Noncontact Evaluation of Articular Cartilage Degeneration Using a Novel Ultrasound Water Jet Indentation System

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Abstract

We previously reported a noncontact ultrasound water jet indentation system for measuring and mapping tissue mechanical properties. The key idea was to utilize a water jet as an indenter as well as the coupling medium for high-frequency ultrasound. In this paper, the system was employed to assess articular cartilage degeneration, using stiffness ratio as an indicator of the mechanical properties of samples. Both the mechanical and acoustical properties of intact and degenerated bovine patellar articular cartilage (n = 8) were obtained *in situ*. It was found that the stiffness ratio was reduced by 44 ± 17% after the articular cartilage was treated by 0.25% trypsin at 37^{o}C for 4 hours while no significant difference in thickness was observed between the intact and degenerated samples. A significant decrease of 36 ± 20% in the peak-to-peak amplitude of ultrasound echoes reflected from the cartilage surface was also found for the cartilage samples treated by trypsin. The results also showed that the stiffness obtained with the new method highly correlated with that measured using a standard mechanical testing protocol. A good reproducibility of the measurements was demonstrated. The present results showed that the ultrasound water jet indentation system may provide a potential tool for the non-destructive evaluation of articular cartilage degeneration by simultaneously obtaining mechanical properties, acoustical properties, and thickness data.

Keywords: articular cartilage, tissue, indentation, ultrasound indentation, water jet, degeneration, osteoarthritis

1. Introduction

Articular cartilage has unique material properties that enable it to perform unique physiological functions over lifetime and under a wide range of loading conditions. The degenerative morphological and structural changes in articular cartilage and subchondral bone, i.e. osteoarthritis (OA), is a complex and progressive musculoskeletal disorder and is common among humans, particularly in the elderly. One of the first signs of early OA is the decrease in superficial proteoglycan (PG) concentration which results in the softening of articular cartilage.[3,5,15-16,37-38,44-45,55,57,64] In contrast to disruption of the collagen fibrils, PG loss may be reversible via appropriate medicines and adequate physical exercises at sufficiently early stage.[16,27,29] Therefore, early diagnosis of OA is important for the prevention of further destruction of the tissue as well as the alleviation of pain and disability. Recent improvements of non-invasive imaging techniques, such as diffraction-enhanced x-ray imaging,[48] microscopic MRI,[17,69] and quantitative MRI[55] have provided more detailed information for better diagnosis of tissue disorders. However, these techniques still lack the sensitivity to detect the cartilage stiffness at the initial stage of degeneration.

Indentation is a widely used technique to measure the mechanical properties of articular cartilage. To perform the indentation on articular cartilage, a number of mechanical, arthroscopic and ultrasound indentation instruments have been developed. Mechanical indentation instruments can provide an accurate and repeatable indentation measurement,[6-9,33,36,49,51,62] but they are not convenient for clinical use. In addition, as mechanical instruments employ a needle probe to penetrate into the cartilage to measure the tissue thickness which is important information for the estimation of stiffness, destruction of the tissue structure might be caused. Arthroscopic indentation apparatuses can perform experiments *in vivo*,[45,52,53] however, they can't provide the tissue thickness thus difficult to provide the accurate elastic modulus of cartilage.

As ultrasound is capable of measuring cartilage thickness and deformation non-destructively, ultrasound indentation instruments have been developed with a combination of a force sensor and an ultrasound transducer.[1,39,63,68,72] By recording indentation force simultaneously, the instantaneous Young's modulus of articular cartilage can be calculated. Since most of the available indentation instruments use a contact way to assess the articular cartilage, tissue damage caused by the measurement instrumentation usually cannot be avoided. Therefore, a diagnostic device which may avoid direct contacts and use low loading forces is desirable to quantify the stiffness of articular cartilage. Besides of the capability to measure the mechanical properties of cartilage, recent studies have reported that ultrasound can also be used for characterizing cartilage structural properties such as surface roughness or fibrillation and PG depletion.[2,19,20,21,22,24,31,54,66] If a probe can measure mechanical properties, acoustic properties, and thickness data for articular cartilage simultaneously,[38] it will be beneficial for the diagnosis of cartilage degeneration.

This study introduced a noncontact ultrasound indentation system that is capable of determining the material properties of the cartilage without a direct contact between the testing probe and cartilage. The key idea was to use a water jet as the indenter and simultaneously as the coupling medium for ultrasound to propagate through. By analyzing the response of articular cartilage to water jet loading and the ultrasound echoes reflected from the cartilage, both mechanical and acoustical properties of articular cartilage could be obtained. Our previous studies have proved that this novel indentation system could successfully measure and map the stiffness of tissue-mimicking phantoms.[42,43] In this paper, the performance of the ultrasound water jet indentation system in evaluation of the softening of degenerated cartilage was tested and validated against a standard mechanical testing protocol, and the potential of the system for the assessment of the cartilage degradation using the change of acoustical properties was also discussed.

2. Methods

2.1 Samples

Eight fresh mature bovine patellae without obvious lesions were obtained from local slaughterhouse within 2 hours post mortem and stored at -20^{o}C before use. During the specimen preparation, the patellae were first thawed in normal saline solution (0.15 M NaCl) at the room temperature of approximately 20^{o}C for 1 hour, then the lateral lower part (1/4 of the patella) of each patella (Figure 1) was cut for the experiment by a bandsaw (Buehler, Lake Bluff, IL, USA), and the bone was cut into a flat layer with a thickness of 3 mm to 8 mm. During processing, the cartilage surface was kept moist with normal saline solution without immersing the sample. All the samples were wrapped with bandage and wetted with normal saline, then stored at -20^{o}C before later use.

Simulated articular cartilage degeneration was achieved by a trypsin digestion for the cartilage (n = 8). Before digestion, the sample was first thawed in normal saline solution at room temperature 20^{o}C for 1 hour; it was then put into a container and immersed in the 0.25% trypsin solution (Gibco Invitrogen Corp., Grand Island, NY, USA). The container was kept for 4 hours inside an incubator (MMM, Medcenter Einrichtungen GmbH, Schulstrasse, DE) at 37^{o}C to induce degeneration of articular cartilage. It has been reported that trypsin reduces the content of PGs in cartilage and a trypsin digestion process is thought to simulate cartilage degeneration.[4,44,57] The concentration and duration of the digestion treatment were selected according to the previously developed protocols.[57] All samples were washed using normal saline solution after the digestion finished.

