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Indentation across interfaces between stiff and compliant tissues ☆

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ABSTRACT

Bone–tendon, bone–ligament and bone–cartilage junctions are multi-tissue interfaces that connect materials that differ by two orders of magnitude in mechanical properties, via gradual variations in mineral content and matrix composition. These sites mediate load transfer between highly dissimilar materials and are consequently a primary site of injury during orthopedic failure. Given the large incidence rate and the lack of suitable surgical solutions for their regeneration or repair, characterization of their natural structure and subsequent replication through tissue engineering is important. Here, we evaluate the ability and accuracy of instrumented indentation to characterize the mechanical properties of both biological tissues and engineered scaffolds with interfaces between materials that contain significant changes in mechanical properties. In this study, finite element simulations and reference samples are developed that characterize how accurately indentation measures the modulus of a material as it varies with distance across a continuous interface between dissimilar tissues with multiple orders of magnitude difference in properties. Finite element simulations accurately predicted discrepancies between the modulus function across an interface observed by indentation and the true modulus function of the material and hence allow us to understand the limits of instrumented indentation as a technique for quantifying gradual changes in material properties. It was found that in order to accurately investigate mechanical property variations in tissues with significant modulus heterogeneity the indenter size should be less than 10 percent of the expected length scale of the modulus variations.

Statement of Significance

The interfaces between stiff and compliant orthopedic tissues such as bone–tendon, bone–ligament and bone–cartilage are frequent sites of failure during both acute and chronic orthopedic injury and as such their replication via tissue engineering is of importance. The characterization and understanding of these tissue interfaces on a mechanical basis is a key component of elucidating the structure-function relationships that allow them to function naturally and hence a core component of efforts to replicate them. This work uses finite element models and exeperiments to outline the ability of instrumented indentation to characterize the elastic modulus variations across tissue interfaces and provides guidelines for investigators seeking to use this method to understand any interface between dissimilar tissues.

1. Introduction

Connective tissues in the musculoskeletal system are subdivided into tendons (bone–muscle interactions in tension), ligaments (bone–bone interactions in tension) and cartilage (bone–bone interactions in compression). These interfaces are a primary site of injury in the musculoskeletal system. In the US alone there are 600,000 rotator cuff tendon surgeries [1], 100,000 anterior cruciate ligament reconstructions [2] and 580,000 surgeries relating to osteoarthritis of the hip per year [3]. Yet, the bulk of current orthopedic tissue engineering efforts have focused on the repair of single homogeneous tissues such as bone [4] or tendon [5]. A small number of authors however, have focussed on the regeneration of the graded interface between bone and cartilage [6–9], or bone and ligaments or tendons [10,11]. Successful replication of the interfaces between different musculoskeletal tissues is essential for restoring proper joint function after injury, as merely juxtaposing ligament and bone during surgery does not result in regeneration of the natural multi-tissue interface [12–14]. In order to satisfactorily replace either the bone–tendon, bone–ligament or bone–cartilage interface after injury, graded tissue engineering scaffolds that

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properly mimic natural interface mechanical properties, will be required (*e.g.* [6–9,11,15–17]). Tissue engineering scaffolds must mimic the natural mechanical properties on both a macroscale to replace function [18,19], and on a microscale to control cell differentiation [20,21]. A prerequisite for a successful replication of these interfaces is a thorough understanding of the chemical and mechanical variations across the natural structure; the study of the mechanical component of this through indentation is the focus of the work contained herein.

Nanoindentation has been widely used to map the elastic modulus of heterogeneous materials [22] including natural materials such as teeth [23], cephalopod beaks [24] and coccinellidae feet [25], or engineering materials such as ceramic composites [26], metal grains [27] and thin film inclusions [28]. These studies frequently test stiff synthetic materials or stiff natural tissues with elastic moduli in the GPa range, as this is the optimal operating region of the majority of commercial nanoindentation instrumentation [29]. For example, Gupta et al. used nanoindentation to measure modulus across a tissue interface, the patella-cartilage osteochondral junction [30]; the tissue was dehydrated prior to testing, bringing the modulus into the optimal range of the instrumented indentation and reducing viscoelasticity [31,32]. The region over which elastic modulus and mineral content changed from the stiff to compliant tissue was found to be approximately 30μm wide. Abraham and Hauch [33,34] used nanoindentation to determine the change in elastic modulus through the insertional zones of human meniscal cartilage on hydrated samples using a spherical tip. In both studies, the transition region was found to be 200–400μm wide, similar to the width of the total tissue transition from tendon to bone through unmineralized and mineralized fibrocartilage; while being the width of the histological tissue transition this is not necessarily the width of the mechanical transition. The ability of instrumented indentation to be used as a tool to quantify small-scale variations in material properties depends on two characteristic lengths scales, the diameter, d, of the contact area between indenter and sample, and the width, W, over which significant changes in elastic properties occur across the sample. The contact patch diameter, d, is related to the indenter radius, R, via the indentation depth, h:

$$d = 2\sqrt{hR} \tag{1}$$

where d/W will be used to define the limits of the ability of instrumented indentation to map mechanical properties across elastic modulus gradients between stiff and compliant materials.

