughness was inversely related to RPI measurements (IDI and TID), but Krege et al contradicted this when they demonstrated that chemically treated bone, that is by demineralisation, drying, or ashing, was shown to have weaker bone toughness when tested mechanically, but had a decreased IDI and TID. This could be due to the inability of the BioDent to generate enough force to damage the tissue. Furthermore, chemical treatment of the bone may not have affected the bone uniformly, and a greater effect may have been found on the surface where the microindentation was carried out. This may have affected results obtained from microindentation more than those from the mechanical testing, and highlights the complex task of comparing RPI measurements with traditional mechanical testing. Both of these studies tested bone in a hydrated state.

Several reports have focused on interpreting RPI parameters. Two, which used the OsteoProbe, have assessed the relationship between BMSi and occurrence of fracture in patients. However, these studies produced differing results, with one finding no significant correlation while the other reporting that patients with a fragility fracture had significantly reduced BMSi compared with patients with no fracture.

Another important issue with the OsteoProbe is the possible harm it may cause to bone. It has been shown that microcracks form during testing, although it is not yet known what effect this may have on a patient’s bone when testing in vivo. Studies have shown no difference in TID between cycles, and that a large part of IDI is achieved in the first cycle which is not affected by increasing the number of cycles. Beutal and Kennedy, however, suggested that 6 N to 8 N of ten to 25 cycles was sufficient for testing, and does not harm bone. Therefore in fragile bone, future studies will need to focus on this point.

Although this review concentrated on human bone, it is worth reviewing results from animal studies. Using RPI, one study on dog and rat bone compared microindentation with three-point bending and axial compression and concluded that IDI was inversely correlated to toughness, as calculated by mechanical testing. A canine study investigating raloxifene, a drug which is used in the treatment of osteoporosis, showed that increasing concentrations of raloxifene significantly decreased IDI compared with untreated dogs. As previous studies have demonstrated that raloxifene increases bone toughness, IDI may therefore be inversely correlated with toughness.

The level of bone hydration has been shown to affect its mechanical properties. The elastic modulus of vertebral cancellous bone, calculated by microindentation, was reduced when the bone was tested hydrated rather than dry. This is in agreement with nanoindentation studies, which have shown that bone has a lower elastic modulus in a hydrated compared with a dry state. Hence, studies comparing indentation with other forms of mechanical testing need to perform both tests in the same state of hydration in order to ensure a fair comparison.
The direction of indentation, which requires taking into consideration the heterogeneity of bone, is an important variable during microindentation. One study showed that the mechanical properties of vertebral bones are affected by both the indentation direction and region, where it was found that the elastic modulus was higher axially than circumferentially, confirming previous studies undertaken using nanoindentation methods.

RPI has been shown to be sensitive to tissue organisation. For example, stiffness is greater in the longitudinal axis than in the transverse direction. Coutts et al measured the level of heterogeneity in RPI to see whether analysing a small part of the bone is representative of the whole. They found that sites on the same bone yield different values, and therefore in order to assess bone mechanical properties at a region of interest accurately, testing must be undertaken at that specific region.

The depth of the indenter is another factor that needs to be considered. Indentation measurements are sensitive to surface roughness at a lamellar level and testing at a higher depth avoids lamellar heterogeneity. If one orientation of lamellae is favoured, there is a risk of overestimating the mechanical behaviour of bone, thus by indenting several lamellae simultaneously, these risks are reduced. Mechanical properties of lamellae vary significantly across the osteon and thus measuring multiple lamellae provides a mean measurement of the overall bone tissue.

The size of indenter tip is also important. One study compared large and small spherical indenter tips (2 mm and 6.5 mm in diameter), reaching differing depths (0.1 mm and 0.65 mm), and resulting in different elastic moduli (206 MPa and 325 MPa, respectively). Increased stiffness is thought to be due to the smaller indenter making contact with the denser bone near to the surface, while the larger indenter made contact with lower density areas deeper in the bone structure. The larger indenter tip engages with more of the porous bone, which reduces the apparent modulus.

In addition, when attempting to measure the elastic modulus of a material, plastic or permanent deformation should be avoided. Microindentation using a sharp tip will cause some plastic deformation of the bone especially at the tip, which may result in calculating the elastic modulus of damaged bone, rather than unaffected bone. Oyen suggested that this can be overcome using a spherical indenter, where inconsequential plastic deformation occurs if the indentation strain is of a lower value than the yield strain of the bone.

Although indentation was originally designed to measure the hardness of a material, it is also possible to calculate the elastic modulus from the data collected. Most studies use the technique developed by Oliver and Pharr which calculates the elastic modulus from the unloading curve of the load-displacement graph. One disadvantage of using the Oliver-Pharr method is that the technique assumes the unloading response is purely elastic. Due to the time-dependent behaviour of bone, unloading is considered viscoelastic, and some studies have tried to correct for this by introducing a creep hold at peak load. This could still affect results, however, and a model that takes into account the viscoelastic properties of bone needs to be considered.

One of the main obstacles facing microindentation as a testing method is recognising what part of the complex hierarchical structure of bone is being tested. Due to the porous nature of cortical bone at the millimetre scale, the apparent elastic modulus at the millimetre scale will be less than the modulus at a smaller material scale, where pores will not affect its stiffness. By combining indentation with high-resolution imaging, displaying the porosity and structure of the bone, a more accurate measurement of the whole bone structure may be calculated. This has been demonstrated by Hengsberger et al, who combined nanoindentation with synchrotron CT, demonstrating a good prediction of stiffness as measured by traditional mechanical testing.

When trying to assess the millimetre-scale elastic properties of bone using indentation, based on this review of the literature, we would suggest using a large spherical indenter tip, as the spherical tip causes less plastic deformation and the larger tip includes some of the porosity of cortical bone at this length-scale.

In conclusion, microindentation has been used to measure bone mechanical properties such as stiffness and toughness, producing several contrasting results. Indenter tip size, indentation depth and method of analysis are among the factors that affect indentation results. Once these variables have been fully evaluated and put in place, including using large spherical indenter tips, indentation should be assessed against other mechanical testing methods so that the reliability and reproducibility of the technique can be determined. Microindentation may be able to provide clinicians with vital bone quality information, potentially allowing a surgeon to customise the operative technique and implant to suit the mechanical properties of an individual patient’s bone.

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Author Contribution

M. Arnold: Study retrieval, Review write up.

S. Zhao: Study retrieval, Review edit.

S. Ma: Image creation, Review edit.

F. Giuliani: Review edit.


J. F. Cobb: Review edit.


Conflicts of Interest Statement

None declared

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