2.2 The Ultrasound Water Jet Indentation System

A noncontact ultrasound indentation system was developed using a water jet as the indenter. As shown in Figure 2, a bubbler was used to eject the water jet by controlling the water flow. The diameter of the water ejecting nozzle was 1.94 mm. A 20 MHz focused ultrasound transducer (GE Panametrics, Inc., OH, USA) was fixed with the bubbler, *i.e.,* the water ejector. The focused ultrasound beam could propagate through the bubbler when it was full of water as the coupling medium. The transducer and the bubbler were installed to a 3-D translating device (Parker Hannifin Corporation, Irvine, CA, USA) which was used to adjust the distance between the nozzle and the specimen surface and to perform the 2-D scanning over a tissue specimen or phantom with a spatial resolution at 1 μm. To focus the ultrasound beam at the specimen surface to obtain the maximal echoes, the distances from the specimen surface to the nozzle outlet and the transducer surface were adjusted to be 5.0 mm and 19.5 mm in current study, respectively. A fixation device was carefully designed to fix the cartilage sample rigidly and keep its surface perpendicular to the ultrasound transducer. As shown in Figure 2, the sample was placed on the head of a tripod (#115, Manfrotto, Italy) which provided a rigid support and meanwhile allowed for 3 degrees of adjustment and was fixed by two clamps to avoid the slipping during test. This fixation device could be easily adjusted and thus was convenient for experimental use.

A pressure sensor (EPB-C12, Entran Devices, Inc., Fairfield, NJ, USA) was used to measure the water pressure within the water pipe. By calibrating the relationship between the overall force applied on a load cell located under the platform and the pressure within the water pipe, the pressure applied on the sample surface could be calculated using the water pressure measured by the pressure sensor.[43] We confirmed that the change of force applied on the tissue could be negligible when the change of distance between the nozzle outlet and the tissue surface was within approximately ± 0.5 mm. When the distance increased, the ultrasound echo reflected from the tissue surface reduced significantly, as the surface moved away from the focal point. In addition, the calibration for the force measurement had to be re-conducted under this situation. A program was developed using Microsoft VC++ to control the 3D translating device and to collect, process and display the ultrasound signals, along with the pressure value, in real time during the indentation process. Thus the movement of the transducer and the acquisition of the radio-frequency (RF) ultrasound signal and pressure data were synchronized. The ultrasound echoes reflected from the articular cartilage under different loading conditions were tracked using a cross-correlation algorithm.[70] The original tissue thickness and the subsequent change of thickness, i.e., the deformation of the tissue under indentation, were derived from the time information. The stiffness ratio, defined as the ratio of the pressure applied on the sample surface to the local strain of the sample, was used as an indicator of the mechanical properties of articular cartilage since it was found well correlated with Young's modulus from previous phantom studies.[42,43]

2.3 Stiffness, Thickness and Ultrasound Reflection Measurement Using Ultrasound Water Jet Indentation

The bovine patella sample was first thawed in normal saline solution at room temperature 20^{o}C for 1 hour, and then it was fixed with its cartilage surface perpendicularly facing the ultrasound transducer. For each sample, three indentation sites were carefully selected through the following procedures: 1) the sample was scanned across by the 20 MHz ultrasound beam to form a sequence of B-mode images, and those regions with strong echoes reflected from both the cartilage surface and the cartilage/subchondral bone interface were selected; 2) three sites in the selected regions were chosen with the distance between each pair of them and the distance from each test site to the edges of the sample both larger than 8 mm; 3) at each test site, fine adjustment of the orientation of the platform was made to obtain the maximal echo amplitude. Such a selection criterion could fulfill the boundary condition of indentation proposed by Galbraith and Bryant[28] and also make the ultrasound beam perpendicular to the cartilage surface. The indentation sites were marked using a permanent marker pen (Lumocolor, Staedtler, Germany).

During the measurements, the thickness and deformation of the sample were determined with the 20 MHz ultrasound by calculating the time of flight and its deflection of the ultrasound signals reflected from the cartilage surface and the cartilage-substrate interface. The inner parts of cartilage were mostly unechoic so it was straightforward to determine the echoes from the interfaces. It has been earlier confirmed using optical measurements that the second ultrasonic reflection was from the tidemark, i.e. uncalcified-calcified cartilage interface.[35,47,66] The cartilage thickness was determined based on a constant ultrasound speed in bovine cartilage $c_{cartilage}$ of 1636 m/s.[56]

The sample was first scanned using the water jet with the pressure applied on the sample surface no more than 1 kPa after the indentation sites were determined. This process last for about 15 minutes which allowed the cartilage swelling caused by the change of the concentration of solution from 0.15 M to approximate 0 M.[67,71] Towards the end of this period, the ultrasound echo reflected from the articular surface was recorded for six times at each selected site for each sample. The peak-to-peak echo amplitudes recorded from the intact cartilage sample A_{intact} and the sample after digestion treatment A_{digest} at the identical indentation site were compared to investigate if there was significant difference between them.

During indentation, the cartilage was first preloaded with a pressure of 20 kPa for 3 seconds, and then it was indented with the pressure increased to approximate 180 kPa within 1 second. The loading and unloading process was repeated for 3 cycles in an indentation. The stiffness ratio of cartilage was determined by calculating the slope of pressure applied on the cartilage to the local strain induced in the loading phase. As suggested by previous researchers, instantaneously induced deformation of a biphasic tissue such as articular cartilage, can be modelled as that of an equivalent incompressible single phase elastic material.[32,46] Therefore, the load-indentation curve was fit by a linear regression to obtain its slope since the strain was small (no more than 2%).

The cartilage sample was immersed in normal saline for 2 hours for full recovery[49] before the next indentation. The indentation was repeated for three times at each selected site, and the mean values of stiffness ratio and thickness were calculated.

2.4 Evaluation of the Effect of Repositioning

The accuracy of the ultrasonic evaluation highly depends on the position of the ultrasound transducer relative to the indentation site. As the samples were repositioned to the previous sites after digestion during the test, the effect of sample repositioning should be evaluated. Two samples were used for this test. The sample was first assessed by the water jet system on the selected test sites and taken away from the platform and immersed into the saline solution for 2 hours. Then it was repositioned to the original place for the repeated test. During this process, the orientation of the platform was kept unchanged and the position of the sample was marked on the platform. The three-dimensional translating device could retrieve the transducer back to the recorded position of each test site. Thus the same test sites were indented. This process was repeated for three times. The obtained stiffness ratio, thickness and peak-to-peak ultrasound echo amplitude of each test site were used to evaluate the variation caused by the repositioning.

