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Quasi-linear Viscoelastic Properties of Fibrotic Neck Tissues Obtained from Ultrasound Indentation Tests in Vivo

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Abstract

Background: Hand palpation is a conventional way to assess and document soft tissue fibrosis. But it is semi-quantitative and subjective, so there is a need to develop quantitative and objective methods for this purpose.

Methods: 105 patients with different degrees of radiation-induced fibrosis of soft tissue of the neck were assessed using an ultrasound indentation method. The force response was reconstructed from the indentation history using a quasi-linear viscoelastic model with four material parameters. The parameters which best curve-fitted the force response with respect to the experimentally measured one, were selected as the viscoelastic properties of the tested soft tissue. These parameters were compared among patient subgroups with different degrees of fibrosis as scored by hand palpation, and also compared with those of a control group of healthy, non-irradiated subjects. Their relation to the rotation range of the neck and the effective Young's modulus, were also assessed.

Findings: Soft tissue with a more severe degree of fibrosis was associated with a larger initial stiffness and a more rapid increase in stiffness under loading. Viscoelasticity parameters can discriminate soft tissue with different degrees of clinical fibrosis and had significant correlation with clinical parameters of fibrosis.

Interpretation: Change of viscoelastic properties is reflection of pathological modifications of components in fibrotic soft tissues. Measurement of viscoelasticity parameters of soft tissue provides a quantitative and objective approach for the researcher and clinician to quantify soft tissue fibrosis.

Relevance

Measurement of the change of viscoelastic properties of soft tissue provides a quantitative and objective approach for researchers and clinicians to quantify soft tissue fibrosis which is one of the most common late effects of radiotherapy.

Keywords: Radiation-induced fibrosis; Hand palpation; Ultrasound indentation; Soft tissue; Tissue mechanical properties; Tissue viscoelasticity

1. Introduction:

Soft tissue fibrosis is one of the most common late effects of radiotherapy. Attempts have been made to increase the probability of eradication of cancer by increasing the radiation dose delivered, but potential accompanying increase in tissue fibrosis, together with other late effects of radiotherapy, is of concern. Assessment of radiation-induced fibrosis is an important issue for the evaluation and comparison of alternative radiotherapy protocols and the efficacy of antifibrotic agents. Although radiation-induced fibrosis has been described and investigated from the molecular and cellar basis (Burger et al., 1998; Rodemann and Bamberg, 1995), clinical diagnosis and documentation have still been limited to hand palpation or other subjective methods to date. Scoring of fibrosis by hand palpation is inherently subjective, at best only semi-quantitative and subject to inter-observer variations.

A clinical method to document soft tissue fibrosis should ideally be noninvasive, reliable, quantitative and objective. Indentation is such a potentially suitable method. Prototype models of such a measurement have been investigated to test the mechanical behavior of soft tissues *in vitro*, *in situ* and *in vivo*. Its test procedure itself is simple, and very much resembles the operation of hand palpation. It has been used by a lot of investigators to extract the mechanical properties of plantar soft tissues for study of foot pathologies (Klaesner et al., 2002) and residual limb soft tissues for a better fitting of prosthetic sockets (Mak et al., 1994; Silver-Thorn, 1999; Vannah and Childress, 1996). A typical indentation system provides the force and indentation information simultaneously during the indentation process. Such an indentation system, with proper designs, is capable of studying different aspects of soft tissue mechanics, such as hysteresis, rate-sensitivity of stiffness, stress-relaxation as well as creep behavior (Ferguson-Pell et al, 1994; Mak et al., 1994; Silver-Thorn, 1999; Vannah and Childress, 1996). Currently, most of the research studies using the indentation method have selected the effective Young's modulus as the quantitative parameter for the tissue material property. Nonlinear elastic and time-dependent characteristics (viscosity) of soft tissues have been noticed in some of the previous studies, but most have not provided quantitative information on them. To neglect the nonlinear and viscoelastic behavior of soft tissues is not appropriate since it is well known that the stress-strain relationship of biological tissues is inherently nonlinear and time-dependent, especially where large strains occur.

It has been demonstrated that when soft tissues undergo structural or pathological degenerations, diseases or healing processes, the nonlinear and viscoelastic behavior of the affected tissues changes as well (Klaesner et al., 2002; Thornton et al., 2000). The change of nonlinear and viscoelastic behavior can be the symptom of systemic alterations of tissue components such as collagen and water contents. If the change of viscoelastic behaviors in soft tissues can be detected by indentation tests, it is for certain beneficial for researchers and clinicians to characterize tissue abnormalities noninvasively.

Almost all the indentation systems afore mentioned are used in laboratory settings or used only for specific parts of the human body. A portable and hand-held ultrasound indentation system has been previously reported (Zheng and Mak, 1996) and demonstrated its applicability in use on a large number of patients in the clinical setting (Leung et al., 2002; Zheng et al., 2000). Instead of using linear variable differential transformer (LVDT), linear actuator or other frequently-used mechanical devices, an ultrasound transducer is utilized in the system to determine the tissue thickness and indentation. Similar ultrasound systems have been developed for the biomechanical assessment of different tissues, such as plantar foot tissues (Cavanagh et al., 1999).

Massive deposition of extracellular matrix and excessive fibroblast proliferation are characteristics of tissue fibrosis (Martin et al., 2000). These characteristics are associated with the change of mechanical properties of soft tissues, where the excessive extracellular matrix components such as collagen and noncollagenous proteins are laid (O'Sullivan and Levin, 2003). In addition, the change of water content in the irradiated tissues associated with the side effects such as fibrosis and edema (Nguyen et al., 1988) may also change their viscoelastic behaviors. Our previous work has demonstrated that the effective stiffness in the fibrotic tissues increased with the severity of fibrosis as determined by hand palpation (Leung et al., 2002; Zheng et al., 2000). The effective stiffness was derived by using a linear elastic model and neglecting the nonlinearity and viscosity of the soft tissue. Therefore it is not known to what extent the fibrotic tissue exhibits nonlinear elastic and viscous properties. In addition, the nonlinear and time-dependent properties of the tissue may cause errors to the measured effective stiffness. For example, different indentation rate may result different effective stiffness. In the current study, we used an extension of the linear elastic indentation solution to include a quasi-linear viscoelastic (QLV) model (Fung, 1981; Zheng and Mak, 1996, 1999) to extract the viscoelastic properties of irradiated neck tissue by using data collected previously in a clinical setting. QLV model is one of the most successful phenomenological models for soft tissues, such as articular cartilage (Woo et al., 1980). It was hypothesized that the QLV model integrated with the indentation solution (Hayes et al., 1972; Zhang et al., 1997) could well describe the nonlinear and viscoelastic behavior of the fibrotic tissue under cyclic loading.

