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Research Paper

Investigating a continuous shear strain function for depth-dependent properties of native and tissue engineering cartilage using pixel-size data

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ABSTRACT

A previously developed novel imaging technique for determining the depth dependent properties of cartilage in simple shear is implemented. Shear displacement is determined from images of deformed lines photobleached on a sample, and shear strain is obtained from the derivative of the displacement. We investigated the feasibility of an alternative systematic approach to numerical differentiation for computing the shear strain that is based on fitting a continuous function to the shear displacement. Three models for a continuous shear displacement function are evaluated: polynomials, cubic splines, and non-parametric locally weighted scatter plot curves. Four independent approaches are then applied to identify the best-fit model and the accuracy of the first derivative. One approach is based on the Akaiki Information Criteria, and the Bayesian Information Criteria. The second is based on a method developed to smooth and differentiate digitized data from human motion. The third method is based on photobleaching a predefined circular area with a specific radius. Finally, we integrate the shear strain and compare it with the total shear deflection of the sample measured experimentally. Results show that 6th and 7th order polynomials are the best models for the shear displacement and its first derivative. In addition, failure of tissue-engineered cartilage, consistent with previous results, demonstrates the qualitative value of this imaging approach.

1. Introduction

Native articular cartilage (AC) has a unique stratified structure and composition, which gives rise to depth-dependent mechanical properties (Buckley et al., 2008, 2010; Buckwalter and Mankin, 1997; Chen et al., 2001; Lopez et al., 2008). Structure and composition also vary during maturation and are thought to influence the function of cartilage *in vivo* (Canal et al., 2008; Hunziker et al., 2007). In contrast, tissue engineered (TE) cartilage that is being developed as a potential treatment for damaged articular cartilage lacks the compositions, structure and mechanical properties of native tissue. Such differences could limit the clinical success of an implanted construct where it must function in the highly loaded environment of a diarthrodial

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joint. Properly functioning TE cartilage may require the depth-dependent zonal architecture and composition of native tissue.

Previous studies in our labs showed failure of TE cartilage under combined cyclic shear and static compressive loads, while native cartilage remained intact when tested under the same conditions (Jayaraman, 2010; Whitney et al., 2010a; Wong and Sah, 2010). Subsequent investigations identified a cell rich (matrix deficient) region in the middle layer of TE cartilage. From these and other studies, it is clear that the depth-dependent properties of TE cartilage do not replicate those of native tissues (Whitney et al., 2010a, 2012a). These observations provided the motivation for investigating depth-dependent properties of native and TE cartilage, which may play an important role in understanding how cartilage function is related to zonal arrangement.

Depth-dependent properties of AC are often evaluated using methods based on optical imaging. Methods that depend on tracking the displacement of low-density markers such as chondrocytes may limit spatial resolution of material properties (Buckley et al., 2010, 2008; Wong et al., 2008a, 2008b). An alternative, with potentially higher resolution, is to track lines photobleached on the tissue (Bruehlmann et al., 2004; Buckley et al., 2010). All of these techniques require cutting the tissue to expose a surface through its thickness, which is then imaged (Bruehlmann et al., 2004; Buckley et al., 2008, 2010; Canal et al., 2008; Hosoda et al., 2008; Schinagl et al., 1997). In all of these approaches, digitizing the displacement of features introduces noise, and computing the derivative of the shear displacement field (*i.e.* shear strain) introduces additional noise into the final estimate of material properties. Typically, some level of smoothing is needed to minimize the effects of noise and produce usable results. For example, Buckley et al. chose to smooth data by using average intensity across rectangular 20 μm deep regions, and then calculated the shear strain using numerical differentiation. Since numerical differentiation increases noise, they introduced weighting schemes that reduced noise (Buckley et al., 2010). Choosing a region that was greater or less than 20 μm, or choosing different weighting factors would have produced smoother or noisier results. Since noise is inherent in experimental measurements and numerical differentiation, determining a level of smoothing that produces accurate results is an ongoing issue (Lesh et al., 1979; Pezzack et al., 1977; Winter et al., 1974).

Based on our previous experience with failure of tissue engineered cartilage and a general interest in mechanical evaluation of native and tissue engineered cartilage, we implemented an experimental approach for determining depth dependent shear properties of cartilage similar to that described in Buckley et al. (2010). Initial measurements showed that the full thickness deformation field for native articular cartilage in shear resembled a smooth, sigmoid-shaped curve. This raised the possibility of modeling the displacement field and shear strain using analytical functions rather than the common approach which is to smooth the data use numerical differentiation. Therefore, the purpose of this investigation was to validate a systematic method for processing displacement data and taking its first derivative. As a result, in this study, we investigate the feasibility of using a continuous, depth-dependent displacement function calculated from high-resolution pixel-dependent displacement data, and calculate shear strain by taking its first derivative analytically, thereby eliminating the noise due to numerical differentiation. This method was validated by comparison with known results.

2. Materials and methods

2.1. *Bovine AC*

Native bovine AC was used during the development of the image processing technique. Samples were harvested from femoral condyle of a 2-year-old cow ($n=7$, Halal Meat, Cleveland, OH). Osteochondral samples were removed from the whole bone using a 6 mm diameter-coring tool. Cartilage disks are then removed from the core using a scalpel, and bisected into semi-cylinders and frozen once prior to use (Fig. 1).

2.2. *Tissue engineered cartilage*

To investigate a possible failure mechanism identified in previous investigations (Whitney et al., 2010a) scaffold-free engineered cartilage constructs were generated. Briefly, rabbit articular chondrocytes were isolated and culture expanded as described (Whitney et al., 2012b). Four cartilage sheets were formed after second passage, by seeding 3.125×10^6 cells, onto porous (10 μm pore diameter) polyester membranes (PET1009030, Sterlitech, Kent, WA) held in place and submerged in medium by custom 4 cm × 4 cm chambers (Weidenbecher et al., 2008; Whitney et al., 2012b). Constructs were cultured for 4 weeks in the chambers and then transferred to 100 mm petri dishes, where they were allowed to float freely for an additional 4 weeks (Whitney et al., 2010a, 2010b, 2012a). The resulting TE cartilage sheet was cut into 4 mm × 6 mm rectangular samples to fit into the heads of holding arms of the apparatus, and frozen.

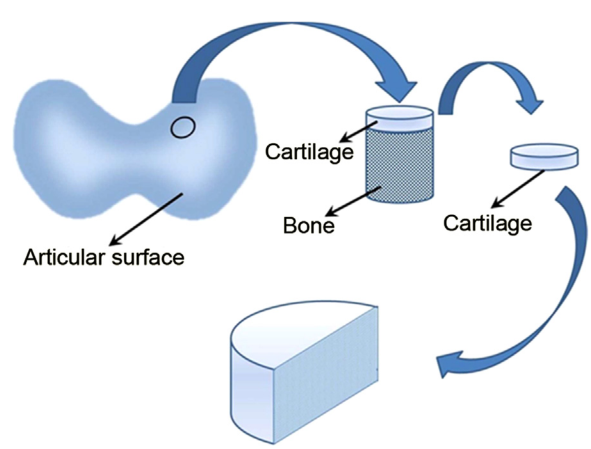

Fig. 1 – Schematic of sample preparation for native AC biaxial mechanical testing. Cartilage disk is removed from the 6 mm diameter bone plug, and bisected into semi-cylinders.