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REPORT

A novel optical coherence tomography-based micro-indentation technique for mechanical characterization of hydrogels

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Depth-sensing micro-indentation has been well recognized as a powerful tool for characterizing mechanical properties of solid materials due to its non-destructive approach. Based on the depth-sensing principle, we have developed a new indentation method combined with a high-resolution imaging technique, optical coherence tomography, which can accurately measure the deformation of hydrogels under a spherical indenter at constant force. The Hertz contact theory has been applied for quantitatively correlating the indentation force and the deformation with the mechanical properties of the materials. Young's moduli of hydrogels estimated by the new method are comparable with those measured by conventional depth-sensing micro-indentation. The advantages of this new method include its capability to characterize mechanical properties of bulk soft materials and amenability to perform creeping tests. More importantly, the measurement can be performed under sterile conditions allowing non-destructive, *in situ* and real-time investigations on the changes in mechanical properties of soft materials (e.g. hydrogel). This unique character can be applied for various biomechanical investigations such as monitoring reconstruction of engineered tissues.

Keywords: mechanical properties; micro-indentation; depth sensing; optical coherence tomography

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1. INTRODUCTION

In nature, the mechanical properties of soft tissue in either human or animal are closely related to their physiological functions. For instance, after injury connective tissue will repair and remodel resulting in an increase of its mechanical strength and a recovery of the original tissue function (Pierard & Lapiere 1997; Wells *et al.* 1999). The concentration and the degree of cross-linkage of collagen fibres have been reported to be related to the toughness and tenderness in meat and fish (Purslow 2002). In tissue engineering of soft tissues, the mechanical properties of the constructs vary dynamically with time depending on the culturing and conditioning environments. Therefore, monitoring the alteration of mechanical properties will greatly enhance our understanding of the bioprocess of the soft tissue constructs.

Depth-sensing micro-indentation has been widely used to characterize mechanical properties of various materials, in particular for soft materials (Briscoe *et al.* 1998). Basically, it measures the reaction forces by imposing certain indentation depths via a rigid indenter in contact with the testing materials. The curve of the indentation depth (or displacement) versus the applied force is normally referred to as the 'compliance', which can be used for deducing the mechanical properties of materials. Recently, a new indentation instrument has been developed in our previous research to characterize biomimetic hydrogel membranes non-destructively and *in situ* (Ahearne *et al.* 2005). In short, the method uses a computerized long-focal charge-coupled detector microscope system which allows the measurement of a side-view image of a suspended circular membrane under the weight of a steel ball. A theoretical model was constructed to quantitatively correlate the viscoelasticity with the time-dependent deformation profile of the membrane. However, the method is only applicable to relatively thin and large membranes and has to evoke specially designed sample holders to minimize any clamping stress applied on the hydrogel membranes. Hence, it is highly desirable to develop an alternative indentation system, which is ideally sample holder-free, for *in situ* characterization of thick specimens in a non-destructive fashion under sterile conditions.

Optical coherence tomography (OCT) is an interferometric imaging method that takes advantage of the short coherence lengths of broadband light sources. Thus, it can scan precisely the deformation images of soft materials (Schmitt 1998; Wang *et al.* 2006). Recent development of OCT allows the mechanical characterization of soft tissue *in vivo* through mapping relative Young's modulus. However, the measurements are in ratio, not the absolute value of the modulus (Khail *et al.* 2005). In this report, we introduce a new characterization technique, which combines the micro-indentation method with OCT imaging modality, to measure the mechanical properties of bulk/thick soft materials under sterile conditions and in a non-destructive manner. The key requirement for such a new micro-indentation technique is to effectively acquire cross-section imaging with high resolution. In principle, other non-destructive imaging methods such as ultrasound and magnetic resonance imaging could be considered as