The purpose of the present study was to extract the QLV parameters as viscoelastic properties of irradiated soft tissues from a population of patients after radiotherapy by using ultrasound indentation tests. Clinical indicators of fibrosis were also obtained and correlated to those QLV parameters.

2. Methods

2.1 The indentation test system

The ultrasound indentation system is mainly comprised of a hand-held indentation probe, an ultrasound pulser/receiver and a personal computer to display and process the collected data (Zheng and Mak, 1996). The probe has an unfocused ultrasound transducer of 5 MHz at its tip, and an in-series 10 N strain gauge load cell. The ultrasound transducer has a flat end with a diameter of 9 mm and serves as an ultrasound emitter, receiver, and indenter at the same time. The original thickness of the soft tissue is calculated using the propagation time of ultrasound from the transducer surface to the soft tissue-bony interface and the sound speed in soft tissues (1540 m/s, Goss et al., 1980). The force response during the indentation is simultaneously sensed by the load cell. The sampling rate for the force response and the indentation is approximately 12.5 Hz.

2.2 Subjects and testing procedure

One hundred and five patients with a mean age of 51 (SD 11) years, who had received radiotherapy to the full length of both sides of the neck, were recruited in the present study. The patients had been followed for at least two years after radiotherapy and had no evidence of cancer at the time of recruitment for the biomechanical indentation tests. The whole study was completed within a 12-month period in the oncology follow-up clinic of the authors' institution. All the patients had been treated for the same type of cancer (nasopharynx cancer) by uniform radiotherapy protocols. The study was approved by the Clinical Research Ethics Committee of the authors' affiliated institutions and written informed consent was obtained from all the patients in the study. In order to compare the viscoelastic properties between irradiated patients and normal subjects, the indentation tests were also conducted on a group of 8 normal healthy subjects with a mean age of 25, (SD 2) years using the same protocols.

The indentation tests were conducted at two standardized reference sites on each side of the neck. These two sites were located 3 cm and 7 cm inferior to the mastoid bone which is a readily palpable reference landmark. These two sites were chosen because they overlie the cervical spine which provides an interface for a consistent reflection of ultrasound signals. The site-dependence, inter-observer and intra-observer variations of a stiffness indicator viz. the effective Young's modulus at these test sites in normal and irradiated subjects had been investigated and reported by the investigators in a previous publication (Zheng et al., 2000). During the test, the subject sat on a chair, with the neck neither extended nor flexed and with the eyes looking forward. The neck was kept in a natural position because it had been demonstrated that the state of muscle contraction did affect the tissue stiffness (Mak et al., 1994). After the subject was properly seated, the ultrasound probe was placed on the defined site and oriented to obtain a maximum reflection peak from the bony interface. The maximum ultrasound reflection indicated that the probe was aligned perpendicular to the underlying bony surface (Zheng et al. 1999). This information was used to maintain a consistent alignment for the probe during the test, as the operator could observe the signal amplitude in real-time during the test. In addition, our previous study demonstrated that effects of the probe misalignment to the load-indentation response could be negligible when the tissue thickness was double of the indentor diameter (9 mm) (Zheng et al. 1999). This requirement could be fulfilled for the neck tissues that we tested in this study.

Before the actual measurement, several cycles of loading and unloading with gentle pressure were performed to precondition the tissue (Mak et al., 1994; Silver-Thorn, 1999) and to ensure that a stable ultrasound reflection signal could be obtained. After a preload of less than 0.5 N was applied on the skin, a load of 5 N or less if the indentation had reached 30% of the original thickness was applied. For each indentation trial, it typically included three cycles of loading and unloading, which were completed within approximately 10 seconds. The indentation rate was controlled in the range of 0.5 to 7.5 mm/s in which a stable manual indentation could be imposed. Fig. 1 shows a typical force and indentation curve from one site of subject #1. For each site, totally 3 trials were conducted and the 3 sets of measurement results were averaged to give the viscoelasticity parameters for the test site.

2.3 Estimation of viscoelasticity parameters

The data reduction method used was similar to the one described previously by the investigators (Zheng and Mak, 1999), but with an improved modification to include the effect of large indentation in the model. If the viscosity of the test tissue is neglected, the indentation force can be written as:

$$P(u) = \frac{2ah\kappa(u)}{1-\nu^2} Eu \tag{1}$$

where a is the radius of the indenter, h is the original tissue thickness, ν is the Poisson's ratio of soft tissue (assumed to be a constant 0.45 in this study), $u = w/h$, is called as indentation ratio (w is the applied indentation), κ is a scaling factor that only depends on u for an indentation at a specific site after a constant Poisson's ratio is set, and E is the Young's modulus of soft tissue. When the soft tissue viscosity is considered, an instantaneous Young's modulus in the quasi-linear viscoelastic form as proposed by Fung (1981) is written as follows:

$$E(u,t) = E^{(e)}(u) \cdot G(t), \; G(0) = 1 \tag{2}$$

where $E^{(e)}(u)$, assumed to be a function of u alone, is called the unrelaxed elastic modulus, and $G(t)$, a normalized function of time, is called the reduced relaxation function. In the current study, the two functions are further simplified to be in the following forms:

$$E^{(e)}(u) = E_0 + E_1 u \tag{3}$$

$$G(t) = 1 - \alpha + \alpha e^{-t/\tau} \tag{4}$$

where E_0 is called the initial modulus, E_1 the nonlinear factor, τ the time constant, and α a viscosity-related constant. According to the data reduction process described previously (Zheng and Mak, 1999), the force response at any discrete time i can be reconstructed from the indentation history $u(j)$ ($0 < j < i$) as:

$$\begin{aligned} P(i) = \frac{2ah}{1-\nu^2} & [\kappa(u(i))[E_0 u(i) + E_1 u^2(i)] \\ & -\frac{\alpha}{\tau}\sum_{j=0}^{i}\kappa(u(i-j))[E_0 u(i-j) + E_1 u^2(i-j)]e^{-j\cdot\Delta t/\tau}\Delta t] \end{aligned} \tag{5}$$

where Δt is the time interval between two adjacent data points. The scaling factor $\kappa(u(i))$ for an arbitrary $u(i)$ can be interpolated from the indentation-dependent values provided by Zhang et al. (1997), and used to compensate the nonlinear effect caused by a large indentation. In our previous study (Zheng and Mak, 1999), κ was assumed to be constant for a different indentation depth and the effect of a large indentation was compensated by changing the initial tissue thickness for each step of indentation. This approach could only be used when the indentation was relatively small. In comparison, the current approach could better compensate the nonlinear effect induced by a large indentation. The effect of friction was neglected in the analysis because the ultrasound coupling gel applied between the transducer and skin surface served as a lubricant.

