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Nanoindentation of hydrated materials and tissues

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ABSTRACT

Nanoindentation techniques have recently been adapted for the study of hydrated materials, including biological materials and hydrogels. There are unique challenges associated with handling and testing hydrated samples. For hydrated materials, a poroelastic or poroviscoelastic analysis, which explicitly treats the fluid flow through the porous material, is used to extract material properties from experimental data. Some key results from recent works using nanoindentation to evaluate hydrated materials are reviewed in the context of these challenges. Finally, as these studies represent relatively recent developments in the nanoindentation field, an outlook for the future is presented, in which it is clear that a consensus is emerging for quantitative evaluation of hydrated materials via a modified nanoindentation approach.

1. Introduction

For more than three decades, nanoindentation testing has been established as an effective tool for measuring the mechanical properties of materials [1–4]. Nanoindentation is a form of depth-sensing indentation (DSI) testing, in which the full force–displacement–time response is monitored during a contact mechanics experiment, which became popular for measuring material properties at depths of tens to hundreds of nanometers. The majority of early nanoindentation studies focussed on the evaluation of the properties of relatively stiff and hard elastic–plastic engineering materials, such as metals, ceramics, glasses and semiconductors. The most commonly reported material parameters are the (plane strain) elastic modulus E' and the hardness H – the resistance to (plastic) deformation. A key enabling breakthrough that led to the widespread adoption of nanoindentation testing was the development of techniques [1,2,5] for the straightforward deconvolution of E' and H from a single nanoindentation test, one typically performed using a sharp Berkovich diamond indenter probe.

The DSI approach was particularly effective for measuring small volumes of material, allowing for quantitative evaluation of thin film mechanical properties [6–8], or for mapping properties across inhomogeneous materials at high spatial resolution [9]. Analyses for elastic indentation of a half-space, based on isotropic elasticity [10] were adapted not just for thin layers and coated systems [6–8] but also for elastically anisotropic materials [11]. In addition to nanoindentation experiments, significant effort has been expended on computational modelling of elastic–plastic indentation in particular [12]. This is true of both "forward" simulations, in which the indentation load–displacement response is predicted for a given set of material properties (E', H, hardening law) and of the "inverse" problem, in which attempts are made to extract properties uniquely from load–displacement data.

Following the establishment of robust nanoindentation techniques for characterising engineering materials, the approach was adapted for testing less stiff materials, including bulk polymers [13], polymer coatings [14,15], and biological materials [16,17]. For reasonably stiff materials, including hard and dehydrated biological materials such as bone [18], tooth enamel [19], and plant seed [20], few experimental or analytical adaptations were required, and nanoindentation testing allowed for the spatial mapping of the elastic stiffness of tooth enamel [21]and for the evaluation of elastic anisotropy in bone [22] based on Berkovich indentation with Oliver–Pharr [2] data analysis assuming elastic unloading. However, three sets of challenges have emerged in the context of hydrated materials, and these have prevented the establishment of a standardized testing routine for nanoindentation measurements of material properties. First, there are inherent challenges due to the fact that the samples are hydrated and the instrumentation was designed for testing dry samples. Second, many-although not all-hydrated materials are significantly less stiff than typical non-hydrated engineering materials. Third, there is no consensus on the appropriate data analysis for interpreting data obtained from tests on hydrated samples. Thus, these three factors taken together have limited the development of routine nanoindentation testing of hydrated materials and tissues, and

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each will be described here in turn. We will conclude with an outlook for the future of nanoindentation of hydrated materials.

2. Hydrated materials

Two types of hydrated materials will be considered here: biological tissues and hydrogels. "Biological tissues" is a broad term encompassing both plant and animal tissues, in which the fundamental make-up of a tissue is biological cells plus extracellular matrix materials [23,24]. The categories of biological tissues can be further sub-divided into "hard" and "soft" tissues, where the words hard and soft do not imply anything with respect to hardness or plastic deformation. Hard tissues, such as bone [18,22], calcified cartilage [25], enamel [19,21] and dentin [26] in teeth, or nacre in sea shell [27], contain significant biomineral content, such as calcium phosphate or calcium carbonate. Soft tissues such as cartilage [28] and artery [29] are non-mineralised in their healthy state. Mammalian tissues have been studied using nanoindentation largely in the context of biomedical applications, informing the community about the disease process in conditions such as osteoporosis [30], and evaluating the effect of drugs or treatments for disease [31]. Other nonclinical research involves elucidating basic structure-properties relationships in natural materials more generally, including both mammalian and non-mammalian animal tissues and plant tissues.

