Study on strain dependence of ultrasound speed in bovine articular cartilage under compression *in vitro*

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Abstract

The change of the ultrasound (US) speed in articular cartilage (artC) under applied strain conditions may induce some measurement errors of the mechanical properties of the artC during both indentation and compression tests using US. In this paper, the strain dependence of the US speed in bovine artC ($n = 20$) under compression *in vitro* was investigated by virtue of using a custom-made US-compression testing system. The US speeds of the artC at the instant after the compression and that after a period of stress-relaxation were estimated under the applied strain ranged from 0 % to 20 %. Moreover, the instantaneous modulus and the modulus after the stress-relaxation of the artC were measured and correlated with the US speeds. There was no significant difference ($p > 0.05$) between the US speeds at the instant after the compression and that after the stress-relaxation, even though there was a discrepancy between the instantaneous modulus and the modulus after stress-relaxation. The US speed was found to be highly correlated to the applied strain ($r^2 = 0.98,\ p < 0.001$) in a quadratic relation and changed by 7.8 % (*from 1581 ± 36 m/s to 1671 ± 56 m/s*) when the applied strain reached 20 %. The results revealed that the strain dependent effect on the US speed in artC should be considered when the US is deployed for the assessment of artC using the compression or indentation test.

Keywords: Strain dependence, Ultrasound speed, Articular cartilage, Cartilage biomechanics, Compression.

Introduction

Articular cartilage (artC) is a firm, resilient and smooth tissue covering the surfaces of freely moving joints for providing low-friction and good wearing resistance of the load-bearing surfaces. During our daily activities, routine physical motion of our bodies involves knee joint flexion that causes the patellar and tibial artCs under stress. Osteoarthritis (OA) which is a complex, common and progressive musculoskeletal disorder leading to clinically identified damage of joints and disability of the patient, was found to be mainly attributed by the high repetitive loading acting to artC (Cooper et al 1994). An increase in the water content (Buckwalter and Martin 1995), depletion of superficial proteoglycans (Buckwalter and Mankin 1997; Arokoski et al 2000) and degradation of collagen fibril network (Kempson et al 1973; Wilson et al 2004) are the early osteoarthrotic changes in artC. Such changes lead to the softening of artC that is considered as the first and most reliable sign of OA. Quantitative assessment of the softening of artC is of vital importance for preventing OA and evaluating the progress of cartilage regeneration. Various kinds of non-invasive imaging techniques, including diffraction-enhanced x-ray (Mollenhauer et al 2002), microscopic MRI (Xia et al 2001) and ultrasound (US) (Zheng et al 2001), have been emerged to provide visual diagnosis of artC in the past decade. However, these techniques are not able to indicate the early degeneration of artC.

Recently, a number of studies have been reported on the use of the mechanical compression or indentation in combination with the US technique for the quantitative assessment of artC. Zheng et al (2001) developed an US-compression system (50 MHz) to investigate the layered biomechanical properties of artC. It was revealed that the compressive moduli of digested and undigested artCs were significantly different. Moreover, this system could also be used to measure the depth-dependent equilibrium compressive material properties of artC (Zheng et al 2002). This technique was later improved for the investigation of the transient measurement and the 2D mapping of the mechanical properties of artC (Zheng et al 2004, 2005). Fortin et al. (2000) used 50 MHz US to measure the transient lateral displacements of artC at different depths under an axial compression with two flat plates. The dimensions of the specimen were in the order of 1 mm. It was demonstrated that the transient depth-dependent Poisson's ratio could be measured using this method. For the deployment of the US indentation, the thickness of artC was measured by using US (Suh et al 2001; Lassanen et al 2002). Saarakkala et al. (2003) have developed a handheld US indentation instrument for the quantitative diagnosis of cartilage degeneration *in vitro* or *in situ*. It was found that both mechanical and acoustic properties of artCs were sensitive to distinguish the enzymatically degraded and normal bovine artCs. Note that a constant US speed was assumed in the above studies. However, it was suggested that the constant US speed may cause inaccurate measurement of artC thickness as well as other mechanical properties since the US speed varies according to the pathological conditions of artC (Jurvelin et al 1995; Yao and Seedhom, 1999).

Heterogeneous structural properties along the depth of artC may cause the variation of the US speed in artC. The depth dependence of the US speed in artC was demonstrated in virtue of the study on the propagation of US through various sections of artC at different depths (Agemura et al1990). The US speed in artC was found to be related to the orientation of collagen fibrils. Moreover, our previous results suggested that the depth-dependence and anisotropy of the US speed in artC should be taken in account for using US in artC measurement (Patil et al 2004). Toyras et al (2003) characterized artC using high frequency US. In their study, different components of artC were selectively digested by enzymes to characterize the relationships among the structural, mechanical and acoustical properties of artC. It was reported that the US speed of full-thickness artC significantly correlated with the equilibrium Young's modulus, the water content and the other artC compositions. Recently, Brommer et al. (2006) showed that the influence of age, site in the joint, and cartilage degeneration in equine artCs on the US speed was significant.

