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A new nonlinear parameter in the developed strain-to-applied strain of the soft tissues and its application in ultrasound elasticity imaging

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Abstract

Strain developed under quasi-static deformation has been mostly used in ultrasound elasticity imaging (UEI) to determine the stiffness change of tissues. However, the strain measure in UEI is often less sensitive to a subtle change of stiffness. This is particularly true for Crohn's disease where we have applied strain imaging to the differentiation of acutely inflamed bowel from chronically fibrotic bowel. In this study, a new nonlinear elastic parameter of the soft tissues is proposed to overcome this limit. The purpose of this study is to evaluate the newly proposed method and demonstrate its feasibility in the UEI. A non-linear characteristic of soft tissues over a relatively large dynamic range of strain was investigated. A simplified tissue model based on a finite element (FE) analysis was integrated with a laboratory developed ultrasound radio frequency (RF) signal synthesis program. Two dimensional speckle tracking was applied to this model to simulate the nonlinear behavior of the strain developed in a target inclusion over the applied average strain to the surrounding tissues. A nonlinear empirical equation was formulated and optimized to best match the developed strain-to-applied strain relation obtained from the FE simulation. The proposed nonlinear equation was applied to in vivo measurements and nonlinear parameters were further empirically optimized. For an animal model, acute and chronic inflammatory bowel disease was induced in Lewis rats with trinitrobenzene sulfonic acid (TNBS)-ethanol treatments. After UEI, histopathology and direct mechanical measurements were performed on the excised tissues. The extracted non-linear parameter from the developed strain-to-applied strain relation differentiated the three different tissue types with 1.96 ± 0.12 for normal, 1.50 ± 0.09 for the acutely inflamed, and 1.03 ± 0.08 for the chronically fibrotic tissue. T-tests determined that the non-linear parameters between normal, acutely inflamed and fibrotic tissue types were statistically significantly different (normal/fibrotic ($P = 0.0000185$), normal/acutely inflamed ($P = 0.0013$), and fibrotic/acutely inflamed ($P = 0.0029$)). This technique may provide a

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sensitive and robust tool to assess subtle stiffness changes in tissues such as in acutely inflamed bowel wall. (E-mail: kangkim@upmc.edu)

Keywords

Ultrasound elasticity imaging; Nonlinear elasticity; Tissue mechanics; Ultrasound speckle tracking; Strain; Crohn's disease

Introduction

In general, the tissue stiffness changes have been important indicators of the development of pathological lesions in soft tissues. In past decades, along with other elasticity imaging modalities, such as magnetic resonance elastography (MRE) (Plewes et al. 2000) and magnetic resonance tagging (tMRI) (Plewes et al. 2000), ultrasound elasticity imaging (UEI) or elastography (Ophir et al. 2002) has been developed for imaging soft tissue elastic properties. UEI has been successfully applied to many diseases including (1) thrombus aging/maturation evaluation in human lower limb deep venous thrombosis (DVT) (Rubin et al. 2003, 2006), (2) breast cancer diagnosis and screening (Garra et al. 1997; Hall et al. 2003; Booi et al. 2006), (3) differentiation of benign and malignant thyroid masses (Lyshchik et al. 2005) and cervical lymph nodes (Lyshchik et al. 2007), (4) prostate cancer detection (Cochlin et al. 2002; Lorenz et al. 1999), (5) renal transplant graft nephropathy evaluation (Weitzel et al. 2004), (6) cardiovascular diseases: characterization of arterial wall stiffness for early atherosclerosis diagnosis (Kim et al. 2004; Weitzel et al. 2005), assessment of myocardial wall stiffness for detection of contractile dysfunction (Perk et al. 2007), atheromata assessment for determining vulnerable plaque (Baldewsing et al. 2007), (7) liver diseases: assessment of the degree of liver fibrosis (Ziol et al. 2005; Castera et al. 2005; Franchis and Era 2007), (8) Crohn's disease (CD): distinguish chronic bowel disease from normal (Kim et al. 2008).

In most elasticity imaging studies, pathologically hardened tissues were successfully distinguished from the normal surrounding soft tissue. However, differentiating subtle changes in stiffness has become an issue with the limited sensitivity of the elasticity imaging techniques and has not been studied extensively. In our previous study of an animal model for Crohn's disease, UEI was only applied to the fibrotic bowel in a TNBS treated rat model (Kim et al. 2008). The in vivo measurements of strain from UEI correlated well with histology and direct ex vivo mechanical measurements of elastic modulus, and demonstrate that the UEI approach can detect stiffness changes as a result of the development of fibrosis with high sensitivity and reproducibility. However, this study only applied to chronic, clearly fibrotic tissues without consideration of early lesion detection, which would be represented by inflamed, edematous bowel (Tersigni and Prantera. 2010).

