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Mechanical behavior measurement of the sheep small intestine using experimental tests

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Key words:
anisotropic constitutive model, fung-type model, nonlinear tissue, modify Mooney-Rivlin model, strain-energy function

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Abstract:

BACKGROUND: There is no consistent data on the mechanical properties of sheep intestine. **OBJECTIVES:** We performed a series of biaxial strain measurement experiments and extracted the constitutive model to describe the mechanical characteristics of the sheep intestinal tissue. **METHODS:** Eleven specimens were obtained freshly from sacrificed sheep and the planar biaxial tests were performed on the tissue specimens by applying simultaneous loads along the circumfer-ential and longitudinal directions. Then the measured data were fitted into the anisotropic four-parameter Fung-type model and also to the modified Mooney-Rivlin model. **RESULTS:** The specimens showed some degree of anisotropy; the stiffer direction is not gener-ally predictable. Some of the specimens were stiffer in the circumferential direction, and the others in the longitudinal direction. However, the average results state the circumferential di-rection as the stiffer orientation. **CONCLUSIONS:** It can be concluded that sheep intestine be-haves normally as a nonlinear anisotropic tissue which is well-characterized by the modified Mooney-Rivlin model.

Introduction

From the anatomical view, the small intestine consists of three adjacent segments; duodenum, jejunum and ileum. The wall of these segments has a multi-layered structure; the four layers are asserosa, muscolaris, submucosa and mucosa. The mechanical p roperties of the small intestine are mainly dominated by the submucosa and the muscular layers, while the other layers contribution is negligible (Egorov et al., 2002).

To estimate intestine mechanical properties, researchers performed uniaxial tensile tests on human intestine and proposed that the mechanical properties of intestine in axial and transversal directions are different (Egorov et al., 2002). The inflation tests have also been applied to porcine intestine (Slatkin, 1999), human duodenum (Frøkjaer et al., 2006) and pig small intestine (Liao et al., 2010). Although the uniaxial tensile test data were presented comprehensively, they are inadequate to determine the mechanical properties, such as nonlinear and anisotropic responses. Moreover, the inflation tests lack the adaptability of a planar test in addition to the fact that the sample sizes in these tests are dependent on the pressure and size of the nozzle (Chong et al., 2005). Meanwhile biaxial planar test is a proper alternative for approaching a better view on the mechanics of soft tissues. Despite the fact that the researchers performed planar biaxial tensile tests on porcine duodenum, jejunum, and ileum and used anisotropic four-parameter Fung-type model and isotropic models in the Neo-Hookean and Mooney-Rivlin forms to describe them (Bellini et al., 2011), there is still a need for an anisotropic model of intestine for a reliable FE analysis in a standard software package. To the authors' knowledge, the anisotropic Fung-type model cannot be used in so many FE packages (e.g. a FSI procedure in a commercial finite-element package named ADINA).

Since the sheep intestine acts similar to human intestine in some diseases (Munday et al., 2006) the goal of this study was to develop an anisotropic constitutive model for the small intestine wall of sheep. By this aim, planar biaxial tests were performed on specimens by loadings applied along the circumferential and longitudinal directions. Then the data of the experimental tests were modeled by the anisotropic four-parameter Fung-type model and the anisotropic modified Mooney- Rivlin Model.

Materials and Methods

Sample preparation: Eleven intestine samples extracted from 6 healthy sheep were used in this study. All the sheep were in the range of 2-4 years old. Tissues were cleaned and stored in physiological 0.9% saline. Tests were completed within 8 hours after extraction. The specimen thicknesses were measured with a micrometer. Deviation of thickness was negligible, so average thickness was used in formulations. The specimens were cut along the longitudinal axis and shaped into squares of 18 mm sides which suits our biaxial test requirements.