To date, the potential change of the US speed under different stress or deformation conditions have not been well examined. The higher the applied stress was, the stiffer the artC stiffness (Bursac et al 1999; Fortin et al 2000; Legare et al 2002). The knowledge of the strain dependent effect on the structural and functional alteration in artC is important for the assessment of artC and the development of theoretical models for compression and indentation tests. Many studies have shown that the strain induced by the mechanical loading could obviously influence the normal function of artC, as well as its structural change such as the breakdown of collagen (Repo and Finlay 1977; Broom 1986; Jeffery et al 1995; Borelli et al 1997; Kerin et al 1998; Quinn et al 1998; Chen et al 1999; Torzilli et al 1999). Such changes may affect the mechanical and acoustical properties of artC, especially in the US speed (Chen et al 1999). The correlation between the stiffness of artC and its US speed has been investigated by several groups (Suh et al 2001; Toyras et al 2003), while few studies have been reported on the relationship between the US speed in artC and the applied strain. Since the change of the US speed caused by the strain effect could directly affect the measurement accuracy of the mechano-acoustical properties in artC, the study on the strain dependence of the US speed in artC is crucial to the deployment of US-compression or -indentation for the assessment of artC. In this study, an US-compression testing system was developed for the simultaneous measurement of the US speed, stress and strain in artC. The relationship between the US speed, the applied strain as well as the modulus in artC were established.

Materials and Methods

Experimental setup

The US–compression testing system used in the present study is schematically depicted in Fig. 1. This system was modified from the US-compression configuration that was shown in our pervious work (Zheng et al 2002). The container was full of 0.15 M saline solution during the experiment. Two unfocused 5 MHz US transducers with a diameter of 8.9 mm (Panamaetrics Inc, Waltham, MA, USA) were used as the specimen platform as well as the compressor for the test such that the specimen with a diameter of 6.4 mm could be placed between two transducers. The saline solution also helped to minimize the friction between the artC sample and the transducer surfaces during the measurement. It was assumed that the US-compression testing system was rigid enough during the compression test so that the deformation was only applied on the specimen. The movement of the compressor was manually driven with a micrometer adjustor installed on the top of the testing device. The axial displacement of the compressor was measured by a linear variable displacement transducer (LVDT) (DFg 5 (guided), Solartron, RS components Inc., Hong Kong). During the compression, the US transducer located at the bottom was used to transmit the US pulses into the artC specimen and the US echoes reflected or scattered with the artC were received by another transducer above the surface of artC. A broadband US pulser/receiver (model 5601A, Panametrics Inc., Waltham, MA, USA) was used to drive the US transducer and to amplify the received US echoes. A 25 N load cell (model ELFS-T3E-5L from Entran, NJ, USA) which was connected in series to the compressor was used to record the compressive force during the test.

The load, displacement, US signals were digitized by two A/D converter cards (NI-DAQ 6024E, National Instruments, USA; Model CompuScope 8500PCI from Gage, Canada) at a sampling rate of 500 MHz installed in the PC. Then, all data was collected and analyzed by using our custom-developed computer program. To improve the ultrasonic signal condition for the cross-correlation algorithm applied later for the signal analysis, the US echo trains were averaged for 30 times to enhance the signal-to-noise ratio. Similarly, the load and LVDT data were averaged for 100 times.

Specimen preparation

Bovine knee patellae without obvious lesions were obtained from a local slaughter shop within 6 hrs of delivery and stored at -20°*C* until further specimen preparation. It has been reported that cryopreservation (Kiefer et al 1989), freezing, and thawing (D'Astous and Foster 1986; Agemura et al 1990; Kim et al 1995) of artC specimens would not affect its mechanical and acoustic properties such as the US speed, attenuation, and backscatter. Fig. 2 schematically presents the specimen preparation configuration which consists of excising the patella into 4 quadrants using a bend saw machine. During excising, it was ensured that the patellae were kept moist with the saline solution. The four parts were named according to the medial or lateral side of the patella as MU (Medial Upper), ML (Medial Lower), LU (Lateral Upper), and LL (Lateral Lower). Full-thickness circular artC disks with a diameter of 6.4 mm were obtained from the artC-bone plugs of the MU quadrant of the patella after detachment from the subchondral bone of artC (n = 20). The artC disks without bone were selected such that the surface of the artC remained flat and parallel to the bottom throughout the whole disk.

Measurement procedure

Measurement for US speed and Young's modulus in artC. The circular artC disks were first thawed for 30 min in the saline solution in the container as shown in Fig. 1. The US transducers acting as the compressor and the specimen platform initially contacted with each other and the reading on the LVDT obtained at this position was recorded as a reference for the measurement of the artC thickness. The specimen was then placed on the transmitting transducer and the compressor was moved vertically down to make an initial contact with the specimen. The reading of the load cell indicated the initial contact of the compressor with the specimen. It was observed that the excised specimens were slightly curled. To ensure the surfaces of the two US transducers in a full contact with the specimen surface, the compressor was further driven by 0.05 mm for each specimen. At this moment, the applied load was normally below 0.001 N at 15 min after the displacement was applied and 0 % compression was assumed. Before further compression, the specimen was allowed to rest in the saline solution for 30 min. Then, stepwise stress-relaxation measurements (step 2.5 % of the artC thickness) were conducted up to a total strain of 20 % with a strain rate of approximately 0.4 %/s. A relatively shorter relaxation time of 15 min was allowed between the steps, in comparison with traditional mechanical measurement of artC for equilibrium parameters, though shorter relaxation time has been used in some earlier studies (Jurvelin et al 1996; Toyras et al 1999; Lyyra et al 1999; Kowhonen et al 2002; Toyras et al 2003; Fortin et al 2003). Using shorter relaxation time, only eight steps were used for testing 20 specimens and thus the overall experiment time could be saved. To test the change of the US speed during the stress-relaxation period, additional 5 specimens were tested with a single compression step of 2.5 % by using the relaxation time of 60 min. US signals were collected continuously at a rate of one frame per two seconds during the compression and stress-relaxation phases. Note that the signal to noise ratio of the LVDT signal was relatively low due to the small deformation applied during each compression. Therefore, data averaging (30 point moving average) was used to enhance the LVDT readings and the related results. The instantaneous Young's modulus and US speed were calculated at the moment with the maximum stress after each ramp compression. The Young's modulus and the US speed after 15 min stress-relaxation were also calculated for compression. In the case of the additional 5 specimens tested with 60 min stress-relaxation, the US speed was calculated for each 5 min.