2.5 Comparison with Mechanical Indentation

To compare with the measurements using the ultrasound water jet indentation, mechanical indentation was also conducted on the same samples using a custom made computer controlled material testing device. This system consisted of a 10N load cell, precision motion controller and a computerized data acquisition system with a cylindrical, plane-ended, impermeable, stainless steel indenter with a diameter of 1.6 mm. The indenter was connected with a load cell (ELFS-T3M, Entran Devices, Inc., Fairfield, NJ, USA, calibrated for a range of 10N) and installed to the three dimensional translating device. During mechanical indentation, the indenter was first moved to the marked test site, and a preload of 0.04 N (a pre-stress of 20 kPa) was applied to the sample. After holding for 3 seconds, the sample was loaded up to a maximal strain of 10%. The loading was recorded by the load cell synchronously with the movement of the indenter by the program and the accuracy of the force collected by the data acquisition card was better than 1 μN. The indentation rate was controlled at approximately 0.2 mm/s.

The Young's modulus of the cartilage was obtained using Hayes' model[32] from the instantaneous load/indentation response of the sample. For a plane-ended cylindrical indenter, at given values of the parameters a/h and v, the relation between the applied force P, and the displacement, ω, is given by

$$E = \frac{P(1-v^2)}{4a\omega\kappa(a/h, v)} \tag{1}$$

where a is the radius of indenter, E Young's modulus, and v Poisson's ratio. Values of the scale factor κ were numerically determined[32,34] The Poisson's ratio of cartilage was regarded as 0.45 for articular cartilage, representing its nearly incompressible property under the indention rate (approximately 2%/s) used in this study. Only the data with deformation less than 2% were used to calculate the Young's modulus, corresponding to the 2% local strain of the samples under water jet indentation. The thickness of cartilage determined from the ultrasound water jet indentation system was used for the determination of the scale factor κ from the given tables by Hayes et al.[32]

2.6 Statistical Analysis

Data were expressed as mean ± standard deviation (SD). For the comparison of various parameters, i.e., the stiffness ratio and thickness obtained by the water jet indentation as well as the Young's modulus measured by the mechanical indentation, paired *t*-test was used to find whether there was a significant difference between the cartilage samples before and after trypsin digestion. The Pearson correlation test was used to investigate the relationship between the stiffness ratio and Young's modulus for both the intact samples and the samples after trypsin digestion. All the statistical analyses were conducted by using the commercial software SPSS (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was used to indicate a significant difference.

The reproducibility of current measurement for stiffness ratio, thickness and ultrasound reflection, were tested using standardized coefficient of variation (sCV),[12,26] which takes into account the biological range of the measured parameters and can be defined as:

$$\text{sCV} = \frac{CV_{RMS}}{4\sigma_{\mu} / \overline{\mu}} \qquad (2)$$

where σ_{μ} is the standard deviation of all samples, $\overline{\mu}$ is the average of the population and CV_{RMS} is the root-mean square average of coefficient of variation of the measured parameter. A smaller value of sCV indicates a higher reproducibility.

3. Results

3.1 Stiffness and Thickness of Normal and Digested Articular Cartilage

Figure 3a shows a typical curve of the loading and unloading cycles obtained from a test site of one cartilage sample. It was noted that the strain curve followed the pressure curve well. As shown in Figure 3b, the data were fitted very well with the linear regression ($r > 0.9$). The data obtained at the loading phase were used to extract the stiffness ratio of the cartilage. For the intact samples, the stiffness ratio and thickness were 18.8 ± 7.5 MPa and 1.75 ± 0.23 mm, respectively. A stiffness ratio of 7.9 ± 3.4 MPa was found for the degenerated samples, and the corresponding thickness was 1.76 ± 0.24 mm. Figures 4 and 5 show the box plots of the stiffness ratio and thickness of the cartilage samples measured before and after the trypsin treatment, respectively. It was found that the stiffness ratio significantly decreased after the sample was digested by trypsin ($P < 0.001$) while no significant difference was observed between the thickness values of the cartilage samples before and after the trypsin treatment ($P = 0.87$).

The reproducibility of the measurement using the ultrasound water jet indentation system was assessed by the standardized variation of three repeated measurements of the stiffness ratio and thickness on each test site of four cartilage samples (number of test sites $n = 12$). The values of sCV were 8.0% for the stiffness ratio measurement and 1.6% for the thickness measurement.

3.2 Ultrasound Reflection Measurements

For the ultrasound reflection measurements, the standardized coefficient of variation for the repeatability test was 4.9% for the peak-to-peak amplitude measurement of all test sites. Figure 6 shows typical A-mode ultrasound signals from the sample before (Figure 6a) and after the trypsin digestion (Figure 6b). A significant decrease of 36 ± 20% of the peak-to-peak amplitude at the articular surface ($P < 0.001$) was found after the cartilage sample was treated by the trypsin, however, no significant decrease of the signal amplitude was found ($P = 0.053$) at the interface of uncalcified-calcified cartilage. The decrease of the ultrasound reflection at the cartilage surface indicated that trypsin induced not only PGs degradation but also small degradation of collagens [38]

3.3 Effect of Repositioning on the Measurement

For the repositioning test, the values of the standardized coefficient of variation were 6.6%, 0.6% and 4.9% for the stiffness ratio, the cartilage thickness and the peak-to-peak amplitude measurements, respectively. The small coefficients of variation demonstrated that the reproducibility of the measurement was good and the effect of repositioning was not significant.

3.4 Comparison with Mechanical Indentation

Using the mechanical indentation system, an instantaneous Young's modulus of 1.59 ± 0.49 MPa was obtained for the native cartilage samples and 0.47 ± 0.13 MPa for the degenerated samples (Figure 7). It was correlated with the stiffness ratio obtained using the water jet system and a good correlation was found between them with $r = 0.87$ ($P < 0.001$, Figure 8). We estimated the Young's modulus of cartilage, E_w, from the obtained relationship between the stiffness ratio and the instantaneous Young's modulus, E, of the cartilage. A Bland-Altman plot (Figure 9) was also used to give an indication of the size of errors between E_w and E. The mean difference $\bar{d}$ was 0 kPa and the standard deviation s was 243.1 kPa. From the Bland-Altman plot, it can be observed most of the differences lied between $\bar{d} - 2s$ and $\bar{d} + 2s$. Such a result was good enough for clinical applications.[13]

It was found that the elastic modulus was significantly reduced after the treatment with trypsin, independent of the measurement methods used. The stiffness ratio was reduced by 44 ± 17% ($P < 0.001$) obtained by the ultrasound water jet indentation system, and the instantaneous Young's modulus decreased by 30.5 ± 7.4% ($P < 0.001$) measured using the mechanical indentation system. The decrease of the mechanical properties might be due to the loss of the PG. As shown in Figure 10, most of the PGs of the sample were digested after the trypsin treatment. However, no significant difference was found between the thickness values of the samples before and after digestion ($P = 0.87$), as measured using ultrasound.

