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Research Paper

Variability in reference point microindentation and recommendations for testing cortical bone: Maximum load, sample orientation, mode of use, sample preparation and measurement spacing

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ABSTRACT

Reference Point Indentation (RPI) is a novel microindentation tool that has emerging clinical potential for the assessment of fracture risk as well as use as a laboratory tool for straight-forward mechanical characterisation of bone. Despite increasing use of the tool, little research is available to advise the set-up of testing protocols or optimisation of testing parameters. Here we consider five such parameters: maximum load, sample orientation, mode of use, sample preparation and measurement spacing, to investigate how they affect the Indentation Distance Increase (IDI), the most published measurement parameter associated with the RPI device. The RPI tool was applied to bovine bone; indenting in the proximal midshaft of five femora and human bone; indenting five femoral heads and five femoral neck samples. Based on the findings of these studies we recommend the following as the best practice. (1) Repeat measurements should be utilised to reduce the coefficient of variation (e.g. 8–15 repeats to achieve a 5–10% error, however

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the 3–5 measurements used here gives a 15–20% error). (2) IDI is dependent on maximum load ($r=0.45$ on the periosteal surface and $r=0.94$ on the machined surface, $p<0.05$), mode of use (i.e. comparing the device held freehand compared to fixed in its stand, $p=0.04$) and surface preparation ($p=0.004$) so these should be kept consistent throughout testing. Though sample orientation appears to have minimal influence on IDI ($p>0.05$), care should also be taken in combining measurements from different orientations. (3) The coefficient of variation is higher ($p=0.04$) when holding the device freehand, so it should ideally be kept supported in its stand. (4) Removing the periosteum ($p=0.04$) and machining the surface of the bone ($p=0.08$) reduces the coefficient of variation, so should be performed where practical. (5) There is a hyperbolic relationship between thickness and IDI ($p<0.001$) with a sample thickness 10 fold greater than the maximum indentation depth recommended, to ensure a representative measurement. (6) Measurement spacing does not appear to influence the IDI ($p>0.05$), so it can be as low as 500 μm. By following these recommendations, RPI users can minimise the potential confounding effects associated with the variables investigated here and reduce the coefficient of variation, hence achieving more consistent testing. This optimisation of the technique enhances both the clinical and laboratory potential of the tool.

1. Introduction

The assessment of bone quality (i.e. its ability to resist fracture) relates to its mechanical, structural and compositional properties (Bouxsein, 2003; Hernandez and Keaveny, 2006). Bone quality has long been used to characterise bone in the laboratory through mechanical testing, structural imaging and compositional analysis. More recently, bone quality (such as analysis of trabecular and cortical structure investigated previously and mechanical assessment of cortical bone focused upon here) has been used in a clinical environment. This emerging assessment of bone quality aims to overcome the limitations of current fracture risk assessment tools, which are classically centred on bone quantity, specifically bone mineral density (BMD). That is, BMD has poor sensitivity, identifying only a small proportion of those who go on to fracture (Schuit et al., 2004; Siris et al., 2004). Bone quality also relates to fracture risk (Nalla et al., 2006; Burstein et al., 1976); therefore the clinical assessment of bone quality may improve future fracture risk prediction.

Reference Point Indentation (RPI) is a newly introduced tool with the potential for clinically assessing bone quality and is already showing some preliminary potential to differentiate between fractured and non-fractured human bone (Diez-Perez et al., 2010; Gueerri-Fernandez et al., 2013). The RPI tool incorporates a hypodermic needle-like reference probe that can be inserted through soft tissue to anchor against the surface of the bone, aiming to avoid the requirement for surface preparation. A small, 350 μm diameter, internal test probe then indents into the bone to measure a material property of the bone, though the exact property being assessed is still unclear (Diez-Perez et al., 2010; Gallant et al., 2013; Granke et al., 2014). The tool currently exists in two forms: the Biodent™ tool, used here, and the Osteoprobe™, used primarily for in vivo studies. With the Biodent ™ system the probe cyclically indents into the bone over a number of force cycles. In contrast, once a preload is achieved, the Osteoprobe™ applies a single rapid impact indentation. To date the two RPI tools have shown some clinical potential following their application in five studies in vivo; in humans (Diez-Perez et al., 2010; Gueerri-Fernandez et al., 2013; Randall et al., 2013; Farr et al., 2014), dogs (Aref et al., 2013) and horses (Randall et al., 2013) where it was reported that subjects, under local anaesthetic, experienced no pain or subsequent complications. The same characteristics that give the device its clinical potential, i.e. testing of a small area and purportedly requiring minimal sample preparation, also mean that the RPI tool has use in the laboratory as a potentially quick and simple means of characterising bone. For this reason, the tool has also been applied to an increasing number of studies examining the mechanical behaviour of human (Granke et al., 2014; Milovanovic et al., 2014; Kaye et al., 2012; Hansma et al., 2008; Hansma et al., 2006; Thurner et al., 2009) and animal (Hammond et al., 2014; Sharma et al., 2014; Setters and Jasiuk 2014; Rasoulian et al., 2013; Bridges et al., 2012; Randall et al., 2009; Gallant et al., 2011) bone, in vitro.

Despite the emerging use of the technique, there has been little published work characterising the variability associated with the device and establishing recommendations for optimally carrying out measurements. Hence, new users of the RPI tools have limited points of reference for selecting optimal parameters and establishing a test protocol. Nevertheless, Bridges et al. (2012) have considered variability with the somewhat similar Osteoprobe™ device that uses a single rapid indentation cycle and does not use a physical reference probe. The study gives recommendations that the probe is within 10° of the normal and that the tip radius is sharper than 10 μm, which are both directly relevant suggestions to RPI testing with the device discussed here (Biodent Hfc™).

Three other publications have also touched on some of the parameters associated with use of the device that may produce variability. Hansma et al. (2008), in one human donor, suggested that removal of the soft tissue did not affect the indentation depth. Though there was a decrease in indentation depth with