To obtain the material constants from the force and indentation data collected experimentally, an iterative optimization process was used. The material constants were selected according to the error indicator defined as follows:

$$S_{err} = \sqrt{\sum_i (P_s(i) - P_e(i))^2} \,/ \sqrt{\sum_i (P_e(i))^2} \tag{6}$$

where S_{err} is the simulation error, named as percentage root mean squared (RMS) error, $P_e(i)$ is the experimentally measured force sequence, and $P_s(i)$ is the numerically simulated force sequence. A Matlab (MathWorks, Natick MA, USA) program was custom-designed to search the optimal material constants. In the optimization process, the time constant τ was deliberately incremented continuously to avoid reaching a local but not global minimum because it was in the exponential form and might induce a complex pattern of simulation error. A previous study had shown that these parameters extracted from lower limb soft tissues were repeatable (Zheng and Mak, 1999).

2.4 Measurement of clinical parameters of tissue fibrosis

Hand palpation scoring. Each side of the neck of the patient was palpated by three independent raters and given a palpation score from 0 to 3. The scoring criteria were as follows: Grade 0, nil or equivocal presence of palpable fibrosis; Grade 1, unequivocal presence of palpable fibrosis of mild degree; Grade 2, moderately severe fibrosis change; and Grade 3, severe fibrosis. According to the palpation score, the patients were further divided into four subgroups: Group 0, Group 1, Group 2, and Group 3. The raters were blind to the ultrasound measurement and did not communicate about their experience of palpation rating throughout the study period. The average score of the 3 raters on each side of the neck was used in the subsequent analysis.

Neck rotation range measurement. This was performed using a protractor positioned horizontally above the patient’s head. During the measurement, shoulder movements were attentively avoided to ensure accuracy. The range of rotation to the left side was taken to reflect the severity of fibrosis of the right neck in the analysis, and vice versa. Small neck rotation range was regarded as a severe degree of neck fibrosis.

2.5 Data analysis methods

Results were presented in the form of mean (standard deviation, SD). After the viscoelasticity parameters were extracted from each test site by three repeated indentation trials, the values of the two test sites on the same side of neck were averaged and the mean values were used to represent the viscoelastic properties of the neck tissue on that side. One-way ANOVA was used to test the difference of viscoelasticity parameters among the four patient subgroups. Post-hoc unpaired *t*-test was used to further test where the difference existed if significant difference was found in the ANOVA analysis. The viscoelasticity parameters were also correlated to the neck rotation range and the effective Young's modulus using a Pearson correlation. The effective Young's modulus was previously defined as an averaged Young's modulus extracted from the overall load-indentation relationship neglecting the nonlinear and time-dependent properties. Viscoelastic results obtained from the patients were also compared with those from the 8 normal subjects using unpaired t-test. Commercial software SPSS (SPSS) was used for all the statistical analyses and in all cases $P < 0.05$ was used as a level of significant difference.

3. Results

3.1 Indentation response

Fig. 1 shows the typical indentation response at one site of Subject #1. The simulated parameters well predicted the force response based on the indentation history. The mean percentage RMS error for all the tests was 0.096 (SD 0.029). Fig. 2 shows the comparison of force-indentation responses obtained from two patient subjects with different degrees of fibrosis. From Fig. 2, the phases of loading and unloading can be well differentiated, which shows the typical hysteresis phenomenon involved in the viscoelastic behavior. The force responses were significantly different in two cases. The same force applied on the tissues with more severe fibrosis induced smaller indentations. This observation, from the perspective of an experienced palpation rater, would be transferred as a feeling to him/her that the palpated tissue was stiffer so that a higher score indicating more severe fibrosis would be given. The mean indentation rate for all the tests in this study was 3.3 (SD 3.0) mm/s, well controlled in the range as previously described in the indentation study of lower limb soft tissues (Zheng et al., 1999).

3.2 Elasticity parameters E_0 and E_1

The mean initial modulus E_0 of the irradiated, fibrotic neck soft tissues in 105 patients was 26 (SD 22) kPa, ranging from 3 kPa (for a patient in Group 0) to 134 kPa (for a patient in Group 3). The mean nonlinear factor E_1 in all patients was 507 (SD 683) kPa, ranging from 19 kPa (for a patient in Group 0) to 4058 kPa (for a patient in Group 3). Fig. 3 shows a comparison of the initial modulus E_0 and the nonlinear factor E_1 in all the patient subgroups with different palpation scores. Although there were overlaps of the elasticity parameters between different patient subgroups, the minimum and maximum values of E_0 and E_1 in each subgroup generally increased with the increase of the palpation score. Table 1 shows the mean E_0 and E_1 in different patient subgroups. One-way ANOVA showed that there was significant difference of E_0 and E_1 among patient subgroups ($P < 0.001$). Post-hoc *t*-test showed that E_0 and E_1 were significantly larger in patients with a higher palpation score ($P < 0.04$), except between Group 0 and Group 1 for E_0.

Fig. 4 shows the correlation of E_0 and E_1 with the neck rotation range. A significantly negative correlation of E_0 (r = -0.50, $P < 0.001$) and E_1 (r = -0.55, $P < 0.001$) with the neck rotation range was found. Fig. 5 shows the correlation of E_0 and E_1 with the effective Young's modulus as derived in the investigators' previous study (Leung et al., 2002). A significant positive correlation of E_0 (r = 0.73, $P < 0.001$) and E_1 (r = 0.83, $P < 0.001$) with the effective Young's modulus. The high correlation of E_0 and E_1 with the effective Young's modulus appeared to indicate that measuring the effective Young's modulus was as "effective" as measuring the E_0 and E_1. But the intrinsic meaning of the two QLV elasticity parameters is different from that of the effective Young's modulus from the mechanical point of view. This issue was discussed in more detail in the discussion section.

3.3 Viscosity parameters τ and α

The mean time constant τ in all patients was 1.34 (SD 1.10) s, ranging from 0.12 s (for a patient in Group 3) to 6.99 s (for a patient in Group 1). The mean parameter α of the irradiated tissues in all patients was 0.42 (SD 0.12), ranging from 0.21 (for a patient in Group 2) to 0.69 (for a patient in Group 0). Table 1 shows the mean τ and α measured in different patient subgroups. There was no significant difference of τ across patient subgroups ($P > 0.05$). For the parameter α, it increased from 0.40 for Group 0 to 0.46 for Group 3, but still the increase was not significant among patient subgroups ($P > 0.05$). No significant correlation was found between either τ or α and the neck rotation range or the effective Young's modulus ($P > 0.05$).