Crohn's disease is an inflammatory bowel disease that is often difficult to diagnose by physical examination as its symptoms are similar to those of other intestinal disorders, such as irritable bowel syndrome and ulcerative colitis (Tersigni and Prantera. 2010). Inflammation occurs in episodic flares in CD, which are part of the waxing and waning course of the disease. Healing between flares allows the intestine to reconstitute its epithelium, but this healing process results in the deposition of fibrotic scar tissue. Repeated cycles of flares and healing often lead to clinically significant fibrosis and stenosis of the intestine, requiring surgery in 20% of patients within 3 years of diagnosis, and in 57% of patients after 10 years of disease. This has a substantial impact on quality of life and medical costs.

When Crohn's patients have abdominal pain and vomiting, this often indicates severe narrowing or stricture of the small intestine. This can be due to inflammation which can be treated with medical therapy, chronic fibrosis which requires surgery, or a mixture of the two. Differentiating fibrotic and edematous thickening of the bowel wall is critically important for diagnosis and follow-up treatment (Tersigni and Prantera. 2010). This is because one is reluctant to operate on an inflammatory stricture since over 80% of patients with Crohn's disease develop recurrence after surgery, and are likely to require more bowel removal, putting them at risk for short gut syndrome. The chronic fibrotic strictures of Crohn's disease, while they contain some inflammation, do not respond to powerful anti-inflammatory medications, so that patients with fibrotic strictures endure the potentially lethal side effects and absorb the significant cost of powerful immune suppressants, with little chance of benefit.

There are no B-mode ultrasound findings that will distinguish between fibrosis and inflammation in Crohn's disease. The ultrasound-based distinguishing characteristics of Crohn's disease are bowel wall thickness, associated abnormalities such as fistulas and abscesses, and qualitative or semi-quantitative estimates of activity using flow imaging, such as power Doppler or contrast agents (Tarjan et al 2000, Schreyer et al 2008). Ultrasound non-linear elasticity measurements can potentially make this distinction, which are presented in this paper.

From our observations with the TNBS treated rat model, there are only small differences in stiffness between acute and chronic bowel damage or normal bowel by conventional UEI, making it challenging to differentiate edematous from fibrotic tissues or normal bowel. However, the causes of the wall thickening and stiffness are different in the two circumstances. Acute inflammatory wall thickening is due to inflammation with high water content, as opposed to fibrotic-tissues, which are thickened because of scar formation. During the initial stages of tissue deformation, the edematous tissue might appear quite linear in their stress/strain response until the water contents are forced out and non-linear subsequently. This results in difficulties with differentiating the edematous tissue from normal tissue in linear region (applied strain less than 10%) and from fibrotic tissue in nonlinear region under higher strain (See Figure2). Therefore, although a stiffness assessment by conventional UEI may not be able to differentiate the edematous from fibrotic tissues or normal tissue with high accuracy using the strain measured at a single-point of deformation, the nonlinear characteristics over a large strain dynamic range of the edematous tissue may be able to make this distinction. These characteristics may be used to identify the stage of lesion development.

In this study, the developed strain inside a targeted lesion over a relatively large dynamic range of the applied strain to the body of the animal was extensively examined. A new method of nonlinear characterization of tissue stiffness change using UEI is proposed and tested. First, a finite element simulation based on a soft tissue model was generated to simulate the relation between the developed strain inside the targeted inclusion and the applied strain within the range of the elastic moduli of tissue in the animal model. Based on the developed strain-to-applied strain (simply called strain-to-strain later in this paper) relations from the simulation, an analytical equation adapted from the Hill equation (Holmes 2006) was proposed and the related nonlinear parameters were optimized for the simulation results. We used this strain-to-strain paradigm because we do not know the force that we apply when deforming tissue using an ultrasound transducer in vivo. Hence, we need an alternative non-linear measure to that observed in a standard stress/strain elasticity curve.

Second, the proposed techniques may identify subtle stiffness changes related to acute inflammation and fibrosis in an animal model. The strain-to-strain relation with the extracted

nonlinear parameter, empirically optimized to the in vivo measurements, successfully differentiates edematous bowel from the chronically fibrotic bowel.

Materials and Methods

(A) TNBS Animal model

A total of 8 female Lewis rats (Harlan Sprague-Dawley, Inc., and weights in 150~180 g) were studied using a well-known model of colitis developed by Morris and colleagues (Morris et al. 1989). The model uses trinitrobenzene sulfonic acid (TNBS)-ethanol enemas to induce a local colitis. Four rats had chronic intestinal fibrosis induced with escalating weekly TNBS-ethanol enemas over 6 weeks. Acute inflammation was induced in another 4 rats with a single TNBS-ethanol enema. Finally, since the TNBS colitis model is localized to the recto-sigmoid portion of the colon, large portions of the proximal colon was normal. Thus all of the 8 rats had normal proximal bowel segments, and we, therefore, define a proximal unaffected segment from each rat as our normal population. This constituted a total of 8 normal bowel segments. The protocol was approved by the University of Michigan Committee on the Use and Care of Animals (UCUCA) and strictly complied with National Institutes of Health Guide for Care and Use of Laboratory Animals. Further details of TNBS treatments are described in the previous study (Kim et al. 2008).