Hydrogels are hydrophilic polymeric materials in which the polymer chains are allowed to swell in water. The polymer can be synthetic or natural in source and the polymer cross linking can be chemical or physical [42]. There are two components to the total water content, water that is tightly bound to the polymer network and water that is free to move through small pores within the polymer network (Fig. 1) [32]. Because of their large water contents, hydrogels are quite biocompatible, and have been used as materials for soft contact lenses [33], coatings on medical devices [34], and wound dressings [35]. It is anticipated that the use of hydrogels in medicine will continue to expand for applications such as drug delivery applications, diagnostics, and in tissue engineering [36]. Hydrogels have also increasingly been used in basic-science biological studies investigating cell-material interactions [37], including those in which gel mechanical properties are varied systematically in order to study mechanical influences on stem cell differentiation [38,39]. The mechanical behaviour of hydrogels has long been recognized as fundamentally important but fundamentally lacking due to the large volume occupied by water [40]. As such, recent studies have considered hydrogel composites [41] to try and improve on the baseline material properties of hydrogels while maintaining their biocompatibility. Nanoindentation has been used to characterise the material properties of hydrogels, which can be difficult to "grip" for traditional mechanical testing due to their compliance and hydrated state [42].

3. Testing hydrated samples

For nanoindentation experiments on hydrated samples, the state of material hydration must be maintained during the test. There are at least three basic ways that this has been done. Samples can be fully hydrated in fluid and then placed into the chamber of the nanoindenter, such that testing takes place quickly before the fluid evaporates. A time-frame for testing can be established by weighing samples at multiple time-points on removal from a fluid bath to check for evaporation. This approach demonstrated no loss of mass for polyacrylamide hydrogels in the first hour in air [43], establishing an acceptable time-frame for testing of just under one hour. Samples of both hydrogels and soft biological tissue have been tested when surrounded by a hydrating foam

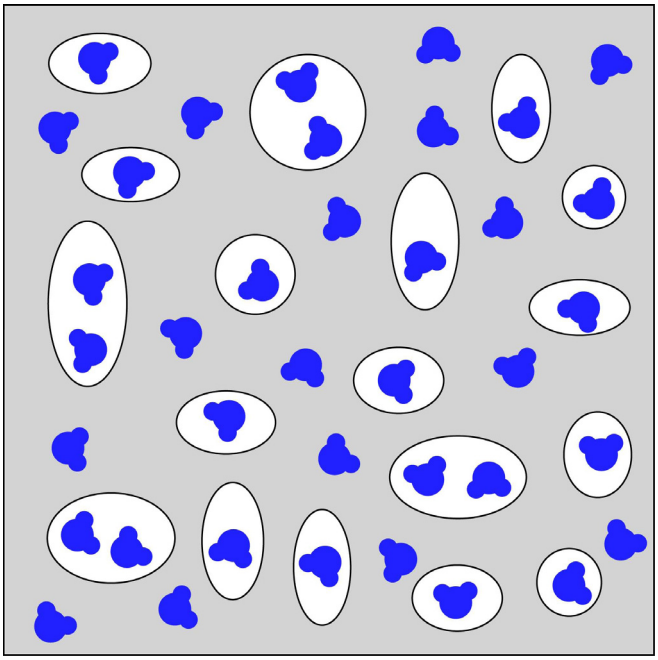

Fig. 1. A schematic illustration of the cross-section a hydrated poroelastic material. There is bound water in the porous solid skeleton and free water in the pore space. On application of a force, the free water moves in response to pressure gradients; the pores are interconnected and accessible to the external fluid environment.

layer, with weight-loss studies demonstrating that the samples were maintained in a hydrated state identical to fully submerged samples over the course of eight hours [29]. Samples can also be tested while fully submerged in fluid [44], although this can cause challenges as capillary forces have been shown to interfere with sample surface detection [45]. A detailed and quantitative exploration of the capillary forces has been performed as a function of indenter geometry [46]. Special indenter probes with longer than typical shafts are often used for fluid-immersed samples, to increase the distance between the fluid and the electronics of the DSI transducer.

A maintenance of hydration state allows for comparisons between nanoindentation results for hydrated versus dehydrated samples of the same material. Bone is approximately 20% water by volume, but a number of studies have demonstrated that dry bone is stiffer than hydrated bone with as much as an order of magnitude difference in elastic modulus [18,22,25,47–50]). A systematic study of the influence of hydration on the same bone samples showed that the differences can in part be explained by differences in probe geometry and data analysis method between different studies [51] (Fig. 2). However, when wet and dry samples were compared using the same probe geometry and data analysis method, plane strain modulus values for wet bone were 60–80% of those observed for the same dry bone [51]. Even more striking, reported hardness values for wet bone were only 30–35% of those for dry bone, illustrating that the total deformations are much greater when the bone is hydrated. This hardness difference was consistent with the observation that total indentation displacement was greater in wet than in dry tooth dentin, giving smaller hardness values and less normalised energy dissipation during the indentation cycle [26]. Most of the literature on bone and tooth nanoindentation considers dry samples, which means the absolute values reported for properties cannot be considered quantitative. Similarly, if even more dramatic, observations have been made for hydrogels, where hydrogels with originally 80% water were three orders of magnitude stiffer when dehydrated in either air or by immersion in ethanol [43].