Calculation of US speed and Young's modulus in artC. A schematic representation of the US echoes passing through the specimen is illustrated in Fig 3(a). The US speed in the artC was determined after the two platens were in contact with the specimen (indicated by the force measured by the load sensor) using the pulse-echo technique. The time-of-flight was determined as the travel time of the rising phase of the US pulse back and forth through the artC specimen, while the artC thickness was determined as the distance between the surface of the two transducers. The distance was accurately measured using the calibrated LVDT fixed on the moving micrometer. The cross-correlation algorithm was used to match the two echoes obtained at each compression level so as to obtain the time-of-flight of US in the artC specimen. In Fig. 3(b), the first echo was selected as the reference using a tracking window and the position of the second echo was automatically searched according to this reference.

Fig. 3(b) shows the echoes obtained under compression levels of 0 % and 20 %. T_2 and T_4 represent the round trip between the transmitting and the receiving, i.e. the round trip inside the artC specimen under two different compression levels, respectively. The artC specimen was compressed up to 20 % of its original thickness at each step of 2.5 %. Therefore, the US speed in artC under these two compression levels can be calculated by Eqs. (1) and (2), respectively, as

$$c_0 = \frac{2d_0}{T_2} \tag{1}$$

$$c_{20} = \frac{2d_{20}}{T_4} \tag{2}$$

where d_0 and d_{20} is the uncompressed and compressed (20 %) thickness of artC, respectively. The artC thickness at 0 % compression was measured as the distance between the surfaces of the two transducers when they were just touching artC. It was noted that the quality of US echo remained almost the same under different compression levels and a good correlation was obtained among echoes.

Data Analysis. One factor ANOVA (SPSS v11.0.0, SPSS Inc., Chicago, IL) was used for analyzing the statistical significance of the difference in the US speeds in artC with different applied strains.

Results

Fig. 4(a) shows a typical stress-relaxation response after a step-wise compression consisting of eight steps with 2.5 % strain for each step. It was observed that the stress-relaxation period of 15 min appeared not enough for the force to reach its equilibrium state when the overall strain was larger than 10 %. A typical stress-relaxation response when the artC specimen was allowed to undergo a relaxation time of approximately 60 min after a single compression step of 2.5 % is illustrated in Fig. 4(b). The results showed that the force value was almost constant after 60 min relaxation with a force change rate of 0.4 ± 0.1 mN/s.

The results demonstrated that the US speed at the moment with the maximum force after each compression was not significantly ($p > 0.05$) different from that measured after 15 min stress-relaxation. Therefore, an average of the US speeds measured at the instantaneous phase and after 15 min stress-relaxation was used for the subsequent analysis. The test on the specimens with 60 min stress-relaxation demonstrated that there was no significant difference between the US speeds measured after 15 min and 60 min relaxation. However, a slight increase of the US speed was observed with the increase of the stress-relaxation time. Table 1 lists the values of US speed and modulus measured at various compression levels. It was found that the US speed in artC changed by 7.8 % (from 1581 ± 36 m/s to 1671 ± 56 m/s) when the compression was changed from 0 % to 20 %. The US speed in artC significantly increased with the increase of the applied strain ($r^2 = 0.98$, $p < 0.001$). A quadratic relationship between the percentage change of US speed in artC and the strain was demonstrated ($r^2 = 0.98$) in Fig. 5.

Fig. 6 indicates the overall stress-strain relationship of artC averaged from the 20 specimens. It was noted that the quadratic functions could well fit the stress-strain relationship of the data collected instantly after the compression ($r^2 = 0.99$) as well as after the 15 min stress-relaxation ($r^2 = 0.99$). From Table 1, the instantaneous modulus changed from 0.21 ± 0.28 MPa to 2.86 ± 2.64 MPa when the applied strain changed from 2.5 % to 20 %. Similarly, the modulus after 15 min stress-relaxation changed from 0.058

± 0.064 MPa to 0.87 ± 0.81 MPa. Even though large individual variation was observed, high correlation was obtained between the applied strain and the instantaneous modulus ($r^2 = 0.99$) as well as the modulus after 15 min stress-relaxation ($r^2 = 0.99$), as shown in Fig 5. It was noticed that quadratic functions could well represent their nonlinear relationship. Moreover, the changing rate of the instantaneous modulus was much larger than that of the modulus after 15 min stress-relaxation. Fig. 7 illustrates that the instantaneous modulus was linearly correlated with the modulus after 15 min stress-relaxation ($r^2 = 0.99$, $p < 0.001$).

It was found that the modulus as well as the US speed both increased nonlinearly with the increase of the strain applied on artC. Fig. 8 represents the correlation between the change of the US speed and the instantaneous modulus and the modulus after 15 min stress-relaxation. Good linear relationships were observed between the change of the US speed and the moduli of artC ($r^2 = 0.97$). Even though the instantaneous modulus and the modulus after 15 min stress-relaxation were dramatically different, the US speed did not show any significant difference.

Discussion

In the present study, the strain dependent effect on the US speed in the bovine patellar artC was investigated. The US speed in artC and the instantaneous modulus as well as the modulus after 15 min stress-relaxation of artC under the applied strain, up to 20 % with each step of 2.5 %, were measured using the US-compression testing system.