Hydrated biological tissues, which are generally more compliant than engineering materials, practically require large indenter tips in order to generate sufficient force on the test equipment in the range of displacements available in commercially available indenters [29]. As a consequence, the size of the contact patch between indenter and sample increases and a larger volume of material is deformed during a single indent. This reduces the spatial resolution of a modulus map across a sample, as the sampling volume of each indent is increased and hence a larger distance is required between successive indents for them to remain independent. As a consequence, transitions between stiff and compliant tissues can appear wider than their true size due to feature blurring introduced by measurement via indentation mapping. In order to demonstrate and quantify this effect, a finite element (FE) model of a line of indents across an interface between stiff and compliant materials is developed. This model is used to show how the function of modulus that is observed, $E_O(x)$, can be different from the true modulus function, $E_T(x)$, of the material, where x is the perpendicular distance from the interface. The difference between true and observed modulus functions is investigated as a function of the width of the true transition in modulus in the sample and the relative size of the indenter contact patch, defined by d/W. Experimental indentation data from a selection of interface samples is used to validate the results of the model. Finally, the FE model is used to analyze literature examples of indentation across biological interfaces and the implications for future studies are discussed.

2. Methods

2.1. Finite element model

A two-dimensional, linear, finite element model of an elastic solid in contact with a spherical indenter was constructed and meshed using ABAQUS 6.11 (Simula, Providence, RI, USA). The solid was modeled as having a gradient in elastic modulus that varied in the transverse direction with a sigmoid shaped logistic function to simulate a continuous interface between two dissimilar materials. The spherical indenter was modeled as a rigid body with one millimeter radius which contacted an elastic solid half space 40 mm wide by 20 mm tall at its top centre (Fig. 1). A refinement study of the width and height of the elastic solid showed that both were sufficiently large compared to indenter radius and indentation depth, respectively, to allow the results to be considered free of edge effects. The elastic solid was rigidly fixed at the base plane with an encastre boundary condition and unconstrained on the top and sides. The simulated indentation depth was chosen to control the characteristic indentation strain, ϵ, [35] where:

$$\epsilon \approx 0.2(h/R)^{1/2} \tag{2}$$

The simulated indentation depth was 0.0625mm, giving a characteristic indentation strain of 5%.

The elastic solid had a continuous quad mesh biased towards the contact point of the indenter. Directly under the contact patch in a 5 mm × 5 mm region the mesh elements were 0.01 mm × 0.01 mm and at the far edges of the sample, the elements were 0.5 mm 0.01 0.5 mm. A mesh refinement study in which the entire solid was meshed with uniform sized elements 0.01 mm square demonstrated that the mesh used for the study did not differ by more than 1% from the solution obtained with the finer, uniform mesh.

The elastic solid had a Poisson's ratio of $\nu = 0.4$, comparable to that of orthopedic biological tissues such as cartilage and bone [36,37]. A range of Poisson's ratio's from $\nu = 0.3$ to $\nu = 0.49$ were also tested in simulation and did not show a significant effect on the results. The transition in elastic modulus across the interface

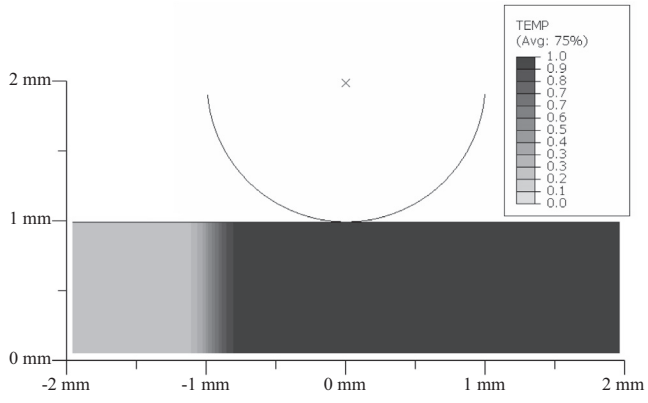

Fig. 1. A typical temperature field in the sample used to induce an elastic modulus field. The light to dark greyscale transition across the sample signifies the temperature change from 0 → 1. The modulus sigmoid in this figure is centered −1 mm from the indentation location.