3.4 Comparison between irradiated patients and normal subjects

For the 8 normal subjects, E_0 and E_1 were 6.6 (SD 1.3) kPa and 56 (SD 22) kPa, respectively. They were significantly smaller than those for the patient group ($P < 0.001$). For the time constant τ, it was 0.24 (SD 0.05) s in the normal subjects, and it was significantly smaller than that for the patient group (mean 1.3 SD 1.1 s) ($P < 0.003$), indicating that the irradiated tissues required a longer time than the soft tissues of normal subjects to reach the equilibrium state after step indentation. For the parameter α, it was 0.53 (SD 0.08) for normal subjects, which was slightly but significantly larger than that for the patient group (mean: 0.42, SD 0.12) ($P < 0.007$), indicating that the irradiated tissues of the patients relaxed to a lesser extent than that for the normal subjects.

4. Discussion

In this paper, a linear elastic indentation solution was extended to include a quasi-linear viscoelastic model to assess the nonlinear elastic and viscous properties of the irradiated soft tissues of the neck. The QLV parameters were obtained from a curve fitting process using force and indentation data collected by a manually-driven hand-held ultrasound indentation probe. The results of the present study showed that our method was more sensitive in discriminating the nonlinear elastic properties of fibrotic tissues than in discriminating the viscous properties among patient subgroups with different degrees of tissue fibrosis judged clinically by hand palpation and other symptoms.

To the best of our knowledge, results on the study of mechanical properties of irradiated soft tissues in the neck region are scarce. Most of the previous studies have focused on the soft tissues in other regions and chosen the effective Young's modulus or effective stiffness as the parameter. Extraction of the effective stiffness is based on a linear load-indentation relationship. A sole effective stiffness is not enough to describe the tissue elasticity when a large indentation is applied (Klaesner et al., 2001). Hence, a model that can better describe the force-indentation relationship in soft tissues other than the linear one is preferred. The current method simplifies the tissue elasticity in the form of an initial modulus plus a nonlinear factor in the QLV model, where the geometrically nonlinear nature of the indentation procedure is compensated by a scaling factor κ depended on the tissue thickness and the indentation depth. The initial modulus is a parameter representing the stiffness when no or infinitesimal indentation is applied. The nonlinear factor is a parameter indicating the speed of increase of stiffness when the indentation increases. Compared with the effective Young's modulus, the initial modulus and nonlinear factor used in this study are extracted in the QLV model in which the effects of viscosity are also considered so that they can better describe the intrinsically nonlinear elastic properties of the irradiated tissue. The results of our present study showed that the mean Young's modulus increased as the indentation percentage increased within the range of 0% to 30% and the Young's modulus within this indentation range in the irradiated tissue was generally larger than that of the normal limb soft tissue (Zheng and Mak, 1999). The tissue with a more severe degree of clinical fibrosis was found to be stiffer initially and the stiffness increased more quickly under loading. The mean difference of Young's modulus between Group 0 and Group 3 increased greatly from the indentation ratio of 0% to indentation ratio of 30%. According to these findings, the initial Young's modulus and the nonlinear factor extracted using QLV model could provide more information than using the effective Young's modulus alone. The results also suggested that it is important to document the initial and the maximum deformation and/or indentation load when the effective Young's modulus is reported alone, as the results significantly depend on these conditions. This issue is particularly important in the comparison of the results reported by different research groups. Similar results have been reported by other investigators using the finite element method (Tonuk and Silver-Thorn, 2003; Vannah and Childress, 1996), but direct comparison between our parameters and their results appeared to be difficult due to the different models and test locations used in the studies. Other forms of elasticity as indicated by more complex stress-strain relationships can also be taken into account in the present QLV model in future studies.

The time constant of the irradiated tissue extracted in this study (1.34 s), which characterizes the length of time required to relax to an equilibrium state after step indentation, was generally smaller than that of the lower limb tissues measured previously (4.69 s, Zheng and Mak, 1999). The difference of the time constant across different patient subgroups was not established in the current study. But when compared with that of the 8 normal subjects (0.24 s), the time constant of the patients was significantly larger. The larger time constant showed that the patient needed a longer time for the soft tissue to relax after it was indented, which was consistently observed by the investigators during the indentation tests on patients. Another QLV parameter α, indicating the level of relaxation at the equilibrium state, was 0.42 for patients. Although there was a slight increase of α with respect to the increase of fibrosis severity, the increase had not reached statistical significance. Compared to the limb soft tissues studied previously ($\alpha = 0.13$), the neck soft tissue of patients relaxed to an equilibrium state with a significantly smaller force response. But compared with the results of the 8 normal subjects ($\alpha = 0.53$), it relaxed to slightly larger force equilibrium. An indentation study on the residual limb soft tissues by Silver-Thorn (1999) showed that stress relaxation in 2 minutes could be 50.6% (i.e. approximately equivalent to $\alpha = 0.51$ in our model) at a mean step indentation ratio of 23% in one subject with amputation. Their value was very similar to that of our normal subjects. However, it should be noted that the viscosity parameters reported by Silver-Thorn (1999) were extracted directly from the force-relaxation tests but the current parameter was extracted indirectly from the cyclic indentation test. In the present study, the difference in mean age between the patient and control groups might induce a bias in the comparison of results. To address this problem, a correlation analysis was conducted between the age of the patients in the range of 29 to 75 and the viscosity parameters, but no significant correlation was found between the time constant and age ($P = 0.45$) and between the parameter α and age ($P = 0.48$). Even no significant correlation was demonstrated between the clinical palpation score and the viscosity parameters in the present study, extracting them was important to improve the reliability for the measurement of the elastic properties in comparison with the case where the viscoelasticity was not considered (Zheng and Mak 1999a). When the effective Young's modulus was extracted alone, it may be affected by the applied indentation rate, as the degree of creep and stress-relaxation depends on the duration of loading or compression.

The mechanical properties of the irradiated tissue measured using the ultrasound indentation test may be affected not only by the tissue fibrosis, but also by other late side effects of radiotherapy such as the lymphedema, fat necrosis and vascular injury (O'Sullivan and Levin, 2003), though these other symptoms are uncommon in the irradiated neck. The significant correlation of E_0 and E_1 with the clinical parameters of hand palpation score and the neck rotation range, and with the effective Young's modulus, suggests that the viscoelasticity parameters are addressing clinically meaningful properties of the irradiated tissue. In the present study, both the elasticity and viscosity parameters of the patients were demonstrated to be different from those of young normal subjects. The main components involved in determining the elastic properties of the soft connective tissue are the type, quantity, structure and cross-linking of collagen fibers (Ottani et al., 2001). We hypothesize that the consistent change of the nonlinear elasticity parameters are due to the consistently uniform change of syntheses and deposition levels of extracellular matrix components such as the collagen and noncollagenous proteins in the skin and subcutaneous tissues (Remy et al., 1991; O'Sullivan and Levin, 2003; Wegrowski et al., 1988). While for the viscous properties, they may tend to be more affected by factors such as the water concentration around the essentially viscoelastic collagen fibers. The change in concentration and rigidity of the water content of the irradiated neck tissues might develop in a rather random manner instead of being closely in parallel with other component changes of fibrosis so that a consistent change of viscosity parameters among patient subgroups could not be established. Exact reasons on these changes need further investigations. Based on the current findings, the QLV parameters represent a significant advance over the effective Young's modulus alone in the characterization of radiation-induced fibrosis and can be potentially introduced as a quantitative tool to assess the tissue fibrosis.

