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Research Paper

Mechanical characterization of oligo(ethylene glycol)-based hydrogels by dynamic nanoindentation experiments

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ABSTRACT

Oligo(ethylene glycol)-based (OEG) hydrogel samples of varying cross-link densities and degrees of swelling were characterized through dynamic nanoindentation testing. Experiments were performed using a non-standard nanoindentation method, which was validated on a standard polystyrene sample. This method maximizes the capability of the instrument to measure the stiffness and damping of highly compliant, viscoelastic materials. Experiments were performed over the frequency range of 1 to 50 Hz, using a 1 mm diameter flat punch indenter. A hydration method was adopted to avoid sample dehydration during testing. Values of storage modulus (E') ranged from 3.5 to 8.9 MPa for the different OEG-hydrogel samples investigated. Samples with higher OEG concentrations showed greater scatter in the modulus measurements and it is attributed to inhomogeneities in these materials. The (E') values did not show a strong variation over frequency for any of the samples. Values of loss modulus (E'') were two orders of magnitude lower than the storage modulus, resulting in very low values of loss factor ($E''/E' < 0.1$). These are characteristics of strong gels, which present negligible viscous properties.

1. Introduction

Hydrogels are hydrophilic polymer networks swollen by water or aqueous solutions, which makes them candidate materials for several biomedical applications that require materials mimicking soft tissues (Peppas et al., 2000; Lin and Anseth, 2009; Hu et al., 2012; Shapiro and Oyen, 2014). As an example, oligo(ethylene glycol)-based hydrogels present characteristics such as non-cytotoxicity, protein repellency, and compatibility with tissues or blood, which created significant interest in exploring their suitability for applications such as controlled drug delivery and regeneration of

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damaged articular cartilage (Lum and Elisseeff, 2003; Gläber et al., 2009; Lin and Anseth, 2009).

When used as tissue replacements, hydrogels are designed to mimic the mechanical behavior of the natural tissue and its response to the complex loading conditions endured by the body (Ahearne et al., 2005; Franke et al., 2007, 2008). In drug or cell delivery applications, mechanical properties are important since the delivery devices must maintain their structural integrity to protect drugs or cells until they are released into the body (Franke et al., 2007). In addition, in applications where gels are used as matrices for the differentiation of stem cells, it has been reported that the elastic properties of the gels strongly influence the lineage specification of mesenchymal stem cells (Engler et al., 2006). Clearly, the successful biomedical application of hydrogels largely depends on the ability to tune, as well as accurately and precisely characterize their mechanical behavior.

A variety of conventional methods have been used to characterize the mechanical properties of hydrogels. Anseth et al. (1996), Peppas and Merrill (1977) suggested the use of tensile tests, together with the theory of rubber elasticity, to evaluate the mechanical response of hydrogels. Dynamic Mechanical Analysis (DMA) was used by Cauich-Rodriguez et al. (1996) to characterize the viscoelastic properties of hydrogel blends over the frequency range of 0.1 to 50 Hz. Additionally, uniaxial confined or unconfined compression has been extensively used due to the ease of sample preparation and simple test methodology (Iza et al., 1998; Thomas et al., 2004; Roberts et al., 2011). While these test methods are among the types needed to investigate the hydrogels constitutive behavior in the time and frequency domains, the wide variety also reflects the fact that each technique has limitations. In the case of hydrogels, test methods can and often do produce unreliable data due to the high compliance and time-dependent deformation of these materials. Their elastic moduli vary from tens of kPa to a few MPa, which requires high-resolution load measurements. In addition, difficulties in the preparation of macroscopic samples (Oyen, 2014), problems with sample fixation during testing (Hu et al., 2012; Oyen, 2014) and the need for testing these materials in the hydrated state (Oyen, 2014) present a number of challenges in generating reliable data that can be used to determine their mechanical behavior (Hu et al., 2012; Oyen, 2013, 2014).