4. Discussion

In this study, the mechanical and acoustical properties of bovine patella articular cartilage were quantified using the noncontact ultrasound water jet indentation system *in situ*. Clinical findings show that during the development of osteoarthritic joint disease, the cartilage swells, the level of aggrecan appears to change, especially that the PG concentration decreases and the collagen network disrupts in the superficial zone.[3,16,23] Such changes in cartilage quality can be determined by stiffness measurement at an early stage,[40] therefore, the determination of the mechanical properties of articular cartilage becomes of great importance in trauma and joint repair surgery. In comparison to other indentation systems, the current ultrasound water jet indentation system offers the advantage of no direct contact between the testing probe and the tissue. Thus, the potential damage to the tissues caused by the testing probe is minimized compared with those contact indentation measurements. Further analysis of cell viability is required to investigate whether any cellular damage might result from the water jet application.[11] In addition, the potential penetration of water into the cartilage should also be investigated and its influence to the tissue should be studied.

According to the results, the ultrasound water jet indentation system demonstrated its ability to effectively assess bovine articular cartilage by measuring both mechanical and acoustical properties as well as tissue thickness. Unlike the ultrasound indentation instrument developed by Laasanen et al.,[38] no additional apparatus was needed in the water-jet measurements when changing from the mechanical to acoustical measurement modes. The repeated measurements of the stiffness ratio, the thickness, and the amplitude echo signal from the articular cartilage were found highly reproducible with low values of sCV (< 10%). The repositioning test showed similar reproducibility, suggesting the system was reliable when used for the assessment of cartilage degeneration.

It was found in the current study that the stiffness ratio obtained using the water jet system correlated well with the instantaneous Young's modulus measured from the mechanical indentation (r = 0.87), and the estimated Young's moduli were well within the range of previous studies.[10,30,61] The Bland-Altman plot indicated that the estimation was acceptable for clinical applications. It was found that it was not easy to keep the pressure as a constant value due to the fluctuation of the flow rate when it was manual controlled. A medical pump will be tried in the future studies to have a better control of the water flow.

A significant decrease was found in both the stiffness ratio and the peak-to-peak amplitude of the echo from the articular surface between the intact and trypsin treated samples. The decrease of the mechanical properties may result from the PG content reduction induced by trypsin treatment.[57] The result of histological measurement revealed that most of the PGs in the digested sample had been lost. More studies will be conducted for the investigation on the relationship between the histological imaging quantification and the water-jet detection of mechanical properties of articular cartilage. The amplitude of the echo signal from the articular surface is related to the ratio of acoustic impedance of cartilage and water. The surface roughness may also control the acoustic scattering, and thereby affect the amplitude of the reflected signal.[2] After the trypsin treatment, the articular surface appeared normal without any fibrillation or other macroscopic signs of degradation. Therefore, the significant decrease of the echo amplitude from the digested articular surface should indicate that trypsin might have not only digested most of PG contents, but also caused slight degradation of collagens.

It is well known that articular cartilage swells when the concentration of the external bathing solution decreases. The swelling is caused by the change of the Donnon osmotic pressure.[25,50] In our test on the articular cartilage, water (nearly 0 M NaCl) was used to eject a water jet as the indenter to deform the cartilage after it was moved away from the normal saline solution (0.15 M NaCl). As reported by Zheng et al.,[71,74] the intact cartilage sample reached its maximal swelling strain (less than 0.1%) with an average swelling duration of approximately 300 seconds when the bathing solution was suddenly changed from 0.15 M NaCl to 0.015 M NaCl. In our experiments, the cartilage sample was scanned under a very low constant pressure by the water jet for 15 minutes before it was deformed. The scan duration should have been long enough to allow the cartilage to equilibrate in the new solution. The strain caused by swelling during the indentation process was small and could be neglected. However, it is better to keep the sample in its physiological conditions to maintain its material properties. Therefore, normal saline solutions will be used in the future instead of water when a medical pump is employed in the system to eject a water jet as an indenter.

One of the main sources of error in the conventional indentation measurements is the imperfect contact between the indenter and the articular cartilage. This may also affect the water-jet indentation. In our measurements, the ultrasound beam was kept perpendicular to the cartilage to maintain the water jet loading being normally applied so as to minimize the error. The test sites were carefully selected by moving the transducer and scanning across the cartilage to find those points with maximal echo amplitude reflected from both the articular surface and the uncalcified-calcified cartilage interface.

Such a selection criterion further ensured the perpendicularity between the ultrasound beam and the cartilage.

Cartilage thickness is an essential clinical parameter for the assessment of cartilage degeneration and necessary for the accurate measurement of the tissue modulus. In this study, the tissue thickness was calculated using a predetermined speed of sound and the time-of-flight in samples. The time-of-flight was determined from the echo signals using a cross-correlation technique. The measurement error of time-of-flight of the ultrasound signal in cartilage may be induced by the distortion of the echoes reflected from different interfaces.[58] In our study, a small strain was applied on cartilage by the water jet ultrasound indentation, and high correlations (R > 0.98) between the echoes were achieved for most of the samples. Therefore, the measurement error caused by the frequency-dependent attenuation may probably be a small value. The uncertainty in determining the thickness was mainly caused by the variation of sound velocity. Previous research work reported a variation of speed of sound between different measurement sites and degenerative states.[41,56,65] The speed of sound is slightly lower in OA cartilage than in normal cartilage[65] and digestion of PGs may reduce the speed of sound in cartilage as well.[63] As reported by Toyras et al.,[65] with considering the variable speed of sound, the use of a predefined speed of sound, 1636 m/s,[56] may induce an error of (mean ± SD) 2.9 ± 1.9% for the measurement of cartilage thickness, which would further induce an error of 0.9 ± 0.6% for the stiffness ratio. These small errors should be clinically acceptable.

The accuracy of the deformation measurement depends rather on the sampling rate of the A/D converter than on the ultrasound frequency. With the 500 MHz A/D converter we used, a resolution of 2 ns, i.e. 1.6 μm, could be reached. Such a resolution is adequate for the determination of cartilage deformation during indentation. Higher frequency (e.g.