With regard to the model used in this study, several issues are worth addressing and paying attention to. The linear indentation solution of Hayes et al. (1972) assumes the tissue layer to be homogeneous, isotropic, linear elastic and the layer is bonded to a half infinite rigid foundation. In our case, the difference of geometry and structure of the skin and subcutaneous tissues is not individually considered in the simulation. Significant curvature of the neck surface, the nonuniform tissue thickness and the finite dimension of the bony substrate under the indentation region may affect the accuracy of the extracted material properties. As for the assumed elastic layer for the soft tissue, a constant Poisson's ratio of 0.45 is assigned, indicating the nearly incompressible characteristics under a fast enough loading rate. Although a constant Poisson's ratio in the range of 0.30 to 0.50 has been commonly used by investigators to study the elastic properties of soft tissues (Klaesner et al., 2001; Mak et al., 1994; Vannah and Childress, 1996), cautions are needed to be taken when this assumption is applied over different body sites, in patients with different severities of fibrosis, in both elderly and young subjects, because the Poisson's ratio may vary from individual to individual and from site to site. To measure the Poisson's ratio *in vivo* is an important and challenging issue for researchers in the mechanical test of biological tissues.

Based on a previous study on the limb soft tissue (Zheng and Mak, 1999), the results obtained by our method were demonstrated to be reproducible and were unique using the defined protocol. According to the previous studies, the intra-operator variability for the QLV parameters of the lower limb tissues was less then 7% (mean/SD) (Zheng and Mak 1999). The intra-operator and inter-operator variability of the effective stiffness of neck tissues was 7.2% and 15.2%, respectively (Zheng et al. 2000). Our QLV analysis was capable of simulating the whole indentation response obtained with different indentation rate (0.5 to 7.5 mm/s) and could extract viscoelastic properties from the indentation tests. It is not sure how our model works when a smaller or larger indentation rate is used, as the viscoelastic properties of biological soft tissues can also depends on the strain rate. This issue should be further investigated before our model can be used for a wider range of indentation rate. It should also be realized that the QLV model used in this study is a phenomenological model that approximates the macro-mechanical viscoelastic behavior and ignores the microstructure and mechanisms that contribute to the observed viscoelasticity. The phenomenological nature of this method may result in the same parameters extracted for tissues with different physiological or pathological conditions. For example, some pathological conditions of the irradiated tissue other than fibrosis, such as lymphedema may also contribute to the increase of tissue stiffness. Investigation using other indentation models such as the biphasic model (Mak et al., 1987) is deserved to extract intrinsic material properties from the indentation test for the irradiated soft tissue in future studies.

5. Conclusion

The force response of the cyclic indentation test conducted using a manually-driven indention system on fibrotic tissues was reconstructed from the indentation history using a quasi-linear viscoelastic model. Viscoelasticity parameters were extracted by an optimization process to minimize the error between the simulated and experimental force. Data from 105 patients showed that increasing severity of soft tissue fibrosis was associated with a more rapid increase in stiffness under loading. Compared with a control group of younger normal subjects, the irradiated soft tissue in patients required a significantly longer time to relax in the indentation process and relaxed to a significantly lesser extent at the equilibrium state. Viscoelasticity parameters can discriminate soft tissues with different degrees of clinical fibrosis, have significant correlation with the clinical parameters of fibrosis, and can serve as an additional approach in the characterization of soft tissue fibrosis.

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References

Burger, A., Loffler, H., Bamberg, M., Rodemann, H.P., 1998. Molecular and cellular basis of radiation fibrosis. Int. J. Radiat. Biol. 73, 401-408.

Cavanagh, P.R., 1999. Plantar soft tissue thickness during ground contact in walking. J. Biomech. 32, 623-628.

Ferguson-Pell, M., Hagisawa, S., Masiello, R.D., 1994. A skin indentation system using a pneumatic bellows. J. Rehabil. Res. Dev. 31, 15-19.

Fung, Y.C., 1981. Bio-viscoelastic solids. In: Fung, Y.C., (eds), Biomechanics: Mechanical Properties of Living Tissues, Springer-Verlag, New York, pp.196-260.

Goss, S.A., Johnston, R.L., Dunn, F., 1980. Compilation of empirical ultrasonic properties of mammalian tissues. II. J. Acoust. Soc. Am. 68, 93-108.

Hayes, W.C., Keer, L.M., Herrmann, G., Mockros, L.F., 1972. A mathematical analysis for indentation tests of articular cartilage. J. Biomech. 5, 541-551.

Klaesner, J.W., Commean, P.K., Hastings, M.K., Zou, D., Mueller, M.J., 2001. Accuracy and reliability testing of a portable soft tissue indentor. IEEE Trans. Neural Sys. Rehabil. Eng. 9, 232-240.

Klaesner, J.W., Hastings, M.K., Zou, D., Lewis, C., Mueller, M.J., 2002. Plantar tissue stiffness in patients with diabetes mellitus and peripheral neuropathy. Arch. Phys. Med. Rehabil. 83, 1796-1801.

Leung, S.F., Zheng, Y.P., Choi, C.Y.K., Mak, S.S.S., Chiu, S.K.W., Zee, B., Mak, A.F.T., 2002. Quantitative measurement of post-irradiation neck fibrosis based on the Young modulus: description of a new method and clinical results. Cancer 95, 656-662.

Mak, A.F.T., Lai, W.M., Mow, V.C., 1987. Biphasic indentation of articular cartilage I: theoretical analysis. J. Biomech. 20, 703-714.

Mak, A.F.T., Liu, G.H.W., Lee, S.Y., 1994. Biomechanical assessment of below-knee residual limb tissue. J. Rehabil. Res. Dev. 31, 188-198.

Martin, M., Lefaix, J.L., Delanian, S., 2000. TGF-β1 and radiation fibrosis: a master switch and a specific therapeutic target? Int. J. Radiat. Oncol. Biol. Phys. 47, 277-290.