The results demonstrated that the sound speed in artC significantly increased as the increase of the compression level. This finding matched with our earlier finding using a ultrasound elastomicroscopy (Zheng et al. 2004). It was found that there was no significant difference between the US speeds in artC during the stress-relaxation period from 15 min to 60 min. Even though using the stress-relaxation time of 60 min can ensure the artC reached its equilibrium state, it is not so practical since it takes eight hours to finish the compression test for each specimen. In practice, the relatively long exposure time of the artC in room temperature might probably cause the degeneration of artC. It was reported that there was a significant difference between the US speeds in normal and degenerated cartilage (Toyras et al, 2003). In this case, the change of US speed caused by the degeneration of artC may dominate that originated from the effect of stress-relaxation time. Therefore, it is reasonable to adopt the stress-relaxation time of 15 min, instead of 60 min, used in the study of the US speed in artC under various strain conditions.

The fluid dissipation speed and the rate of fibril stiffening are the two main factors governing the instantaneous modulus and the modulus after the stress-relaxation (Li et al 2003). For the compression test on artC at the strain rate of 0.4 %/s, the collagen fibrils stiffened quickly with the flow-dependent lateral expansion and the water has little time to dissipate. At this moment, the artC tended to resist the immediate fluid exudation by undergoing a slight volume change. That resulted in the quick growth of the instantaneous stiffness of the artC. The water then gradually flowed out during the stress-relaxation. Subsequently, the fluid pressure that supported the majority of the applied stress decreased obviously, resulting in a significant decrease in the artC's stiffness. These are the explanation on why the instantaneous modulus is always higher than that after 15 min stress-relaxation (Fig. 5). Consistent with the earlier studies (Li et al 2003), the instantaneous modulus and the modulus after the 15 min stress-relaxation increased with the increasing applied strain (Fig. 5). It was found that a quadratic function can better represent the relationship between the moduli of artC and applied strain. That non-linear relationship could be explained by the inhomogeneous and anisotropic material properties of artC from the superficial layer to the deep layer along its axial direction, as illustrated in the stress-strain relationship (Fig. 6).

Previous literatures revealed that the mechanical properties (Suh et al 2001; Patil et al 2004), cartilage composition (Joiner et al 2001) and the degeneration level (Myers et al 1995; Toyras et al 2003) of artC were highly correlated to its US speed. It has been reported that the US speed was significantly related to the modulus at equilibrium and the dynamic conditions of artC. The higher the modulus was, the higher the US speed in artC (Toyras et al 2003). This study was in a good agreement with the previous findings that linear relationships between the change of the US speed and the instantaneous modulus as well as the modulus after 15 min stress-relaxation were established. However, it was surprisingly to notice that there was no significant difference in the US speed measured at the immediate moment after the compression and that captured after 15 min stress-relaxation, even though the instantaneous modulus and the modulus after 15 min stress-relaxation showed a great discrepancy. There was no volumetric change of artC in the stress-relaxation period. In consideration of the composition of artC, only the fluid which mainly consisted of water flowed out from artC. An obvious decrease in the water content in artC would affect the US speed since the US speed was highly dependent on the water content in the artC. A decrease in the water content would cause an increase in the US speed (Toyras et al 2003). This might imply that the effect of the change of the water content in the artC on the US speed compensated that of the change of artC's stiffness. Consequently, the increase and the decrease in the US speed caused by the changes of the water content and the artC's stiffness, respectively, could be balanced. Moreover, a slight increase of the US speed was observed with increasing stress-relaxation time ranged from 15 min to 60 min. The reason was probably due to the stress redistribution of the collagen fibrils after the water fully ran out from the artC. Approaching to the equilibrium state, the collagen fibrils bore nearly 100 % of the applied load after stress redistribution and that resulted in the further stiffening of the artC.

It was demonstrated in this study that the US speed increased by 7.8 % when the applied strain was changed from 0 % to 20 %. If a constant US speed was used in the US indentation (Zheng and Mak 1996; Toyras et al 2003), the potential errors caused by this assumption on the artC thickness and indentation measurement would be in the same percentage range. Toyras et al (2003) reported that use of constant US speed in artC for the measurement of artC modulus could generate an error of approximately 1 - 7 % on the thickness of artC and dynamic modulus measurement. Considering the strain-dependent US speed demonstrated in artC, the potential error should be larger. It is therefore suggested that the strain dependent effect on the US speed should be taken into account in the future in the experimental studies as well as theoretical modeling of artC, such as US indentation, US compression, and mechano-acoustic assessment. In particular, the quadratic relation between the US speed and the applied strain would give the guideline for the correction of the US speed during the assessment of artC.

The US speed in the full-thickness artC measured under 0 % compression in this experiment was 1581 ± 36 m/s. It was significantly smaller than those measured in other experiments in our previous studies (Patil et al 2004). It implied that some systematic error might exist in the measurement of the initial US speed (0 % compression) using the US compression system. In the US compression method, two flat unfocused US transducers with a frequency of 5 MHz were used for the contact measurement. This configuration was different than that used in other experiments, where a 50 MHz focused US transducer was used for non-contact measurement. Further studies are required to find out what causes the difference in the measurement of the US speed between the two systems. The main focus in using this US compression method was to demonstrate the strain-dependence of the US speed in artC. Therefore, the percentage change of the US speed was used to avoid the influence of this potential systematic error.