50 MHz) ultrasound transducers would enable a higher axial resolution, and thereby improve the resolution of thickness measurements. Utilizing a high frequency ultrasound transducer in current system may also help to measure the layered material properties of articular cartilage without damaging its biomechanical integrity, for the analysis of zonal variation in articular cartilage biomechanical properties.[57,73] This will potentially enable the system to identify the grade of degeneration of the cartilage.

The ultrasound water jet indentation system could be easily used for the A-mode reflection measurement. The condition of the cartilage sample could be differentiated based on the change of the amplitude of the A-mode echo signals reflected from the articular surface. Recent studies on the ultrasonic evaluation of articular cartilage have suggested that quantitative ultrasound imaging could sensitively diagnose degeneration of articular surface and changes in the subchondral bone, related to early osteoarthritis[18,31,59,60] In addition to the A-mode measurement, this system is capable of performing scanning to obtain B-mode ultrasound images which would provide additional, visual and quantitative information of the cartilage and subchondral bone structure and composition[41]. However, to obtain a deeper insight of the acoustic properties of articular cartilage, it is necessary to develop a theoretical model which could describe satisfactorily the behaviour of ultrasound in cartilage.

Based on the present results, it is concluded that the ultrasound water jet indentation system is capable of providing quantitative evaluation of both mechanical and acoustical properties of intact and degenerated articular cartilages. Compared to other ultrasound indentation devices used to assess cartilage, this new system could minimize the risk of surface damage to the cartilage caused by mechanical loading therefore provided a nondestructive way to measure the cartilage degeneration. However, the system needs to be further improved before it can be clinically used to assess the cartilage degeneration, such as the miniaturization of the water jet ultrasound probe for arthroscopic use, better water pressure control mechanism, and use of physiological saline.

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Figure Captions:

Figure 1. The lateral lower (LL) quarter of normal bovine patella without obvious lesions was cut as the sample for experiments.

Figure 2. Diagram of the noncontact ultrasound indentation system using water jet compression. The water jet was used as the indenter and focused high-frequency ultrasound was employed to monitor the deformation of the cartilage.

Figure 3. (a) A typical curve of the loading and unloading cycles obtained from a test site of one cartilage sample using the water jet indentation system. (b) The relationship between the pressure and strain which was fitted by a linear regression model.

Figure 4. Box plot of the stiffness ratios of the cartilage samples obtained using the ultrasound water jet indentation. Significant difference was found between the stiffness ratios of intact and degenerated samples ($P < 0.001$). The box represents the inter-quartile range. The upper and lower limits of the box indicate the 75th and 25th percentile. The horizontal line in the box represents the median.

Figure 5. Box plot of the thickness of the cartilage samples obtained using the ultrasound water jet indentation. No significant difference was found between the thickness values of the samples before and after trypsin digestion ($P = 0.87$).

Figure 6. Typical A-mode ultrasound signals from (a) the control sample and (b) the digested sample. The first echo indicates the cartilage surface and the second echo indicates the calcified-uncalcified cartilage interface.

Figure 7. Box plot of the Young's modulus obtained using mechanical indentation. It was found that the Young's modulus significantly decreased after trypsin treatment ($P < 0.001$).

Figure 8. Correlation between the stiffness ratio obtained using the ultrasound water jet indentation system and the Young's modulus obtained from the mechanical indentation for all the cartilage samples, both intact and degenerated ($r = 0.87$, $P < 0.001$).

Figure 9. Bland-Altman plot to test the agreement between the estimated Young's modulus of cartilage samples by ultrasound water jet indentation and the measured modulus by the mechanical indentation.

Figure 10. Representative histological images of articular cartilage stained with Safranine O (red in coloured images) and fast green of a typical sample: (a) intact status; (b) after trypsin treatment.

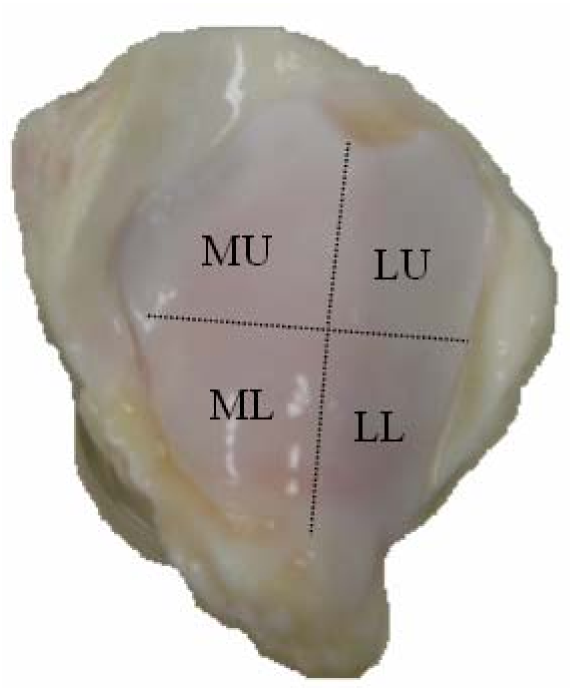


Figure 1.

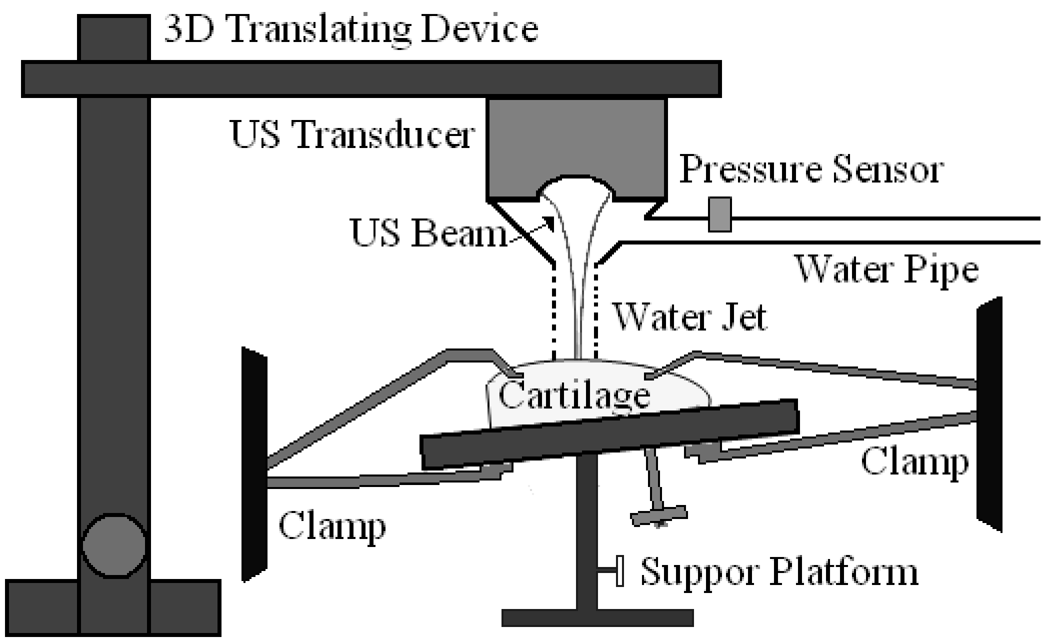


Figure 2.