Nguyen, T.D., Panis, X., Froissart, D., Legros, M., Coninx, P., Loirette, M., 1988. Analysis of later complications after rapid hyperfractionated radiotherapy in advanced head and neck cancers. Int. J. Radiat. Oncol. 14, 23-25.

Ottani, V., Raspanti, M., Ruggeri, A., 2001. Collagen structure and functional implications. Micron 32, 251-260.

O'Sullivan, B., Levin, W., 2003. Late radiation-related fibrosis: pathogenesis, manifestations, and current management. Semin. Radiat. Oncol. 13, 274-289.

Remy, J., Wegrowski, J., Crechet, F., Martin, M., Daburon, F., 1991. Long-term overproduction of collagen in radiation-induced fibrosis. Radiat. Res. 125, 14-19.

Rodemann, H.P., Bamberg, M., 1995. Cellular basis of radiation-induced fibrosis. Radiother. Oncol. 35, 83-90.

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Silver-Thorn, M.B., 1999. In vivo indentation of lower extremity limb soft tissues. IEEE Trans. Rehabil. Eng. 7, 268-277.

Thornton, G.M., Leask, G.P., Shrive, N.G., Frank, C.B., 2000. Early medial collateral ligament scars have inferior creep behaviour. J. Orthop. Res. 18, 238-246.

Tonuk, E., Silver-Thorn, M.B., 2003. Nonlinear elastic material property estimation of lower extremity residual limb tissues. IEEE Trans. Neural Sys. Rehabil. Eng. 11, 43-53.

Vannah, W.M., Childress, D.S., 1996. Indentor tests and finite element modeling of bulk muscular tissue in vivo. J. Rehabil. Res. Dev. 33, 239-252.

Wegrowski, J., Lafuma, C., Lefaix, J.L., Daburon, F., Robert, L., 1988. Modification of collagen and noncollagenous proteins in radiation-induced muscular fibrosis. Exp. Mol. Pathol. 48, 273-285.

Woo, S.L.Y., Simon, B.R., Kuei, S.C., Akeson, W.H., 1980. Quasi-linear viscoelastic properties of normal articular cartilage. J. Biomech. Eng.-Trans. ASME 102, 85-90.

Zhang, M., Zheng, Y.P., Mak, A.F.T., 1997. Estimating the effective Young's modulus of soft tissues from indentation tests-nonlinear finite element analysis of effects of friction and large deformation. Med. Eng. Phys. 19, 512-517.

Zheng, Y.P., Leung, S.F., Mak, A.F.T., 2000. Assessment of neck tissue fibrosis using an ultrasound palpation system: a feasibility study. Med. Biol. Eng. Comput. 38, 497-502.

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Zheng, Y.P., Mak, A.F.T., 1996. An ultrasound indentation system for biomechanical properties assessment of soft tissues in-vivo. IEEE Trans. Biomed. Eng. 43, 912-918.

Zheng, Y.P., Mak, A.F.T., 1999. Extraction of quasi-linear viscoelastic parameters for lower limb soft tissues from manual indentation experiment. J. Biomech. Eng.-Trans. ASME 121, 330-339.

Zheng, Y.P., Mak, A.F.T., Lue, B.L., 1999. Objective assessment of limb tissue elasticity: development of a manual indentation procedure. J. Rehabil. Res. Dev. 36, 71-85.

Figure Captions:

Fig. 1. A typical indentation and its curve fitting results for Subject #1 (Group 3). The original tissue thickness was 22.5 mm. The maximum indentation was 14.1% of the original tissue thickness. The simulated QLV parameters were E_0 = 59.5 kPa, E_1 = 1278.9 kPa, τ = 0.132 s, α = 0.577. The Pearson correlation between the simulated and experimental force was 0.998 with a simulated percentage RMS error of 0.045.

Fig. 2. Force-indentation responses of two test sites in two patients having different palpation scores. Subject #2 was in Group 0 and subject #3 was in Group 3.

Fig. 3. Boxplot of (a) the initial modulus E_0 and (b) the nonlinear factor E_1 in different patient subgroups. 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. The box and the whiskers together indicate the area in which all observations are found, unless outliers (○) are present. Outlier is defined as a value which is located more than 1.5 times the inter-quartile range below the lower quartile or above the upper quartile. "**" represents a significant difference ($P < 0.04$) in comparison with the patient subgroups with a lower palpation score.

Fig. 4. Correlation of (a) the initial modulus E_0 and (b) the nonlinear factor E_1 with the neck rotation range for the patients.

Fig. 5. Correlation of (a) the initial modulus E_0 and (b) the nonlinear factor E_1 with the effective Young's modulus for the patients.

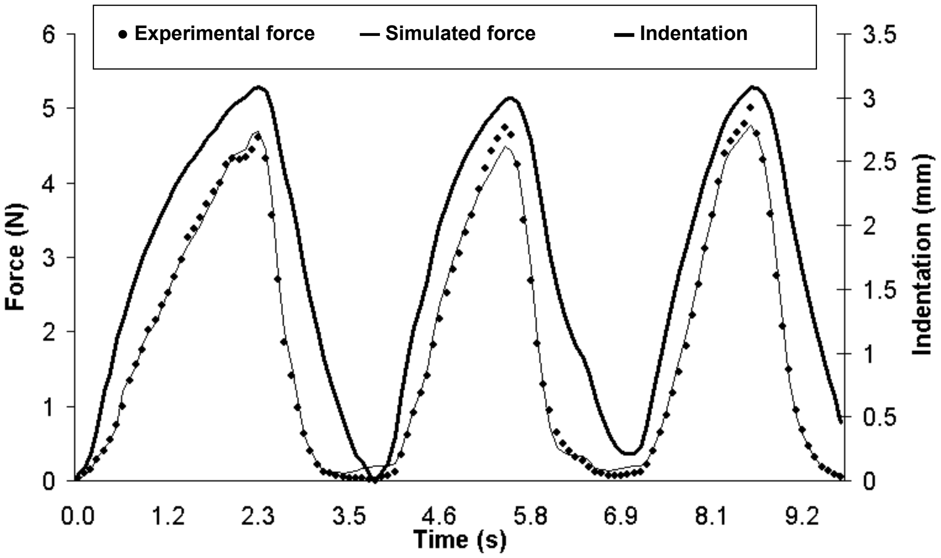

● Experimental force
— Simulated force
— Indentation
Force (N)
Indentation (mm)
Time (s)
0
1
2
3
4
5
6
0
0.5
1
1.5
2
2.5
3
3.5
0.0
1.2
2.3
3.5
4.6
5.8
6.9
8.1
9.2

Fig. 1.