(B) Ultrasound RF data collection

In vivo ultrasound (US) in-phase/quadrature (I/Q) data were acquired at a frame rate of about 60 frames per second (depending on depth) by using a linear array ultrasound probe (L10-5, centered at 6.5 MHz, ZONARE Medical System, Mountain View, CA, USA) connected to a commercial ultrasound scanner (Z. one, ZONARE Medical Systems, Mountain View, CA, USA). Ultrasound data sets were continually collected while the abdomen was being pushed by the ultrasound probe to develop about 10% average strain over a total of 3 seconds. The animals were anesthetized with ketamine (36 mg/kg) and xylazine (3.6mg/kg) during the exam. The ultrasound probe was placed on the anterior abdominal wall of the rats, and was also used to compress the rat's abdominal wall and underlying tissue. A mild preload on the abdomen in the transverse plane was used to displace luminal stool and gas while reducing image shadowing artifacts. The transducer was fixed to a laboratory designed deformation device to produce a uniform displacement on rat's shaved abdomen. The overall experimental setup is similar to the previous study (Kim et al. 2008).

(C) Phase-sensitive two-dimensional (2D) speckle tracking for strain estimates

The beam-formed I/Q data were derived from the raw data stored in the scanner's channel domain memory. Radio frequency (RF) data samples were reconstructed from the IQ data considering the time-frequency compensation parameters and sound speed to improve the data quality (Mo et al. 2007). A phase-sensitive correlation-based 2D speckle tracking algorithm was then applied to the reconstructed RF data to estimate the displacement between frames (Lubinski et al. 1999). Frame-to-frame displacements were estimated from the position of the maximum 2D correlation function of the baseband complex signals derived from the RF data. The search kernel of the speckle tracking was set to be about the ultrasound speckle size. The ultrasound speckle size was estimated to be 0.25 mm in axial direction and 0.5 mm in lateral direction from the 2D correlation function of the baseband signals. The frame-to-frame displacements were then accumulated over a number of frames covering the entire deformation to estimate the total displacement. With accumulation of frames, the strain signal-to-noise (SNR), which is defined as average over standard deviation of the displacement, is enhanced by reducing any uncorrelated errors in the strain estimates (Lubinski et al. 1999). The axial normal strains were then calculated by taking the spatial

derivative of the accumulated axial displacements with respect to the axial direction using centered numerical differentiation (Lubinski et al. 1999). As accumulated axial strains changed with the number of US frames acquired, the regions of interest (ROIs) for normal/fibrotic areas or normal/acute/chronic tissue types, and the reference of the rat's pelvis were marked by a clinical ultrasound expert (JMR) in the original B-Scan images. The corresponding ROIs were used to estimate the developed strains by averaging strains over the selected ROIs for normal/acute/chronic tissue types, the relationships between the developed strains and the number of acquired US frames were determined from the accumulated axial strain maps for every acquired RF sequence. The overall averaged strain estimated from the pelvis during a deformation is used to represent the applied average strain based on the assumption that the tissue overlying the pelvis is generally similar from animal to animal. Due to the much smaller size of the targeted tissue area in the bowel compared to the body of the animal, these stiffness differences should not affect the overall applied strain estimates.

The overall applied strain is simply a relative height change of the body after deformation, estimated from the displacement of the pelvis. The overall applied strain calculation is as follows: (1) Determine the position of the pelvis from the first initial B-scan image; (2) At every US frame, estimate the displacement of the rat's pelvis from the RF sequence; (3) The overall strain was used as an applied strain for every scan with the relationships between the applied strain and the US frame number determined from RF sequences. Note these estimates are on the same section of deformation from the beginning to the end (first frame to the last frame) on the RF sequence. The variance with potential sound speed changes on different sections should not be significant. Normalized strains for normal/acute/chronic were determined from the developed strain over the ROI divided by the applied strain at each US frame, usually around 50 frames total, or a total overall developed strain of less than 15% in the linear range.

The normalization of developed strain to the applied overall strain is a necessary procedure to standardize the data between different scans and animals where the applied strain can vary. Given the nonlinearity of the targeted tissue responses to the applied strain, it is important to observe this behavior relative to the applied strain, not just as a function of time. Although the deformation protocol was designed to increase applied strain monotonically, it can vary with the operator, and it is important that the nonlinear characteristics of the targeted tissue be referenced to the applied strain as well as time.

(D) Direct mechanical measurements

The tissue samples (both normal and treated bowel for every rat) were harvested after the final B-scan ultrasound imaging and IQ data collection. A commercial soft tissue compression test device (MicroElastometer, Model 0501, ARTANN Laboratories, Inc, West Trenton, NJ, USA) was used to measure the elastic modulus of the excised normal bowel and treated bowel samples (Egorov et al. 2008). The device measures the displacement vs. applied force when a test sample is under of compression. The applied force is measured by a strain gauge (Acculab VI-200, Acculab, Edgewood, NY, USA) mounted under the object plate. The measurable force range is up to 2N with a precision of 0.1mN. The device employs a pressing stamp with a rectangular cross-section of 8.88 mm by 3.98 mm. The position of the compression stamp is controlled by a hybrid linear actuator (HIS 35N67-12-405, Haydon Switch & Instrument Inc., Waterbury, CT, USA). The travel distance per step was set for 3 μ m. Each sample was measured twice for statistical analysis. Between each measurement, the sample was soaked in normal saline for a few minutes to allow it to regain its elasticity. The test samples were cut into 10mm by 10 mm with height varying from 1mm to 4 mm (average value: 2.8mm $\pm$ 1.5mm from all 32 samples for 8 rats).