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Figure

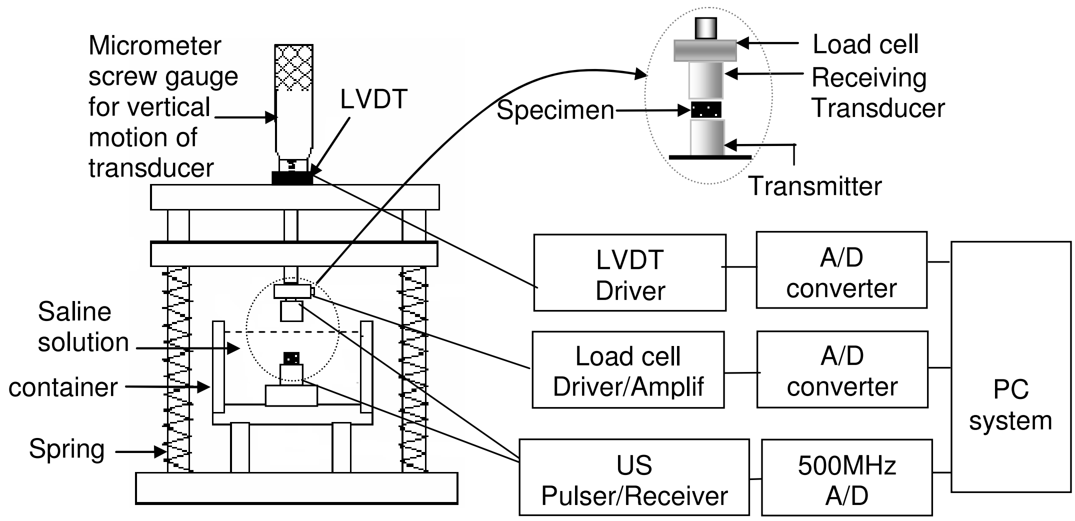

Fig. 1 The US-compression system for the study of the strain dependence of the US speed in artC.

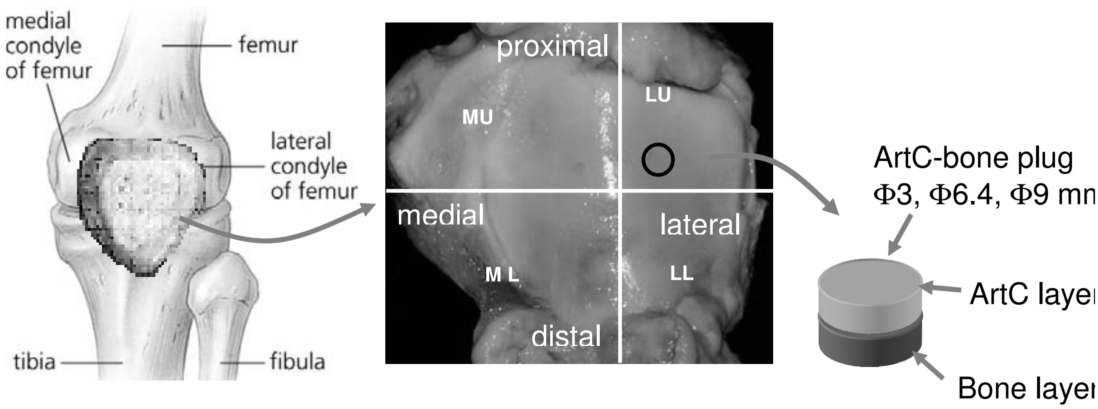

Fig. 2 A schematic representation of the artC specimen preparation configuration.

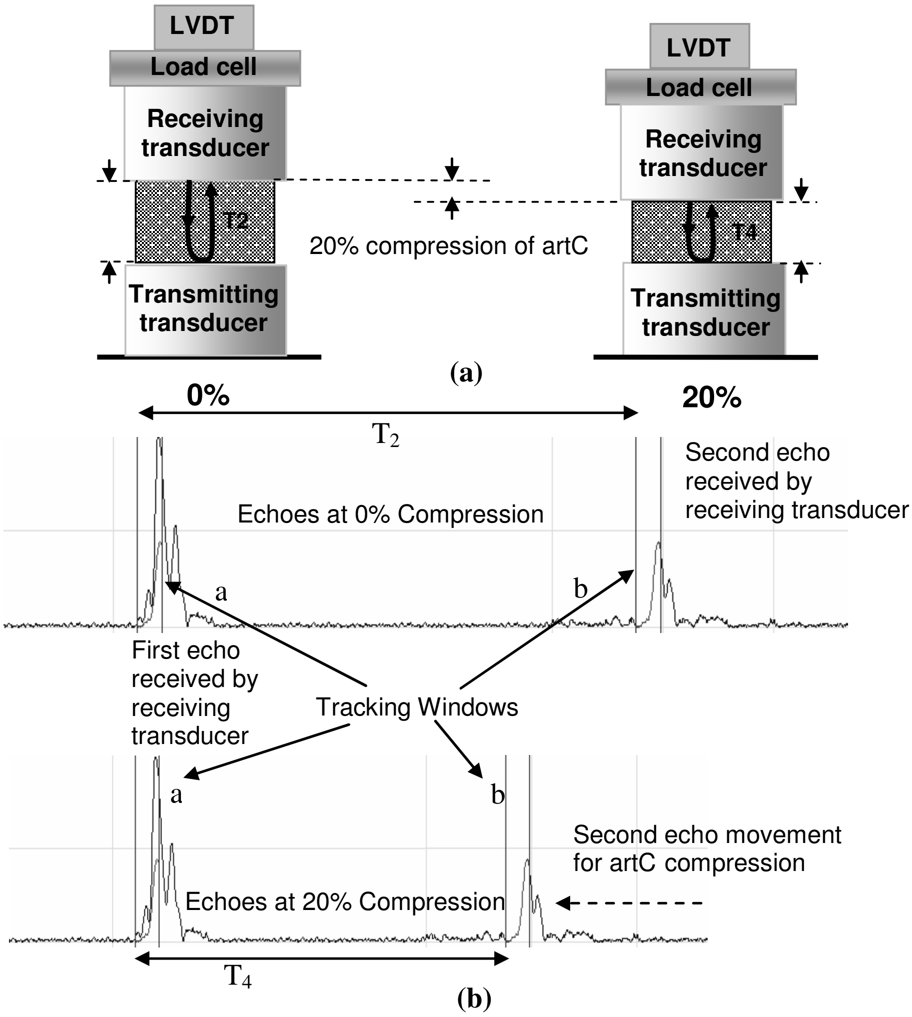

Fig. 3(a) A schematic representation of the US echoes passing through the specimen and (b) Typical US echoes received by the US receiving transducer. The cross-correlation algorithm was used to match the two echoes obtained at each compression level. The echo indicated by ‘a’ represents the reference signal and the echo indicated by ‘b’ was located by the cross-correlation tracking. The echo to be matched was overlapped with the reference echo.