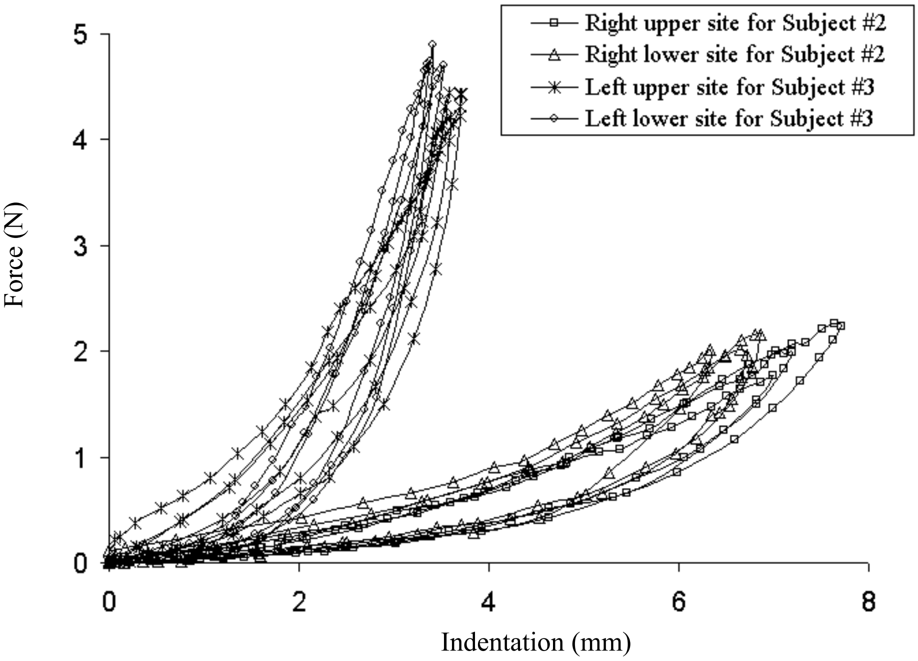

Right upper site for Subject #2
Right lower site for Subject #2
Left upper site for Subject #3
Left lower site for Subject #3
Force (N)
Indentation (mm)
0
1
2
3
4
5
0
2
4
6
8

Fig. 2.

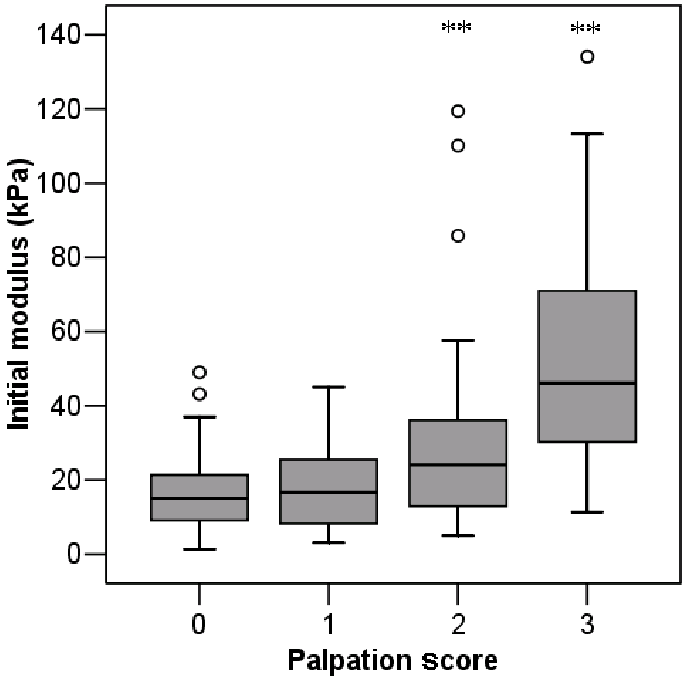

140
120
100
80
60
40
20
0
Initial modulus (kPa)
**
**
0
1
2
3
Palpation score

Fig.3. (a)

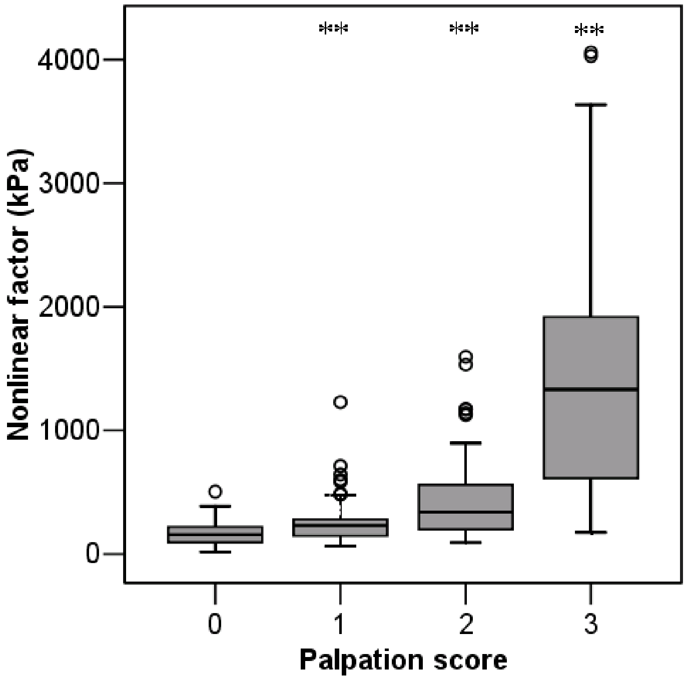
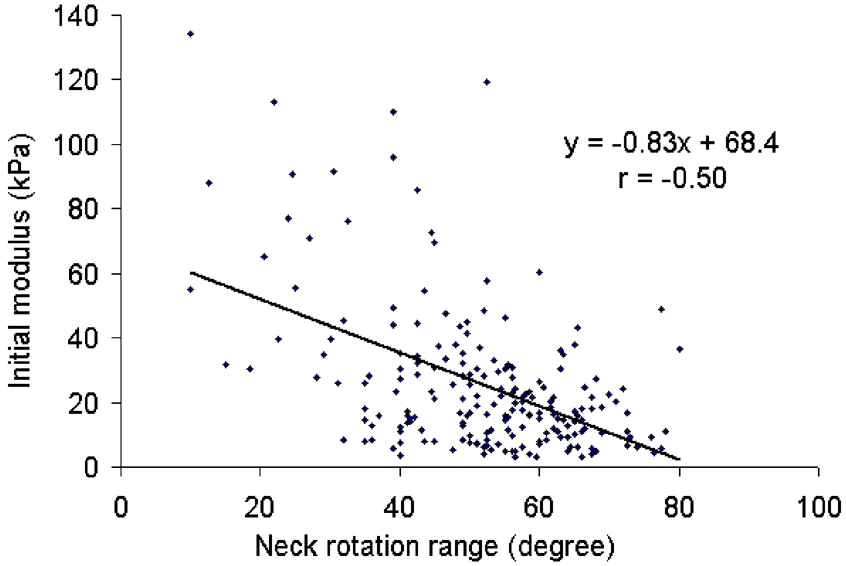
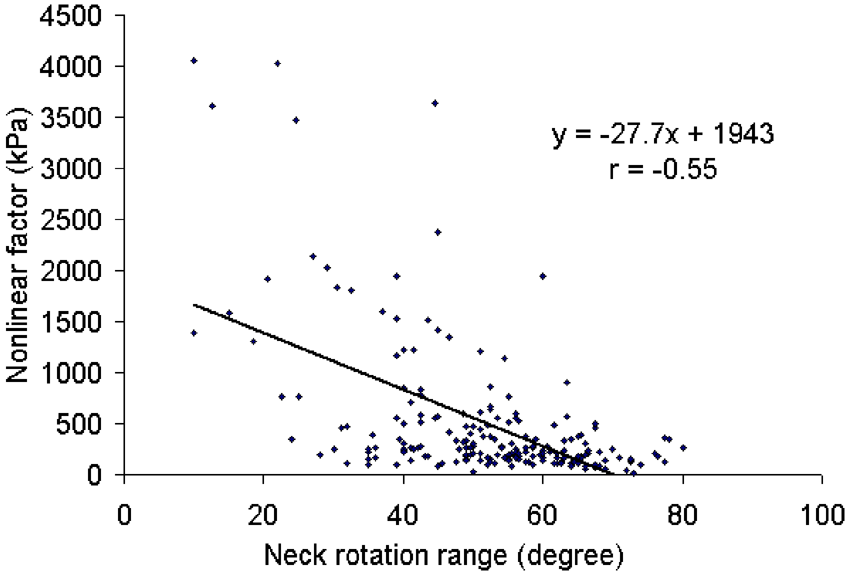