Recently, instrumented nanoindentation has arisen as an interesting technique to characterize the mechanical properties and viscoelastic behavior of soft materials such as polymers, gels and biological tissues (Ebenstein and Pruitt, 2004, 2006; Oyen, 2006; Franke et al., 2007; Deuschle, 2008; Herbert et al., 2008, 2009; Kaufman et al., 2008; Kaufman and Klapperich, 2009; Liu et al., 2009; Oyen and Cook, 2009). In this technique, a sample is locally compressed by an indenter with known geometry, while load, displacement and time are constantly recorded and then used to calculate materials properties (Oyen, 2014). Advantages of using nanoindentation include (i) the ability of testing small volumes of materials with spatial resolution in the nm to μm range, enabling the characterization of heterogeneities typical for biological materials (Tomkoria et al., 2004; Tai et al., 2007; Oyen et al., 2008), (ii) the ability to control and/or measure very low forces (sub-μN), displacements (sub-nm) and changes in stiffness (<1 N/m), which are crucial for testing compliant samples (White et al., 2005; Deuschle, 2008), (iii) the avoidance of extensive macroscopic sample preparation (White et al., 2005; Oyen and Cook, 2009; Oyen, 2014), (iv) the possibility to characterize materials in a variety of different deformation modes by changing the time scale, indenter tip geometry and loading conditions (Oyen and Cook, 2009) and (v) the possibility of testing samples in the wet state (Zysset et al., 1999; Galli et al., 2008) or even immersed in a liquid (Lundkvist et al., 1996; Bushby et al., 2003; Bembey et al., 2006; Mattice et al., 2006; Franke et al., 2011; Hu et al., 2012; Nayar et al., 2012).

Like all testing techniques, however, nanoindentation has its limitations. Since it was primarily developed to test elastic and elasto-plastic materials (Oliver and Pharr, 2004) (which are much harder than gels), the application of this technique to characterize soft samples offers unique challenges, and requires adaptation of standard testing procedures. In particular, surface detection is a critical problem in testing highly compliant materials. Especially when indenters of varying cross-section are used (such as pyramids and spheres), the point of first contact between the indenter and the sample must be correctly determined, since the contact area as a function of contact depth is needed for the calculation of elastic modulus and hardness. In standard nanoindentation methods, initial contact is usually identified as the point when a small increase in force or stiffness is detected. Although this works well for hard materials such as metals or ceramics, even small forces can lead to extensive displacements in soft materials, thereby leading to zero-point errors and, consequently, to wrong determinations of contact area and material properties (Kaufman and Klapperich, 2009; Oyen, 2013). Furthermore, the large displacements usually imposed during mechanical loading of compliant materials require the tip area function to be calibrated for large indentation depths. This requires reliable reference materials characterized by a similar compliance as the samples to be tested (Kaufman et al., 2008). Apart from these "extrinsic effects", the intrinsic time-dependent deformation of hydrogels and how it can be characterized using nanoindentation is also an experimental challenge (Ebenstein and Pruitt, 2004; Franke et al., 2008; Kaufman and Klapperich, 2009; Oyen and Cook, 2009; Shapiro and Oyen, 2014).

Time-dependent deformation of hydrogels results from a combined effect of the intrinsic viscoelasticity of the polymer matrix and the poroelasticity associated with the flow of a liquid through the porous polymeric network (Hu et al., 2012; Oyen, 2013, 2014). Nanoindentation data is therefore normally analyzed using viscoelastic or poroelastic models (Oyen et al., 2007; Galli et al., 2008; Kaufman et al., 2008; Hu et al., 2012; Shapiro and Oyen, 2014), being most experiments performed in the time domain, i.e. by creep or stress-relaxation tests (Oyen, 2007; Galli et al., 2008; Kaufman et al., 2008; Hu et al., 2012; Shapiro and Oyen, 2014). Nevertheless, for applications such as articular cartilage regeneration, the investigation of material properties in the frequency domain is also of great interest, since such soft tissues are daily submitted to a wide range of frequencies during regular walking (Franke et al., 2008). To date, however, only a few