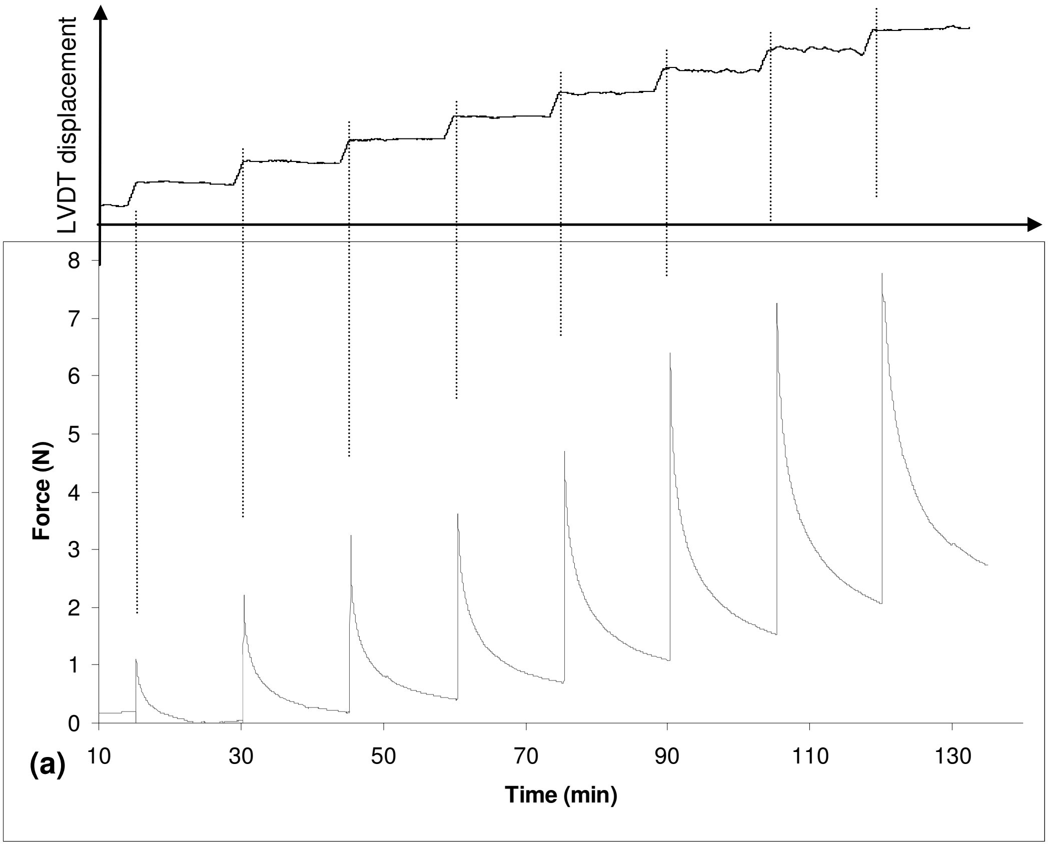

Fig. 4(a) Typical behavior of artC in step-wise stress-relaxation experiment as a function of time in the unconfined compression and the corresponding displacement data by the LVDT sensor.

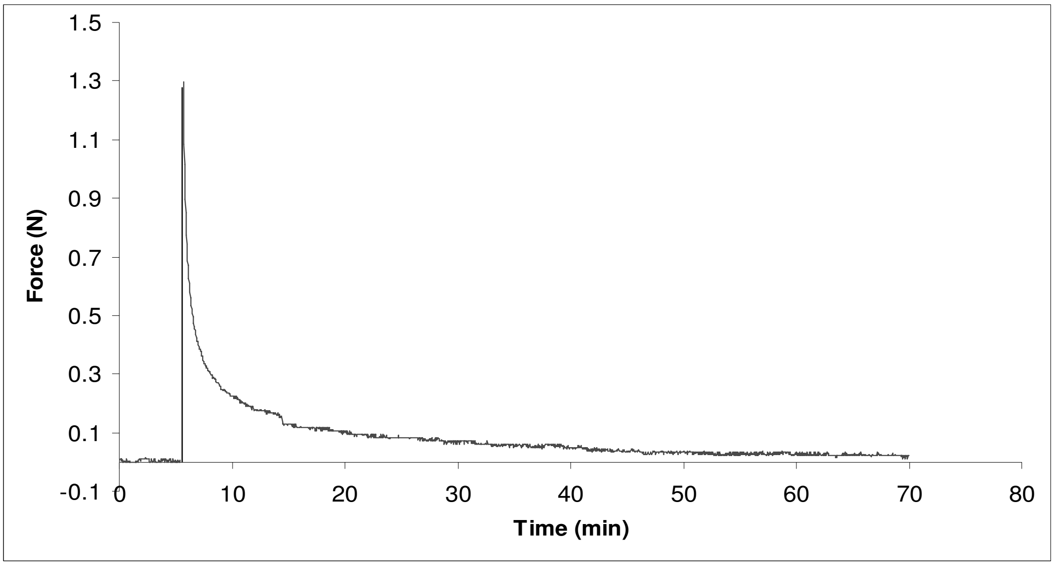

Fig. 4(b) The typical stress-relaxation response of a single step compression when the artC specimen was allowed to relax for 60 min.