4000
3000
2000
1000
0
Nonlinear factor (kPa)
**
**
**
0
1
2
3
Palpation score

Fig. 3. (b)



140
120
100
80
60
40
20
0
Initial modulus (kPa)
y = -0.83x + 68.4
r = -0.50
0
20
40
60
80
100
Neck rotation range (degree)

Fig.4. (a)



4500
4000
3500
3000
2500
2000
1500
1000
500
0
Nonlinear factor (kPa)
y = -27.7x + 1943
r = -0.55
0
20
40
60
80
100
Neck rotation range (degree)

Fig. 4. (b)

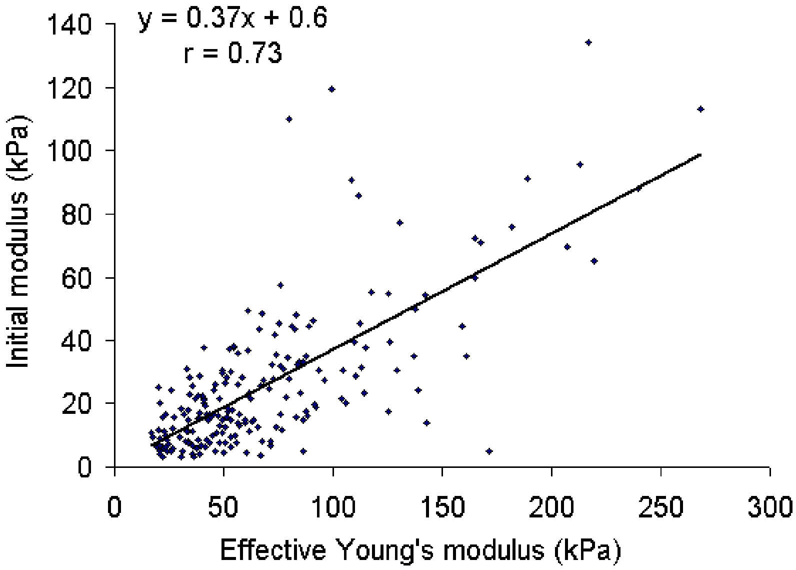

y = 0.37x + 0.6
r = 0.73
Initial modulus (kPa)
140
120
100
80
60
40
20
0
0
50
100
150
200
250
300
Effective Young's modulus (kPa)

Fig. 5. (a)

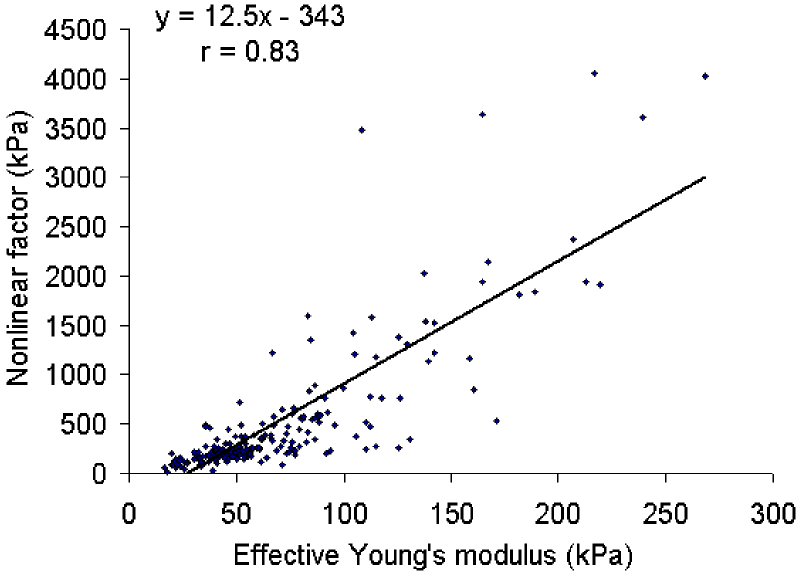

y = 12.5x - 343
r = 0.83
Nonlinear factor (kPa)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0
50
100
150
200
250
300
Effective Young's modulus (kPa)

Fig. 5. (b)

Table 1

QLV parameters extracted in four patient subgroups

Patient subgroups		Score 0 (*n = 24)	Score 1 (n = 35)	Score 2 (n = 28)	Score 3 (n = 18)	Overall mean
E_0 (kPa)	Mean	16	19	28	50	26
	SD	11	11	23	31	22
E_1 (kPa)	Mean	181	266	450	1501	507
	SD	108	187	355	1117	683
τ (s)	Mean	1.35	1.52	1.17	1.24	1.34
	SD	0.90	1.32	0.95	1.08	1.10
α	Mean	0.40	0.42	0.42	0.46	0.42
	SD	0.11	0.12	0.12	0.13	0.12

*: *n* represents the number of patients in each subgroup

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