(E) Histology

The tissue samples from the distal colon for 8 rats were collected immediately following the ultrasound RF data acquisition. The harvested tissue samples were dissected into two 10mm by 10mm pieces for direct mechanical measurements and histology. Samples for histology were put into 10% buffered formalin, paraffin embedded, underwent hematoxylin and eosin (H&E), trichrome, and α -smooth muscle actin (SMA) staining, and assessed by pathologists. The process for histology is detailed in our previous study (Kim et al. 2008).

(F) Finite-element analysis based on nonlinear soft tissue model

To numerically simulate the relation between the developed strain of the target inclusion and the applied average strain, a finite element (FE) based nonlinear soft tissue model was developed using a commercially available software package (ABAQUS, Dassault Systemes, Rhode Island, United States). Figure 1 presents the schematic of the FE model. A circular inclusion in a uniform rectangular tissue block is modeled, while a rigid transducer was used to deform the sample with equal displacement boundary conditions (DBC) (Doyley et al. 2000). The 2 dimensional (2D) plane strain problem was solved quasi-statically using 40 steps to obtain a total axial displacement of 8 mm out of the initial height of 40 mm, which results in is 20% total strain with 0.5% strain for each deformation step. The boundary condition at the bottom surface was set as roller (no axial displacement) and all other surfaces were set as free. The contact between the surface of the transducer and top surface of the tissue block was modeled as frictionless, since we use ultrasound gel between the transducer and the tissue in the experiments. The surface between the inclusion and the surrounding tissue block were “tied” so that there is no relative motion between those surfaces. The model was meshed using regular 8-noded plane strain elements with reduced integration. The differences between the FEM model and rat experimental results were discussed in later of this paper.

A hyper-elastic nonlinear material model was employed for the background material surrounding the target inclusion. The polynomial model which has been widely used in modeling soft tissues like breast was used (Samani and Plewes 2007). The strain density for such model is given as follows:

$$U = \sum_{i+j=1}^N C_{ij} (I_1 - 3)^i (I_2 - 3)^j, \quad (1)$$

where U is a selected strain energy function, and I_1 and I_2 are the first and second strain invariants respectively, $N=2$, C_{ij} represents the hyper-elastic material parameters, which determine the intrinsic nonlinear elastic properties of the tissues. The material parameters, C_{ij} , were optimized to obtain similar contrast between inclusion and background as observed in the in vivo rat experiments. Identical values of C_{ij} was used for all the three cases, so that the comparison would not include the effect of the surrounding material and is only a function of the tissue (inclusion) types. The anatomical structures of the rat are complicated and incorporating all the details in the simulation is impractical and unnecessary considering the scope of this study. Instead, the entire belly was considered as one homogeneous tissue with a representative stiffness, which might not vary between animals within the scope of this study.

Three inclusions with different stiffness were considered based on their contrast in strain to the surrounding material: type I inclusion mimicking the normal colon tissues, type II inclusion mimicking the acutely inflamed tissues, and type III inclusion mimicking the chronically fibrotic tissues, where stress-strain curves of the excised tissues (Figure2) were used as inputs to the inclusion model and the hyper-elastic parameters were adjusted until

the developed strains inside the inclusion and surrounding material become similar to the strains from the in vivo measurements. Further details are described in Results.

A 1-D linear array centered at 6.5 MHz with a fractional-bandwidth of 60% with a Gaussian-type envelope was used to generate 2-D US RF data. To construct the US images, a 2D point spread function (PSF) was evaluated which closely mimics the transducer used in this study. Each frame of digital RF data was then produced with 40 MHz sampling frequency by convolving the 2D PSF with the US scattering function. The scattering function was defined as the random distribution of US scatters over the entire tissue area. For each time-step of the FE simulation, the displacements of each of the scatters were interpolated from the nodal solution and added to the pre-deformed coordinates to obtain the deformed scattering function. The speed of sound was assumed to be 1540 m/s in soft tissue. The scatter density was set as 3 scatters per wavelength. Each RF image with size of 60mm × 40mm (corresponding to 3636 axial samples × 256 beams) was generated at each time-step of the deformation. (Kaluzynski et al 2001).