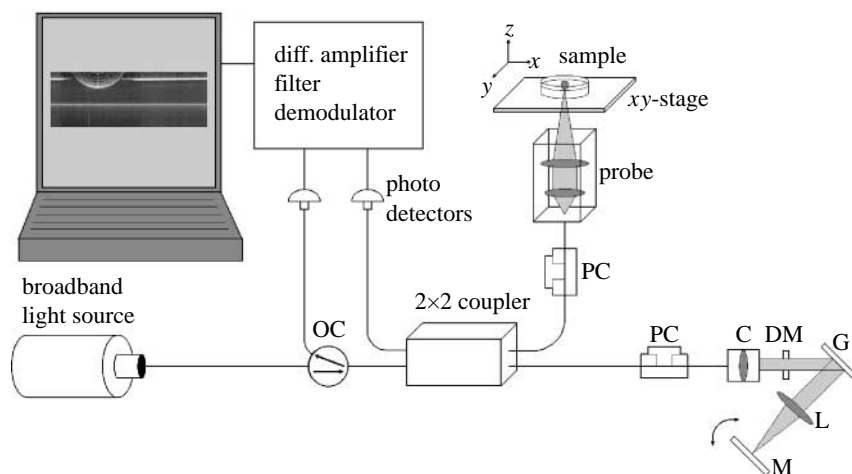


Figure 1. Schematic of the time-domain OCT system setup used for the current investigation. The hydrogel specimen in a Petri dish placed on a xy -stage was set in OCT light path. The sample arm beam went through the bottom of the Petri dish. OC denotes the optical circulator; PC, the polarization controller; C, the collimator; DM, the double pass mirror; G, the grating; L, the optical lens; M, the reflecting mirror; and D, the detector.

alternative modalities. But either low resolution or expensive cost of these two alternative means deter their applications for the new micro-indentation system. Confocal and multi-photon microscopes can provide up to 200 μm penetration depth cross-section images with high resolution. However, they depend on the fluorescent labelling of the specimens. The easy set-up, high imaging resolution, ability to image highly scattering tissues and non-destructive scanning features enable OCT to be a perfect candidate to facilitate the imaging acquisition for the new micro-indentation technique in mechanical characterization for opaque hydrogel and tissue engineered constructs. In this report, the OCT-based micro-indentation instrument has been applied to measure the deformation of hydrogels under indentation of a constant weight ball. Simple analyses were also developed to estimate their mechanical properties such as the value of Young's modulus and effective viscosity. Here, we use agarose gel as a model material to demonstrate how the new technique is feasible for characterizing soft biomimetic/biological materials. The estimated Young's modulus of the hydrogels has been validated independently by the conventional micro-indentation technique.

2. EXPERIMENTAL

2.1. Optical coherence tomography indentation system

Figure 1 shows a schematic of the OCT indentation system set-up and its working principles. A benchtop fibre-based time-domain OCT system was used in this study which employed a 1300 nm superluminescence diode with a bandwidth of 52 nm. The light source yielded a 14 μm axial resolution in free space, or 10 μm in the tissue if the mean refractive index of bulk tissue is assumed to be 1.4 (Tuchin 2000). The OCT image was scanned at 100 Hz with the frame consisting of 400×350 pixels, corresponding to a physical size of the image at $5.8 \text{ mm} \times 2.9 \text{ mm}$ ($x \times z$). The signal-to-noise ratio of the system was

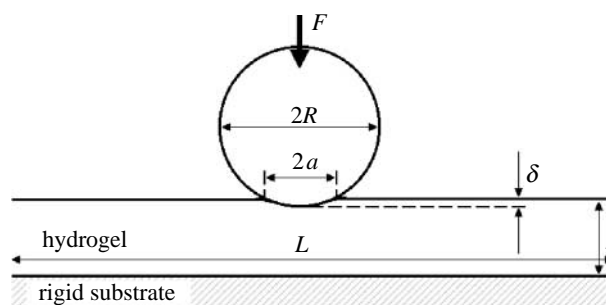


Figure 2. Schematic of fundamental principle of spherical indentation of a hydrogel sample.

evaluated to be 90 dB for the current investigation. The detailed description for the system can be found in the literature (Wang *et al.* 2001; Yang *et al.* 2006).

An xy -stage has been used to obtain precise images through specimen width (y -direction), which enabled three-dimensional scanning, and therefore the accurate measurement of the maximum displacement. The current version of our OCT-indentation instrument has a high force resolution and displacement resolution as accurate as 10 μN and 15 μm , respectively, and the penetration depth of sample scanning is approximately 2 mm. In addition, a conventional homemade depth-sensing micro-indentation instrument was used for independently validating the mechanical properties. Its detailed set-up and analyses were reported elsewhere (Khoo *et al.* 2003).

2.2. Materials preparation

A model hydrogel, agarose, has been used in this study. A heated agarose solution (3 ml) was poured into a small plastic Petri dish (35 mm in diameter and with a refractive index of 1.57). After setting, a thin layer of deionized water was poured on top of the agarose to prevent dehydration. There was no extra swelling. The hydrogels formed were 2–4 mm thick. Agarose concentrations of 0.5, 1 and 2% were used to examine properties of hydrogels. A stainless steel ball was