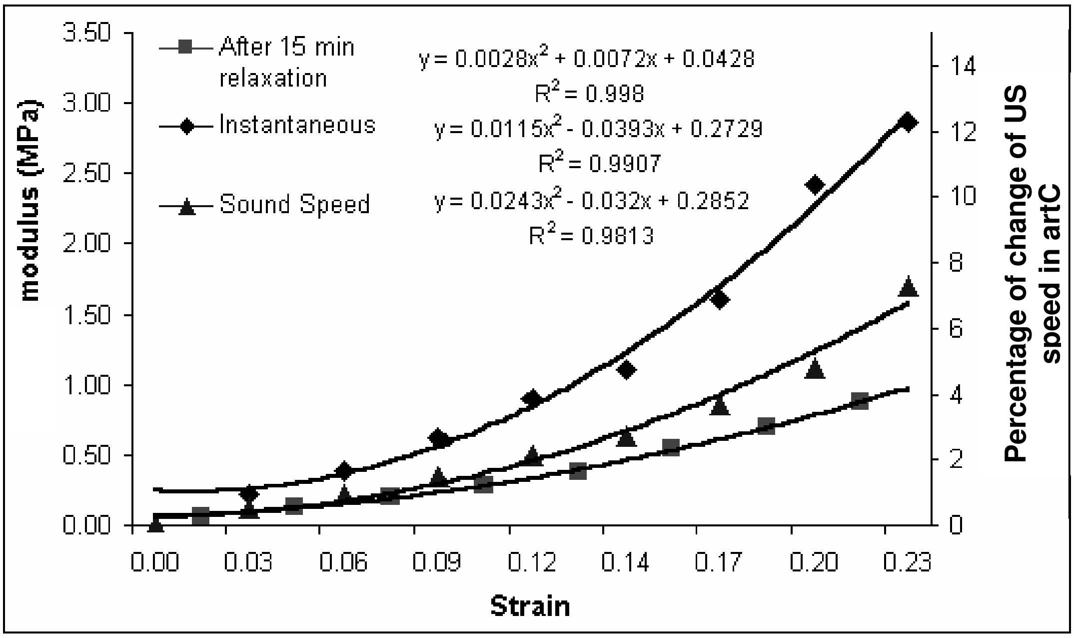

Fig. 5 The relationship between the applied compression on artC and the percentage changes of the US speed, the instantaneous moduli, and the moduli obtained after 15 min stress-relaxation. A quadratic function was used to fit each set of data. Large variation among individual specimens was observed. To make the figure easier to read, the error bar had not been included, the reader can refer to Table 1 for the SD for each data set.

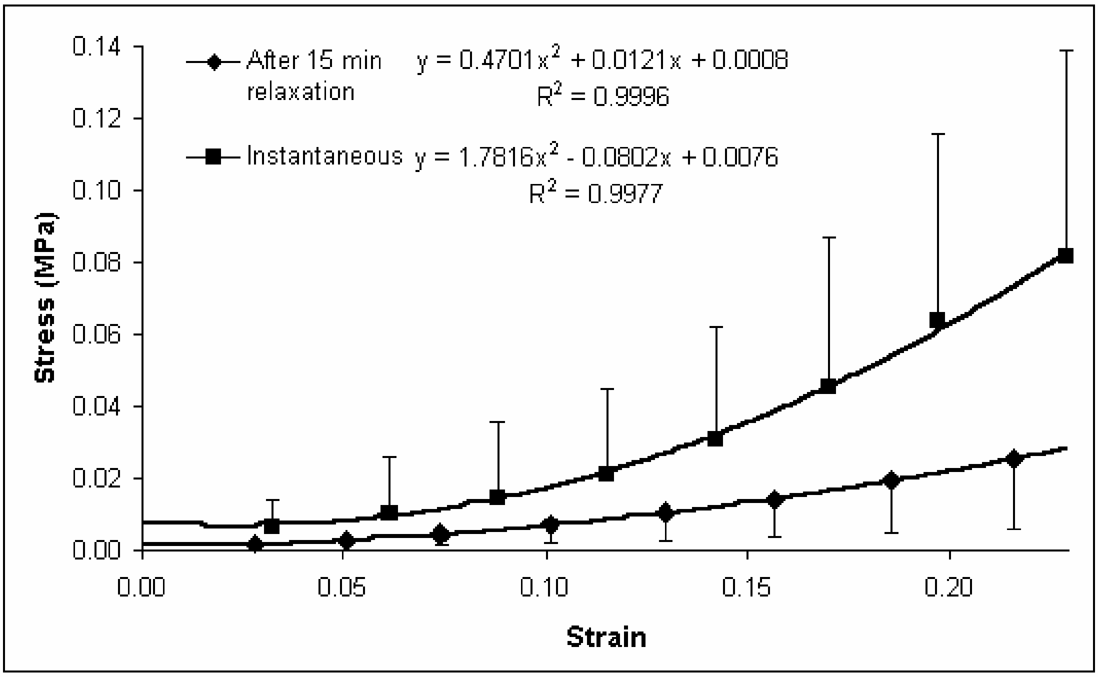

Fig. 6 The relationship between the strain and the mean instantaneous stress as well as stress after 15 min stress-relaxation. The error bars represent the SD of the stress at each strain level among the 20 specimens.