(G) An empirically derived nonlinear equation

From both experimental data and FE-based simulation data, the developed strain-to-applied strain relation exhibits non-linear characteristics over the wide range of strains. To differentiate the target tissues with different stiffness, empirical curve fitting was performed by employing the Hill equation, which was originally proposed for a muscle contraction study (Holmes 2006). This equation was chosen over other general candidate models for its best fit to our simulations and measurements for all cases of different tissue types. For example, a standard sigmoid model fits well to FE simulation for a type I inclusion and in-vivo strain-to-strain curves for the normal colon tissues. However this model does not fully describe the strain-to-strain relations for type III inclusions and in-vivo chronically fibrotic tissues. Also with its simplicity with a relatively small number of independent parameters, the adopted Hill equation is used in this study to describe the strain-to-strain relation as follows:

$$\varepsilon_d = \frac{A\varepsilon_a^n}{k^n + \varepsilon_a^n}, \quad (2)$$

where ε_a is the applied strain to the surrounding tissues, and ε_d is the developed strain in the target inclusion, and n is the model order, where k and A are arbitrary constants. Note A only scales the curve and k contributes to the onset of the strain-to-strain relations, and it also often depends on slightly different experimental conditions such as small variance in preloading of the tissues and/or surface roughness of the tissues in this type of experiment.

In our application, the order n determines the unique feature of the developed strain-to-applied strain curve of each inclusion and separates the inclusions with different stiffness from each other. As further described in Discussion and Conclusions, all three types of the tissue do not completely differentiate from each other at the initial stage of loading (lower deformation loading) or later stage of loading (higher deformation loading) where the tissues experience hardening effects by applied loading. However, the non-linear order n uniquely characterizes the strain-to strain non-linearity, separating all three types of the tissues. Based on these empirical observations in conjunction with simulation results, only n was chosen as a discriminator between different tissue types in this study. The arbitrary constants, k and A , which govern the onset shift of the curve and the amplitude of the tail of the curve, respectively, were first determined to best match both lower and higher deformation loading. Then optimized fitting for highest correlation was performed to find unique n for each data set using Levenberg-Marquardt algorithm in MATLAB from MathWorks. This procedure was identical for both simulation results and in vivo measurements. Since the optimization

algorithm could be affected by the initial guess, a sensitivity study was performed to support the solution for n found through the optimization procedure above. The test were performed 10,000 times using initial values with (0,1) uniform random distributions for all three parameters, generating the distribution of model order n ; for example, (1) n was complex (422/10,000), (2) n was negative (4306/10,000), (3) n was greater than or equal to 4 or less than 0.5 (7/10,000), and (4) the remainder of the estimated values of n were close to 2 (5265/10,000) for the normal rat bowel tissues from the acutely inflamed rat. Neglecting nonphysical solutions such as complex or negative values, solutions for n fall in the range of 0.5 to 4 centered at 2 with narrow distribution, which support the optimized solutions obtained for this case. Using similar process, the optimized nonlinear order n was confirmed for each simulation results and in vivo data.

(H) Statistical analysis

A standard two-tailed unpaired t-test was performed for the nonlinear parameter by using Excel from Microsoft. Note a difference between means with a $p < 0.05$ was considered significantly different.

Results

(A). Nonlinear FE simulation

A 2-D plane strain problem was formulated as shown in Figure 1 for the numerical phantoms of the representative cases of type I, type II and type III inclusions.

Figure 2 illustrates the typical stress-strain curves measured by the Microelastomer for the excised for normal (solid), acutely inflamed (dotted), and chronically fibrotic (dashed) tissues from the rats.

For FE simulation, these strain-stress curves in Figure 2 were used as input to the inclusion model. In order to obtain similar elasticity contrast in the FE simulation to that of the in vivo animal experiments, the hyper-elastic parameters of the surrounding material, C_{ij} , were adjusted such that the ratio of the overall strain from the height change of the surrounding background and the local average strain in the inclusions is similar to the ratio obtained from in vivo ultrasound data, i.e., the ratio of the overall strain from the height change of the body and the average strain inside each tissue types. The average strain developed in the region of interest for the chronic tissue type was about 18% ($\pm 5\%$) under the applied average strain of 10% estimated from the displacement of the pelvis. The hyper-elastic parameters, C_{ij} , were adjusted until the targeted inclusion developed 15% strain when the applied strain reached 10%. The non-linear coefficients for the hyper-elastic function were then set to be: $C_{01} = C_{10} = 0.3$, and $C_{11} = 2.25$, $C_{20} = 3.8$, and $C_{02} = 4.7$ for the fibrotic tissues. The hyper-elastic parameters for the other tissue types were almost identical. Therefore, the hyper-elastic parameters, determined above for the fibrotic tissues were used for all other cases in this study. This made the simulations consistent with the same background materials for inclusions with different stiffness.

Accumulated strain estimates over the entire deformation from 2D speckle tracking were compared with FE solutions in Figure 3. The bottom row represents the strain maps from 2D speckle tracking for (d) type I inclusions, (e) type II inclusions, and (f) type III inclusions, and the top row (a) (b) and (c) represents the strain map from FE solutions for the same inclusions, respectively. Overall, the strain maps from the speckle tracking compare well with the strain maps from the FE solutions. The average strain in every inclusion between the FE simulation and speckle tracking were also comparable. On the average, the relative error in average strain estimates between speckle tracking and FE simulation for every inclusion was less than 5%.