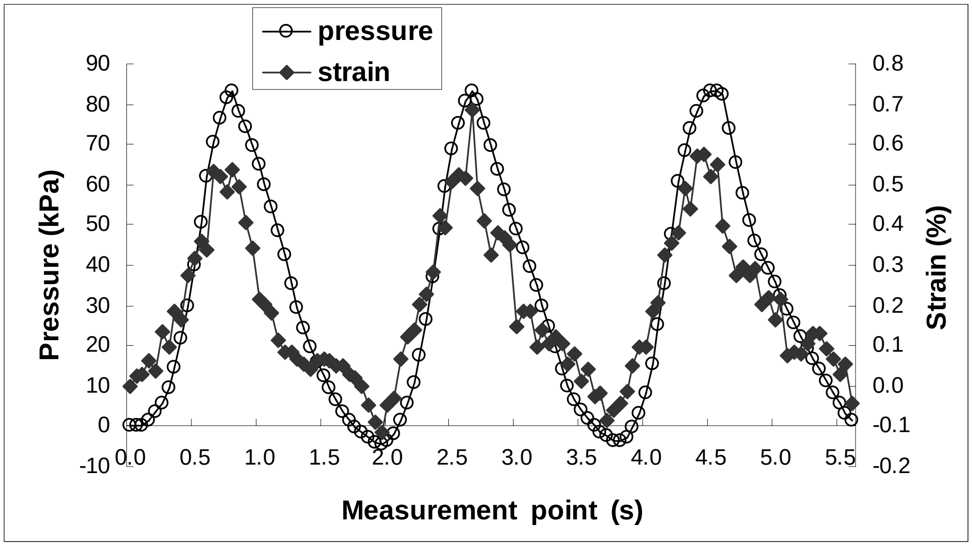
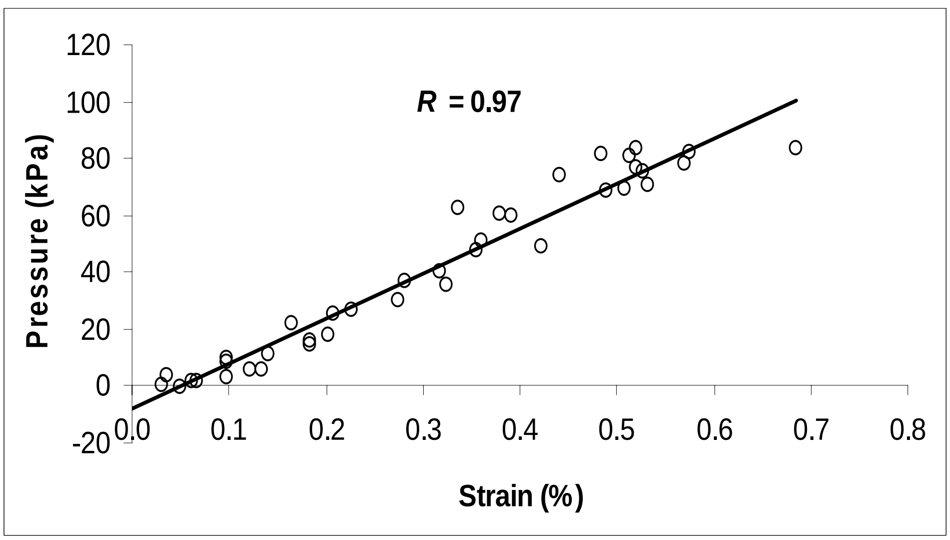


Figure 3a.



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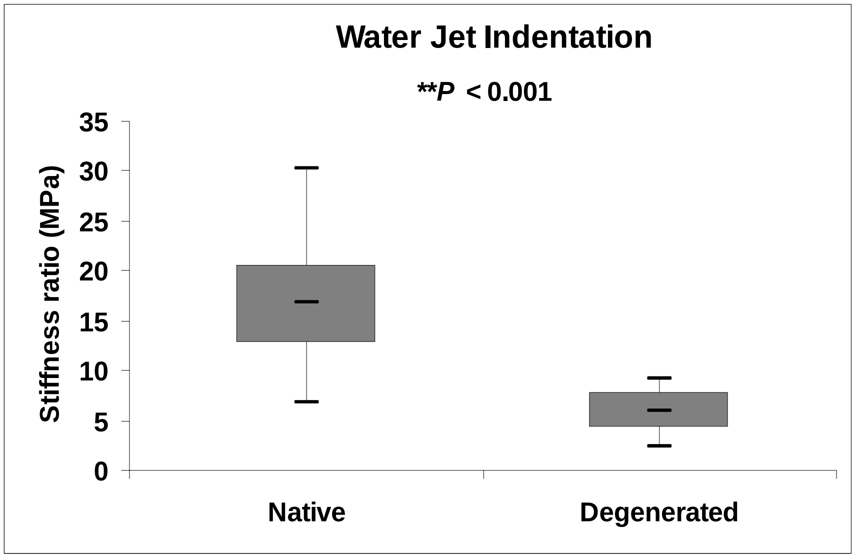