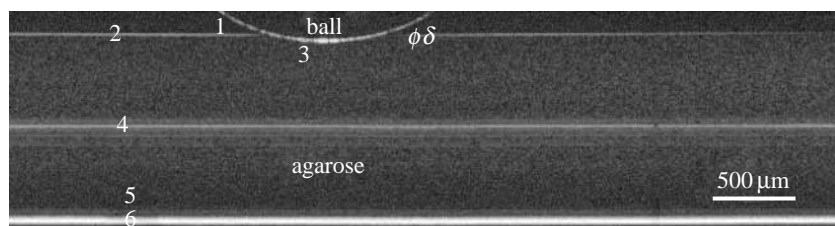


Figure 3. A typical image of OCT-scanning at the maximum indentation for an agarose sample with 1.0% concentration. On the figure different interfaces have been marked as (1) air/metal, (2) air/gel, (3) metal/gel, (4) artefacts due to internal reflections, (5) gel/plastic and (6) plastic/plastic within the Petri dish.

placed on the centre of the gel. Stainless steel balls of diameter 2 mm (with weight 31.8 mg or 3.1×10^{-4} N) and 2.5 mm (with weight 62.5 mg or 6.1×10^{-4} N) were used for 1% agarose gels and 2.5 and 3 mm (with weight 108.1 mg or 10.6×10^{-4} N) balls used for 2% agarose gels. By a pre-set programme, the OCT scan (x - z cross-section) was initiated immediately across the ball (y -direction) with a step distance of $15 \mu\text{m}$ at ambient temperature. To avoid the reflection of light from the metal ball, the sample beam was placed underneath the Petri dish which contained the gel, while the Petri dish was placed on an xy -stage which has a window for the light beam through the samples. With the aid of a red visible light, the scan plane was focused on the top surface area. Thus, the whole thickness of the bottom layer of the Petri dish (0.9 mm thick) could not appear in the OCT images due to the penetration limit of OCT scan. The creep experiments were performed using a 4 mm diameter steel ball (with weight 254 mg or 2.5×10^{-3} N) on 0.5% agarose only. The OCT images were collected every minute for 1 h.

3. THEORETICAL ANALYSES

Figure 2 shows the fundamental principle of micro-indentation. When a spherical indenter (of a radius R) is in contact with the testing material, an indentation depth δ is created by applying a force F (mN) via a spherical indenter. For the current case, the applied force is equal to the weight of the balls placed on the gels (the weight values given in §2.2). If δ is small when compared with the hydrogel depth h (approx. 10%), the effect of the backing substrate can be neglected. Furthermore, if the lateral dimension of the tested materials, L , is much larger than the contact radius, a , then the ‘half-space’ assumption can be applied (Johnson 1985). In our case, which has $\delta/h < 7\%$ and $L/a > 12$, Hertz contact theory (Hertz 1882) can be applied for correlating the indentation depth with Young’s modulus, E , of the materials and the relationship of these parameters can be expressed as

$$a = \left(\frac{3}{4} \frac{1-\nu^2}{E} FR \right)^{1/3}, \quad (3.1)$$

where ν is the Poisson ratio and its value is assumed to be 0.5 for hydrogel materials (Anseth *et al.* 1996).

For modelling the creep deformation, a simple analysis based on the Zener viscoelastic model has been applied. The Zener model, which consists of one viscous constant (the effective viscosity η) and two

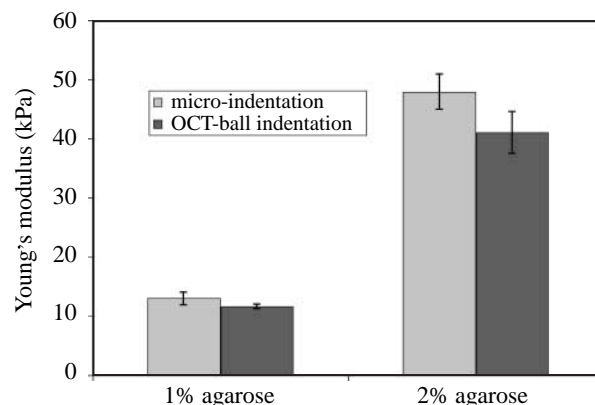


Figure 4. Young’s modulus of agarose determined by two independent methods: the OCT-indentation and the depth-sensing micro-indentation.

elastic constants E_1 and E_2 , is applicable to describe the experimental data (Fung 1993). By measuring the central displacement against time, the time-dependent modulus can be determined and the effective viscosity can then be calculated by applying the following set of equations (Ahearne *et al.* 2005):

$$\frac{\varepsilon(t) - \varepsilon(0)}{\varepsilon(\infty) - \varepsilon(0)} = 1 - e^{-t/\mu_\sigma}, \quad (3.2)$$

$$E_2 = \left(\frac{\varepsilon(\infty)}{\varepsilon(0)} - 1 \right) E_1, \quad (3.3)$$

$$\mu_\sigma = \frac{\eta}{E_1} \left(1 + \frac{E_1}{E_2} \right), \quad (3.4)$$

where $\varepsilon(t)$ is the deformation strain at time t , $\varepsilon(0)$ is the initial deformation strain and $\varepsilon(\infty)$ is the final deformation strain.