In Figure 4, the accumulated average strain in every inclusion is plotted over the-applied average strain, which is exactly known in the FE simulation by pre-setting the total strain to 20% in 40 steps. The accumulated average strains from 2D speckle tracking for the type I (circles), type II (diamonds), and type III (pluses) inclusions are overlaid on the corresponding FE solutions in solid lines. The type III inclusion generates overall less strain to the same amount of applied strain as expected. More notably, the type I and type II inclusions exhibit non-linear relations in strain-to-strain plots, while the type III inclusion maintains a straight line.

(B) In vivo animal experiments

(1) Chronic/fibrotic tissue type—The accumulated axial normal strain map over the 50 frames along with B-scan images for a representative chronically fibrotic case are depicted in Figure 5. In Figure 5(a), the region enclosed by the dashed blue line indicates the ROI for the normal tissues, and the region enclosed by the dashed red line indicates the ROI for the fibrotic tissues. The dashed green box indicates the pelvis, which is used as the reference to obtain the average applied strain. In Figure 5(b), the corresponding strain maps in the ROI are overlaid on the top of the B-scan images indicating the relative changes of the stiffness. The diseased fibrotic bowel developed less strain reflecting the increase in stiffness compared with the normal bowel of the rat.

Figure 5(c) shows the accumulated average strain inside normal (solid line) and fibrotic (dotted line) bowel plotted against US frame number. The dashed-dot line presents the monotonically increasing applied average strain estimated from the displacement of the rat's pelvis. In Figure 5(d), the same accumulated strains for normal and fibrotic bowel in 5(c) were plotted against the applied average strain. Note that the overall characteristics of the fibrotic bowel in the developed strain-to-applied strain relation are differentiated from that of the normal bowel, which were observed in the FE simulation.

(2) Acute/inflammatory tissue type—The accumulated axial normal strain over the 50 frames along with B-scan images for a representative area of acutely inflamed bowel is depicted in Figure 6. In Figure 6(a), the region enclosed by the dashed blue line indicates the ROI for the normal bowel, the region enclosed by the dashed red line indicates the ROI for the acutely inflamed bowel. The dashed green box indicates the pelvis, which is used as the reference to obtain the applied averaged strain. In Figure 6(b), the corresponding strain maps in the ROIs are overlaid on the top of the B-scan images indicating the relative changes in stiffness. The acutely inflamed bowel developed less strain reflecting an increase in stiffness compared with the normal bowel.

Figure 6(c) illustrates the accumulated strain inside normal (solid line) and acutely inflamed (dotted line) bowel plotted against US frame number. The dashed-dot line represents monotonically increasing applied average strain estimated from the displacement of the rat's pelvis. In Figure 6(d), the same accumulated strains for normal and acutely inflamed bowel in 6(c) were plotted against the applied averaged strain.

(3) Non-linear curve fitting for the normal, acutely inflamed and chronically fibrotic tissues—In rat study, the results from all 4 rats in the fibrotic tissue type exhibit very similar developed strain-to-applied strain relationships as seen in to the representative plot in Figure 5 for a selected rat subject. The results for all 4 rats in the acutely inflamed tissue type also show very similar developed strain-to-applied strain relationships, as seen in the representative plot in Figure 6. All of the normal bowel measurements of 8 rat subjects in the untreated region also exhibited very similar strains.

Figure 7 presents the fitted curves of the equation (2) to the data for the acutely inflamed bowel (b) $\epsilon_d = 45.2\epsilon_a^{1.5}/8.6^{1.5} + \epsilon_a^{1.5}$, fibrotic bowel (d) $\epsilon_d = 80.2\epsilon_a^{1.03}/19.2^{1.03} + \epsilon_a^{1.03}$, and corresponding nearby bowel (a) $\epsilon_d = 47.8\epsilon_a^{1.9}/4.3^{1.9} + \epsilon_a^{1.9}$ and (c) $\epsilon_d = 57.8\epsilon_a^{2.0}/4.0^{2.0} + \epsilon_a^{2.0}$, respectively. The fitted curves are overlaid on top of experimental data. The non-linear parameter corresponding to the order n of equation (2) was determined to be 1.50 for the inflamed (b), 1.03 for the fibrotic (d), and 1.9 (a) and 2.0 (c) for the normal rat bowel. Note the accumulated strain data points are arbitrarily parsed for presentation clarity. It also should be noted that the non-linear parameter n from the curve fittings to the equation (2) based on the FE simulation results also show distinct differences between the inclusions with different stiffness, where n is equal to 1.6 for the type I inclusion mimicking the normal bowel, 1.3 for the type II inclusion mimicking the acutely inflamed bowel, and 0.9 for the type III inclusion mimicking the chronically fibrotic bowel.