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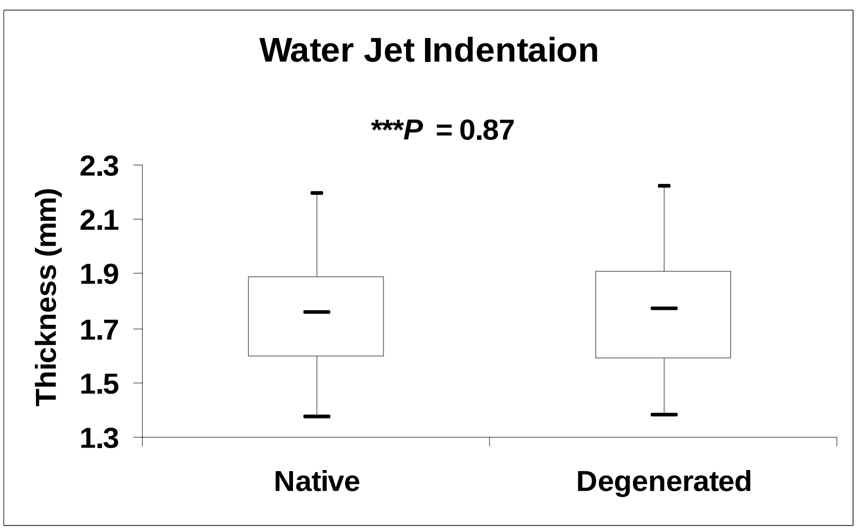

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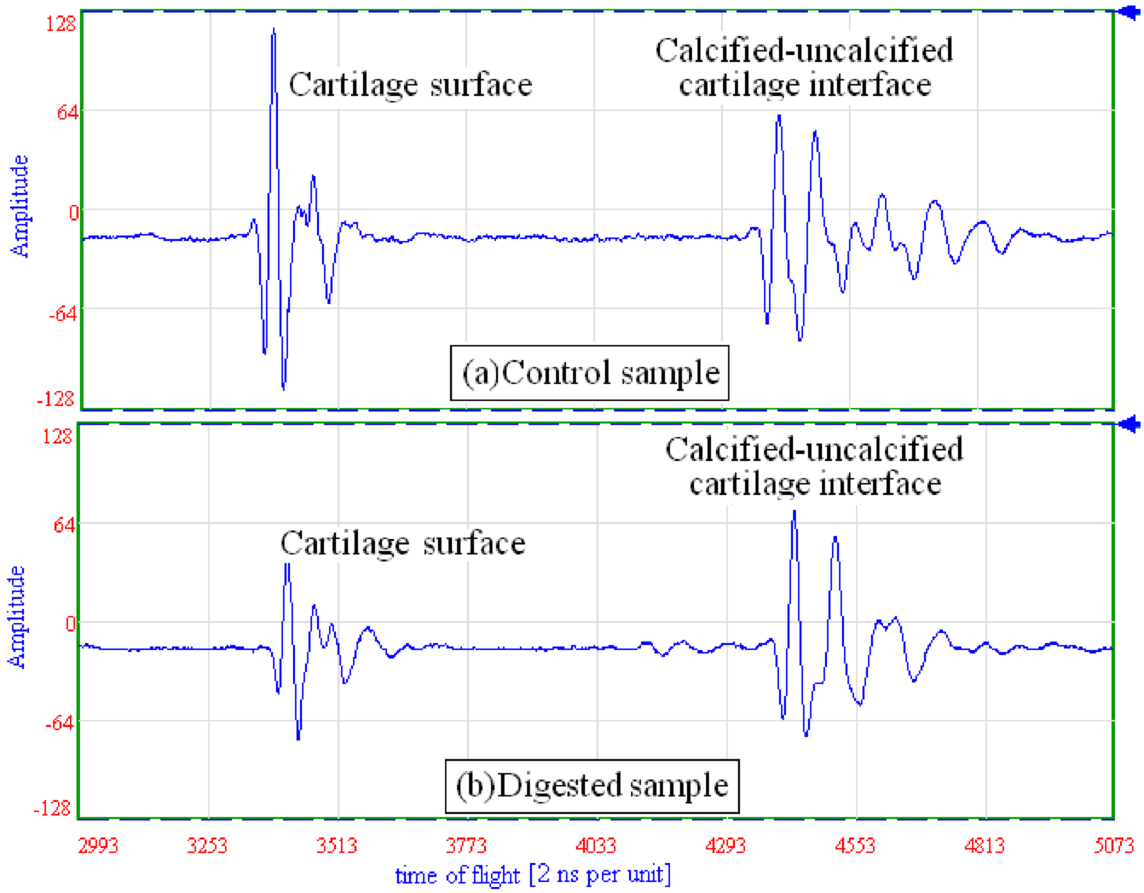

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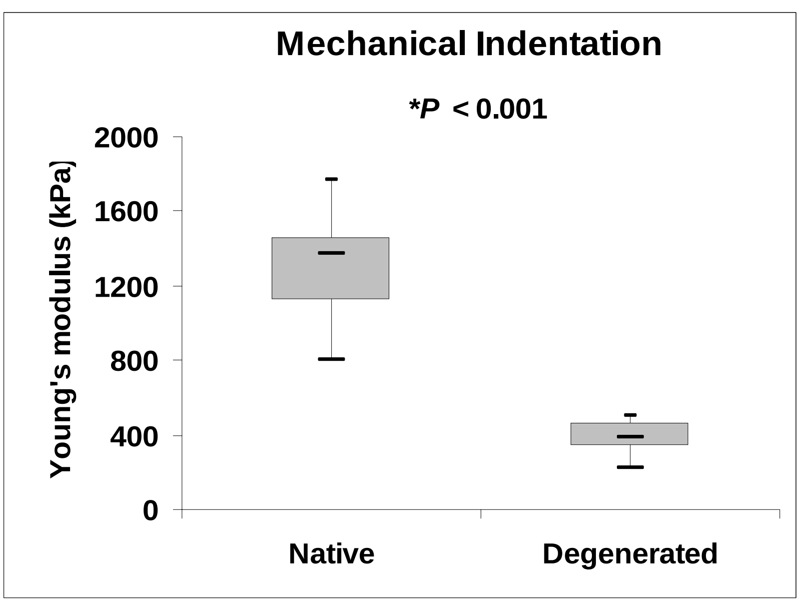