4. RESULTS AND DISCUSSION

Figure 3 shows a typical OCT-scanned image at the maximum indentation for a hydrogel sample with 1.0% agarose concentration. The video clip of the whole indentation process can be found in the electronic supplementary material. The image shows that the hydrogel surface around the indenter has slightly rippled during the indentation. The displacement in this particular set was around $100 \mu\text{m}$. Figure 4 shows Young’s modulus of agarose determined by two independent methods: the OCT-indentation and the depth-sensing micro-indentation. Young’s modulus

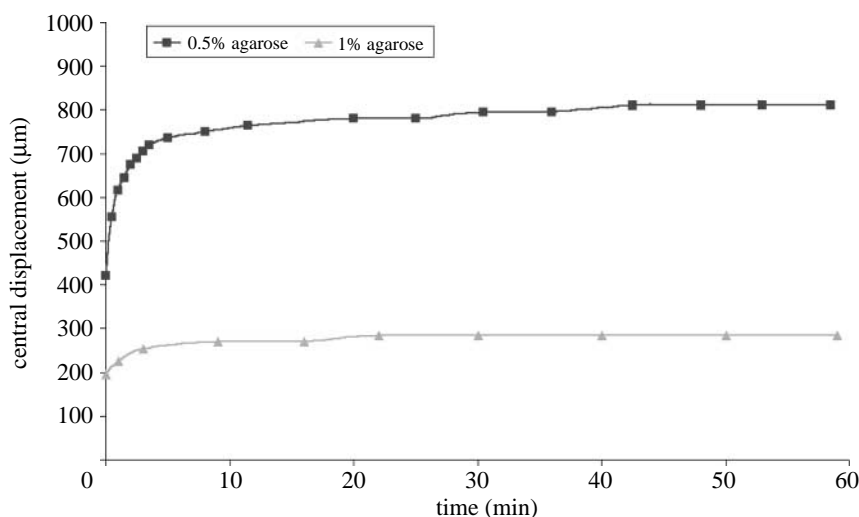


Figure 5. The central displacement δ against time (creep test) of the sample with 0.5 and 1% agarose concentrations. The ball diameter is 4 mm.

measured by the former method was consistently lower than that measured by the latter. However, the discrepancy between the moduli measured by these two methods was less than 14%. Such a difference was presumably due to the size effect of the indenter; currently the indenter radii of the conventional micro-indentation and OCT-based system are approximately 200 μm and 1 mm, respectively. In addition, the displacement resolution of the current OCT system may impose a certain, but minor, limitation on the accuracy of the elasticity measurement. Nevertheless, our results are also comparable with the magnitude of those measured by other methods such as dynamical mechanical analyses (Chen *et al.* 2005). In our future endeavour, a submicrometre displacement resolution may be achieved by incorporating a femtosecond fibre laser source imaging system (Nishizawa *et al.* 2004).

Figure 5 shows the plot of the central displacement δ against time, which is also known as a creep test. It is notable that δ initially increased with time and then reached a plateau after approximately 70 min. The creeping central displacement can be correlated with viscoelasticity by using the Zener viscoelastic model (§3). However, it is worth pointing out that the value of η for the current samples with 0.5% agarose concentration was approximately 0.26 (MPa s) which is one order magnitude lower than that of an alginate hydrogel (Ahearne *et al.* 2005). This implies that agarose, when compared with alginate, is a more elastic rather than viscoelastic material. The typical results of the agarose samples with 1.0% concentration are also shown in figure 5 and the central displacements are less than those of the 0.5% concentration, and also much less variant against time. Hence, the gels of 1.0% (or above) agarose concentration can be considered as nearly elastic materials.

5. CONCLUSIONS

The technique reported here has explored a new non-destructive method of online measurement of mechanical properties for soft tissues under sterile conditions, which will be invaluable for monitoring various

biomechanical/biochemical processes. Our initial results show Young's moduli of agarose gels determined by the new method are comparable with those measured by other conventional techniques such as depth-sensing micro-indentation and dynamical mechanical analyses. When compared with these conventional techniques, the current technique can perform the *in situ* measurements with much less disturbance on the samples under sterile conditions. The measurements also show agarose is a more elastic rather than a viscoelastic material, especially for the gels with higher agarose concentration (more than 1%). The OCT-based micro-indentation method also demonstrates the capability to measure viscoelasticity of soft materials by performing a creep measurement.

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