The average non-linear parameter n for each animal group was determined to be 1.96 with standard deviation of ± 0.12 for the normal tissue type, 1.5 with standard deviation of ± 0.09 for the acutely inflamed tissue type, and for 1.03 with standard deviation of ± 0.08 for the chronic tissue type, as shown in Figure 8. T-tests determined that the comparisons of non-linear parameters between normal, acutely inflamed, and fibrotic tissues were statistically significantly different (normal/fibrotic ($P = 0.0000185$), normal/acutely inflamed ($P = 0.0013$), and fibrotic/acutely inflamed ($P = 0.0029$)). The three different tissues were clearly separated by the non-linear parameter n with no overlap of the standard deviations. In contrast, the conventional normalized average strain obtained at one state of the deformation at an applied strain around 5% does not fully differentiate the acutely inflamed bowel from the chronically fibrotic bowel with the normalized average strain (2.7 with standard deviation of ± 0.4 for acutely inflamed tissue type, 2.6 with standard deviation of ± 0.98 for chronic tissue type, 5.6 with standard deviation of ± 1.8 for the normal bowel). T-test determined that normalized strain between normal/fibrotic tissue types and normal/acutely inflamed tissue types were significantly different (normal/fibrotic ($P = 0.024$), normal/acutely inflamed ($P = 0.022$)), but not for the acutely inflamed/fibrotic case ($P = 0.744$).

Discussion and Conclusions

A new nonlinear elastic parameter of the soft tissues is proposed and tested using a FE based nonlinear soft tissue model and in vivo animal model. To examine the nonlinear characteristics of the tissues over a relatively large dynamic range of strain, a simple nonlinear soft tissue model was established using FE methods where an inclusion of an infinitely long cylinder was embedded in a homogenous surrounding tissue block. Within the range of the stiffness of biological tissues in the animal model, including chronically developed fibrosis, acutely inflamed, and normal bowel tissues, the developed strain inside the inclusion vs. applied strain exhibit overall similar behaviors to those observed in the animal study. As shown in Figure 4, the developed strain-to-applied strain curve for type I, type II, and type III inclusions both from FE model and speckle tracking demonstrate similar trends with those of normal, acutely inflamed, and fibrotic bowel in the animal model, respectively, presented in Figures 5 and 6. The soft inclusion develops strain quite linearly at the beginning stage of the loading until the developed strain reaches the point where the material experiences nonlinear hardening effects, resulting in the saturation of the strain development (Erkamp et al. 2004). The type III inclusion develops overall less strain monotonically over the entire strain dynamic range of interest. The type II inclusion exhibits almost the same strain as the type I inclusion, i.e. linearly at the onset of the loading up to a few percent of strain. Later, the strain, developed in the type II inclusion deviates from that of the type I inclusion, producing less strain and eventually exhibiting slight saturation of the strain, but distinct from the monotonic strain increase in the type III inclusion.

It is notable that the strain developed in the type II inclusion is hardly differentiated from the strain developed in the type I inclusion at the beginning stage of the loading up to a few percent of strain, while at the later stage of the loading where the type II inclusion experiences tissue hardening, it is more difficult to separate it from the type III inclusion. These trends were also observed in the direct mechanical measurements on the excised tissues as illustrated in the stress-strain curves in Figure 2. The monotonic increase in the stress-strain relations for the normal (type I inclusion) and acutely inflamed (type II inclusion) bowel are very similar at the beginning stage of the loading, while the acutely inflamed bowel approach the fibrotic bowel (type III inclusion) in the stress-strain relation as they develop further strain and experience tissue hardening.

In the animal model, in vivo strain estimates by speckle tracking also exhibit overall similar trends as illustrated in Figures 5 and 6. These characteristics actually make it challenging to identify the acutely inflamed bowel using conventional UEI estimates at a fixed applied strain. At the lower deformation loading, monotonically increasing strain common for normal, acutely inflamed, and chronically fibrotic bowel makes it very difficult to differentiate the different groups. In addition, the relatively low signal-to-noise ratio of UEI at mild deformation loading in vivo makes it difficult to detect the acutely inflamed bowel using UEI. At higher deformation loading, the signal-to-noise ratio becomes large enough to estimate strain with less error, however, the stiffness of the acutely inflamed bowel tends to get closer to the chronically fibrotic bowel as inflamed bowel experiences non-linear tissue hardening after the edematous water contents are forced out. This might be one of the fundamental limitations of the conventional UEI in identifying acutely inflamed, edematous tissues. It is believed that the accumulated water contents in the thickened edematous tissue due to inflammation would make the tissues linear and appear softer until the liquid inside is squeezed out and the tissue then becomes non-linear and stiffer producing less strain to the applied deformation loading. This also could be further explained from the point of view of poroelasticity (Konofagou et al. 2004; Berry et al. 2006a and 2006b), which will be further investigated.

It should be noted, as shown in Figure 4, a Type II inclusion could appear hard like a Type III inclusion at higher strains or soft like a Type I inclusion at lower strains. This is reflected by our in vivo results where the normalized strains at 10% applied strains for the acutely inflamed and chronically fibrotic bowel are almost identical (2.7 with standard deviation of ± 0.4 for acutely inflamed tissue type, 2.6 with standard deviation of ± 1.0 for chronic tissue type). They could only be differentiated by their responses in non-linear analysis over the large dynamic range of strains.