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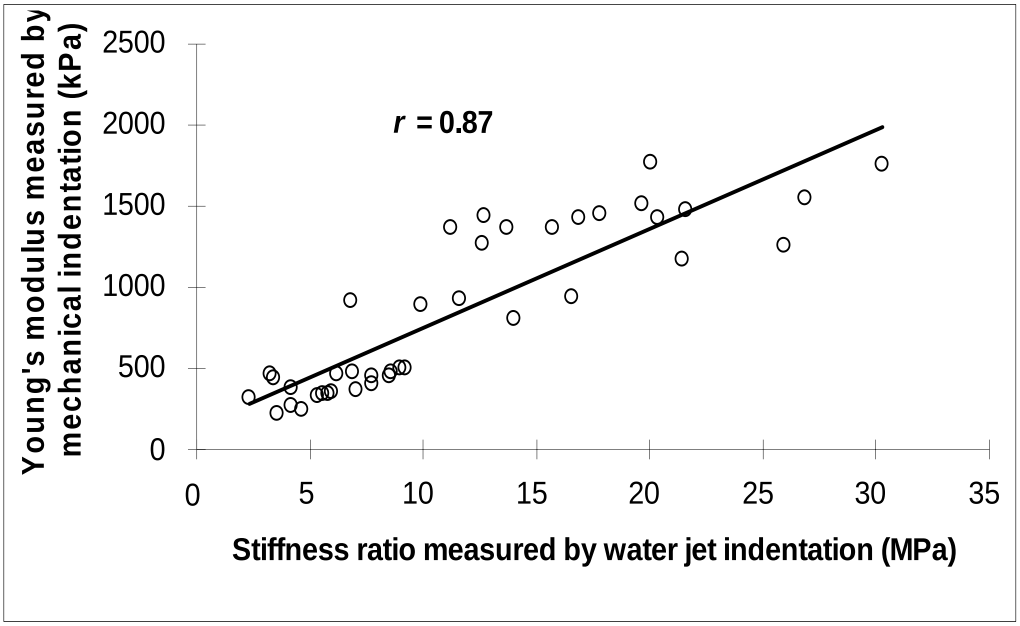
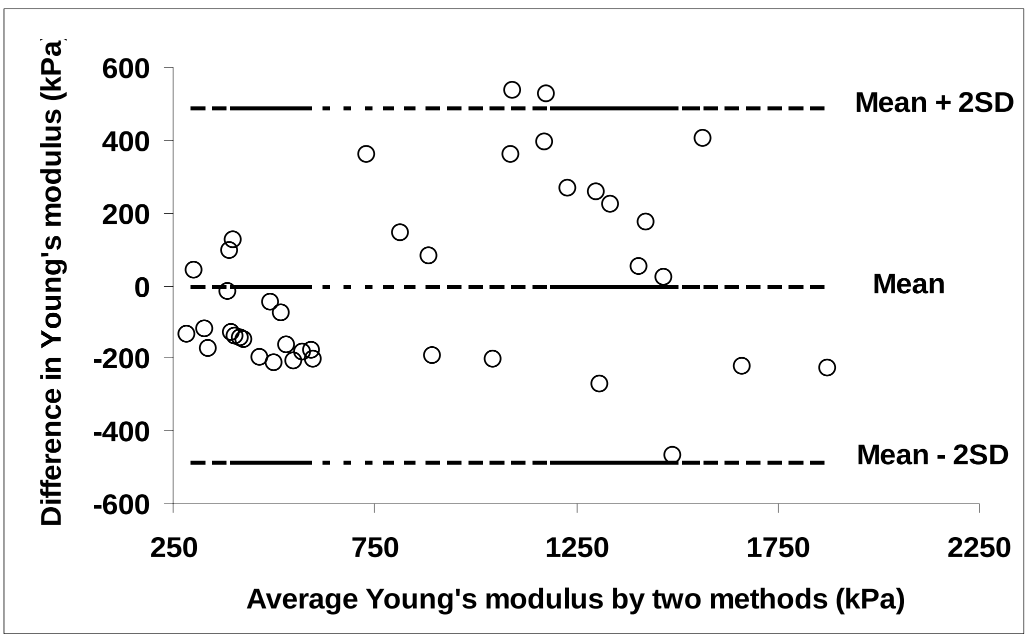


Figure 8.



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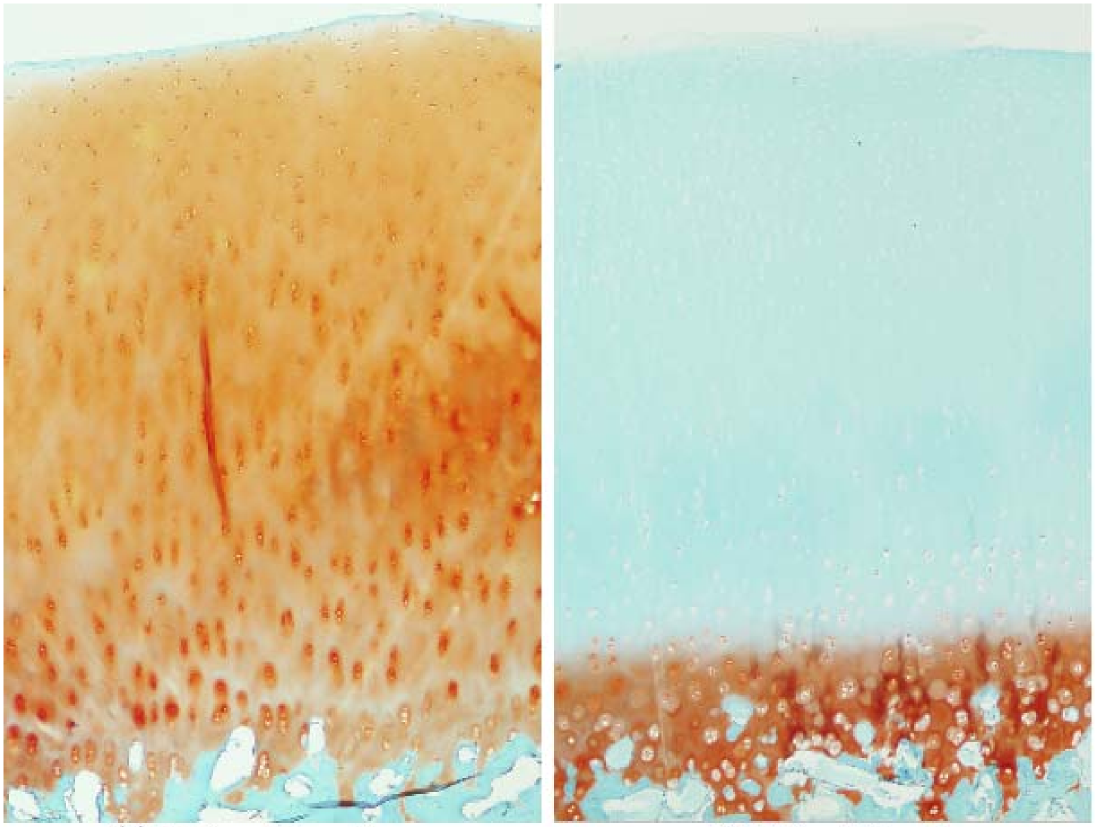

(a) Before Digestion (b) After Digestion

Figure 10.