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Research Paper

Towards a standardized reference point indentation testing procedure

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ABSTRACT

We study the reference point indentation (RPI) technique which has a potential to directly measure mechanical properties of bone in patients. More specifically, we tested 6 month swine femoral cortical bone at mid-diaphysis region to investigate the effect of several testing variables on the RPI outputs. They include the force magnitude, preconditioning, variation within a sample and between samples, number of cycles, indentation surface (transverse versus longitudinal, polished versus unpolished), and micro-computed tomography radiation exposure. The force magnitude variation test shows that all RPI parameters increase linearly with the increasing force magnitude except the indentation distance increase which shows a cubic trend with a plateau for force magnitudes between 4 N and 8 N. Preconditioning does not affect the trends for a force magnitude variation test. The cycle variation test shows that most RPI parameters reach either a maximum or minimum at 15–20 cycles. Transverse surface measurements are more consistent than the longitudinal surface measurements, but a rough surface and periosteum on the longitudinal surface could account for this difference. Exposure to the micro-computed tomography radiation in general does not have effect on the RPI measurements. For the 6 month swine femoral cortical bone, testing using 6 N force and 20 cycles with preconditioning on an unpolished longitudinal surface is recommended. This study advances our knowledge on how the RPI testing variables influence the RPI outputs and provides guidance on the RPI measurements. It may also serve as a framework for developing a standardized testing procedure for the RPI technique.

1. Introduction

Reliable prediction of bone strength is an outstanding medical challenge. A novel microindentation technique, known as the reference point indentation (RPI) technique (Hansma et al., 2008, 2006; Randall et al., 2009), has potential to directly measure cortical bone properties in patients (Diez-Perez et al., 2010; Gueerri-Fernandez et al., 2013). The RPI technique consists of a reference probe, which stays on the bone surface, and a test probe, which moves relative to the reference probe while indenting the bone (Fig. 1). The RPI technique involves successive indentation cycles which create local damage in the bone. Published studies on the RPI technique have either compared the RPI outputs obtained by testing bone in different conditions (e.g. healthy versus diseased) or studied relationships between the RPI outputs and properties of cortical bone

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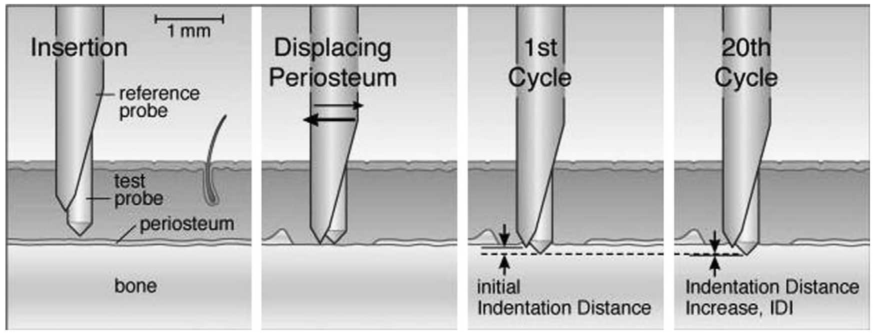

Fig. 1 – Diagram of an RPI test (Diez-Perez et al., 2010).

measured using traditional mechanical tests (Diez-Perez et al., 2010; Gallant et al., 2013; Hansma et al., 2008; Randall et al., 2009; Rasoulian et al., 2013). While comparing outputs does not necessitate a standard testing procedure, such a standard would be beneficial towards developing relations between the RPI outputs and mechanical properties of bone. This is due to the fact that various factors such as the force magnitude, preconditioning, number of cycles, variation within and between samples, indentation surface, and radiation exposure can influence the RPI outputs. Thus, in this paper we are investigating the effect of the above factors on the RPI outputs to provide guidance on the RPI testing. Our goal is to develop the framework for a standardized testing procedure for the RPI technique.

2. Materials

All samples were prepared from 6 month swine femurs provided by the Meat Sciences Department at the University of Illinois at Urbana-Champaign. Seven femurs from four different animals were used for this study. One femur was used for each test except for the sample variation tests that used two femurs to compare the data obtained by testing cortical bone from two different animals. The femurs were wrapped in a phosphate-buffered saline (PBS) soaked gauze and stored frozen at $-20\,^{\circ}$C until analysis. Bones were allowed to thaw for 24 h at 4 °C before sample preparation and testing. Samples were taken from the mid-diaphysis region of femurs. Each sample was divided into four quadrants: posterior, medial, anterior, and lateral. Transverse and longitudinal surfaces were prepared. The longitudinal surface is the outer surface of the bone and the transverse surface is the surface created by cutting the bone perpendicular to bone's long axis. The longitudinal surfaces were cleared from soft tissue and either polished or remained unpolished, depending on the test, while all transverse surfaces were polished. The polishing procedure involves a succession of finer grit sandpapers and polishing cloths and powders. The polishing stages were as follows: P180, P280, P400, P800, P1200, P2400, and P4000 sandpaper followed by micron cloth/powder combinations of 1 μm/1 μm, 0.25 μm/0.3 μm, and 0.25 μm/0.05 μm. Bone samples were kept wet during the RPI testing by placing few drops of PBS on the bone surface just prior to the RPI testing. Additional sample preparation details specific to each test are described in the Methods section. The BioDent™ Hfc reference point indentation instrument (Active Life Scientific, Inc., Santa Barbara, CA) was used for all microindentation tests.

3. Methods

3.1. *Data analysis*

For each comparison test the following RPI outputs were obtained: the first indentation distance (ID1), first creep indentation distance (CID1), first unloading slope (US1), total indentation distance (TID), indentation distance increase (IDI), average creep indentation distance (AvCID), average energy dissipated (AvED), average unloading slope (AvUS), and average loading slope (AvLS). Definitions of these quantities are given in Table 1. Figs. 1 and 2 provide graphical representations of the RPI technique and the RPI parameters. With the exception of the preconditioning, radiation exposure and sample variation test, one-way analysis of variance (ANOVA) was used to determine if the RPI outputs were significantly different for the studied variable in each comparison test. A Tukey test was then used to determine which means were significant. A confidence level of 95% ($p<0.05$) was considered statistically significant. For the radiation exposure test, a two-parameter *t*-test with the same confidence level was used. The statistical analyses were performed using ORIGINPRO v9.0 statistical analysis and graphing software (OriginLab Corp., Northampton, MA).

3.2. *Force variation*

The effect of force magnitude on the RPI measurements was investigated using a 2.5 cm long section of the mid-diaphysis of a femur. The only factor varied was the indentation force. The force magnitudes included 2 N, 4 N, 6 N, 8 N, and 10 N. The number of cycles was kept constant at ten cycles and the indentation frequency was 2 Hz. The surface that was indented was polished using the procedure described in Section 2. Indents were performed on the polished longitudinal surface in the anterior and medial quadrants. These quadrants were chosen due to the already smooth nature of these surfaces before polishing. Ten indents were made along the length of the sample for each force magnitude. The indents were spaced approximately 2 mm apart.