In this study, the developed tissue model is simplified in two perspectives. The model includes a well defined geometry of the inclusion and homogeneous surrounding tissue block, while the anatomy of the rat is more complicated. However, it is observed that the relatively soft abdomen of the rat absorbs the applied strain more or less uniformly avoiding any serious stress concentration around the bowel tissues of interest. The developed model also assumes a purely elastic model and does not include viscosity. Since edematous tissue is believed to be viscoelastic, it would be more desirable to apply a visco-elastic model to study the mechanical properties of the acutely inflamed tissues. However, it will still be challenging to assess the viscosity of the tissues using conventional UEI in vivo as liquid will be easily pushed away during the onset of deformation or with mild preloading, which often is required in UEI practice in vivo.

Here we used a simplified nonlinear hyper-elastic tissue model that exhibits the representative nonlinear characteristics of the tissues with different stiffness contrast to the background over a large strain dynamic range. It is true that the accumulated strain at some

point of the applied loading (strain), as shown in Figure 4, can differentiate the inclusions with different stiffness. For example, the developed strains normalized to an applied strain of 10% in type I, type II and type III inclusions are 2.3, 1.8, and 1.5, respectively, thus differentiating the three tissue types. However, such a distinction becomes more difficult in vivo where strain estimates contain many more uncertainties, including (a) animal-to-animal variability, (b) ultrasound imaging and data acquisition system noise, and (c) out-of plane motion due to free hand deformation and non-uniform anatomical structure. These were not considered in this simulation.

To quantitatively describe the nonlinear characteristics of the tissues with different stiffness contrast in the developed strain-to-applied strain relations, numerical curve fitting was attempted. A very common equation for asymptotic relationships is known to be the Michealis-Menten equation or rectangular hyperbola (Holmes 2006), which is a special case of the Hill function with order 1. This equation has been employed in various different fields, including ecosystem modeling (Hassell 1978) and biochemical characterization and molecular modeling (Chu et al. 2009). The unique asymptotic relationships among the variables were common characteristics in all these applications and were sufficient for this study (Holmes 2006). Curve fitting using Equation (2) was tested in simulation, supporting the fact that inclusions with different stiffness contrast can be differentiated with the variable n . Based on the empirically optimized variable n , normal, acutely inflamed, and chronically fibrotic bowel were successfully differentiated with variable n values of 1.96 ± 0.12 , 1.50 ± 0.09 , and 1.03 ± 0.08 , respectively with no overlap of the standard deviations. T-tests determined that the non-linear parameters between normal, acutely inflamed, and fibrotic tissue types were statistically significantly different (normal/fibrotic ($P = 0.0000185$), normal/acutely inflamed ($P = 0.0013$), and acutely inflamed/fibrotic ($P = 0.0029$). In contrast, the normalized strain using conventional UEI at average applied strain about 10% was estimated to be 5.6 ± 1.8 , 2.7 ± 0.4 , 2.6 ± 1.0 for normal, acutely inflamed, and chronically fibrotic bowel, respectively, leaving the acutely inflamed bowel within the range of chronically fibrotic bowel. The stiffness of the acutely inflamed bowel was clearly separated from the chronically fibrotic bowel in the stress-strain relation from the direct mechanical measurements using the excised tissues. The fibrosis scores from the histopathology, which are not presented in this paper, also support the difference between two tissue statuses.

For future clinical applications, estimating the applied strain may be challenging. For the animal study, the average strain was estimated with reasonable errors from the displacements of the pelvis or spine. However, it may be difficult to apply the same technique to the human subjects due to limited imaging depths. However, the use of lower frequency transducers will still permit strain estimates to be made at much greater imaging depths. Objects such as the pelvic wall, spine, and large posterior muscle groups such as iliopsoas should provide references for mean strain estimates in humans very similar to those performed in rats. This will be a topic for study in the future.

This paper presents a new nonlinear parameter to detect and characterize tissue mechanical property changes, especially a subtle change in stiffness such as those seen in acutely inflamed bowel. The proposed nonlinear fitting of the developed strain-to-applied strain relation was tested using a simple FE based nonlinear soft tissue model. Using a rat TNBS treated model of the inflammatory bowel disease, in vivo feasibility was successfully shown with some limitations, differentiating normal, acutely inflamed, and chronically fibrotic bowel with no overlap. This method can be applied to other applications of ultrasound elasticity imaging with the proper adjustment of the involved nonlinear parameters in Equation (2) depending on the stiffness contrast of the tissues of interest. A relatively easy adaptation to the human subject study is also expected. This technique may provide a

sensitive and robust tool to assess subtle stiffness changes in tissues such as in acutely inflamed bowel wall.

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Figure 1.
Schematic diagram of finite element tissue modeling and simulation.

Figure 2.
Direct mechanical measurements of the excised tissues from the rats.

Figure 3. Comparison of the strain maps from FE simulations with those from the US speckle tracking.

Figure 4. Average strains developed inside the inclusion vs. the applied strain from US speckle tracking and corresponding FE simulations

Figure 5. Strain maps and the developed strain-to- applied strain relations for typical chronically fibrotic bowel.

Figure 6. Strain maps and the developed strain-to- applied strain relations for typical acutely inflamed bowel.

Figure 7. Developed strain-to- applied strain relations for three tissue types of normal, acutely inflamed and chronically fibrotic rat bowel.

Figure 8. Nonlinear parameters for three types of normal, acutely inflamed and chronically fibrotic bowel.