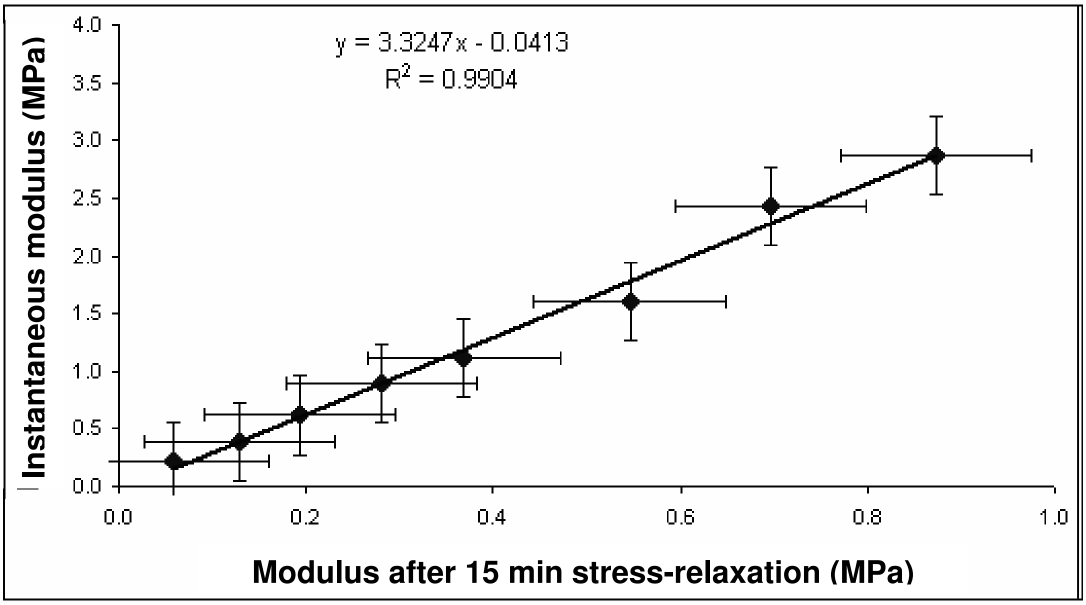

Fig. 7 The relationship between the instantaneous modulus of artC and the modulus obtained after 15 min stress relaxation. The error bars represent the SD of the results of the 20 specimens.

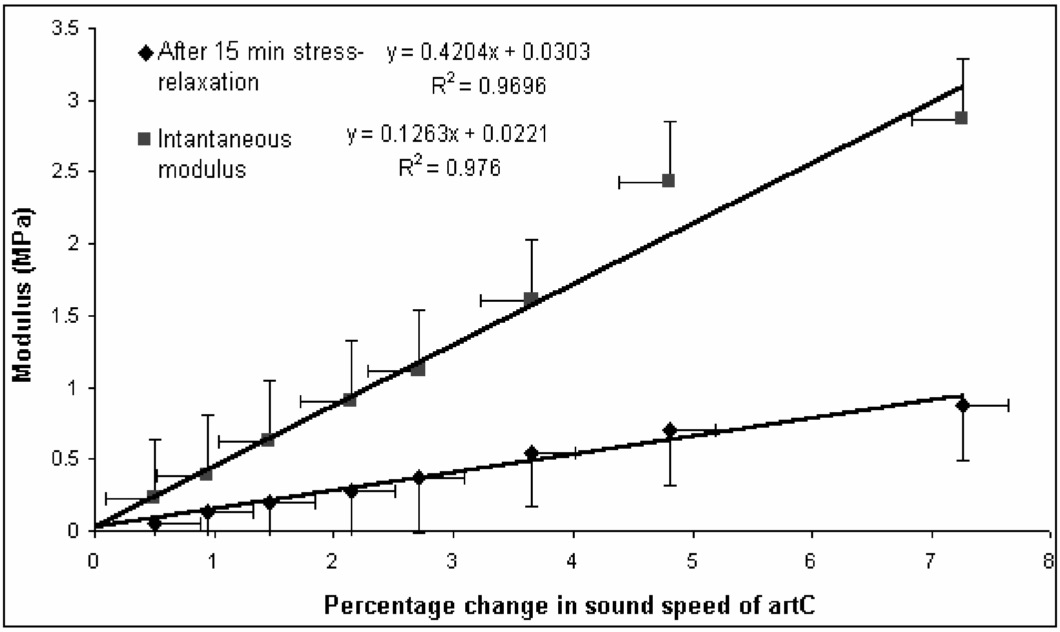

Fig. 8 The relationship between the change of the US speed in artC and the instantaneous modulus and the modulus after 15 min stress-relaxation. The error bars represent the SD among the 20 specimens.

Table

Table 1. Averaged values (n = 20) of the US speed, the instantaneous modulus and the modulus after 15 min stress-relaxation for the 8 compression levels ranged from 2.5 to 20%.

		% artC compression (n = 20)								
		0	**2.5**	**5**	**7.5**	**10**	**12.5**	**15**	**17.5**	**20**
US speed (m/s)	**Mean**	1581	1585	1593	1605	1616	1626	1641	1659	1671
	SD	36	43	46	42	44	44	47	49	56
Instantan--eous Modulus (MPa)	**Mean**		0.21	0.39	0.62	0.90	1.11	1.60	2.42	2.86
	SD		0.28	0.53	0.86	1.05	1.10	1.50	1.92	2.64
Modulus after 15min of stess-relaxation (MPa)	**Mean**		0.05	0.13	0.19	0.28	0.37	0.54	0.70	0.87
	SD		0.06	0.08	0.16	0.21	0.29	0.37	0.51	0.81