Biaxial mechanical characterization: A planar biaxial test device with strain measurement capability was utilized to obtain stress/strain correlation along the longitudinal and circumferential axes of the specimens (Figure 1). In order to preserve the mechanical properties, samples were kept wet at temperature of 37◦C by use of a temperature controlled water bath. The clamps of the system were able to directly hold samples with dimensions ≥15×15 mm2 without damaging the tissue. After placement of the specimens on the device, they were preloaded with 0.01 N loading along both axes in order to obtain meaningful measurements. The loadings were applied with the rate of 0.02 mm/s through four microstepper motors in both

directions and the tensile forces were measured by two UMAA 2 kgf load cells. For the purpose of deformation measurement of the tissue, a USB digital microscope camera was used (300X zoom, 30 Hz and resolution of 480×640). The data of digital microscope camera were processed with

ImageJ package to obtain stretch in each direction. Due to small dimensions of samples there was no way to use ink markers in stretch (displacement) measurement. Initial distance between the ends of each clamp was considered as the reference measure of sample length in both directions.

The stress-strain curve for each specimen was obtained in two axes (the (11) corresponding to the circumferential direction and the (22) to the longitudinal direction). The experimental stresses for the samples were computed as follows:

(1) $\sigma_{11}^{exp}=\lambda_1(F_{11}/l_2t)$

(2) $\sigma_{22}^{exp}=\lambda_2(F_{22}/l_1t)$

where λ_1 and λ_2 are the stretch ratios, F_{11} and F_{22} are the forces measured by the load cells, t is the thickness of the samples, and l1 and l2 are the unloaded widths of the samples in the two directions.

Constitutive model: In this study, small intestine was modeled as an incompressible, homogeneous and hyperelastic material. These assumptions confirm the existence of a strain energy function W which is the criterion for the stored energy in the materials as a result of the deformation. By use of the strain energy function, the stresses can be computed from the strains as follows (Sun et al., 2003; Humphrey et al.,1990; Humphrey et al.,1990):

(3) $S_{ij}=PC^{-1}+2(əw/əC_{ij})=PC^{-1}+(əw/əE_{ij})$ (I,j=1,2,3)

(4) $C_{ij}=F_{ij}^{T}F_{ij}$

(5) $E_{ij}=(1/2)(C_{ij}-I_{ij})$

where S_{ij} is the second Piola-Kirchhoff stress tensor, p is the Lagrange multiplier introduced to enforce incompressibility, C_{ij} is the right Cauchy-Green deformation tensor, Eij is the Green-Lagrange strain tensor, Iij is the identity unit tensor and F_{ij} is the deformation gradient tensor which can be described as F= ∂x/∂x' in which x' and x are the position of material point in the reference and current configuration, respectively.

The Cauchy stress tensor σij is calculated as follows:

(6) $\sigma_{ij}=PI+2F_{ij}(əw/əC_{ij})F_{ij}^{T}=PI+F_{ij}(əw/əE_{ij})F_{ij}^{T}$

According to the strain energy function and investigating the hyperelastic models (Fung et al.,1979; Holzapfel et al., 200; Holzapfel et al.,1996; Holzapfel et al., 2004; Holzapfel et al., 2005) two appropriate constitutive models were chosen to express the mechanical properties of the intestine, as these models have been used previously for other soft tissues.

The first model utilized was a Fung-type model (Fung, 1991) which is able to describe the anisotropic behavior of tissue. In the Fung model, the strain energy density is given by:

(7) $W(Q)=0.5c(e^{Q}-1)$

where $Q(E)={}_{a1}E_{11}^{2}+{}_{a2}E_{22}^{2}+2{}_{a3}E_{11}E_{22}$, and c, a1, a2 and a3 are constitutive parameters.

The Cauchy stress components in the two directions were then calculated as follows:

(8) $\sigma_{11}=\{0.5c\lambda_1^{2}[a_1(\lambda_1^{2}-1)+a_3(\lambda_2^{2}-1)]*\text{-}\exp((1/4)[a_1(\lambda_1^{2}-1)^{2}+a_2(\lambda_1^{1}-1)^{2}+2a_3(\lambda_1^{2}-1)\ (\lambda_2^{2}-1)]\}$

(9) $\sigma_{22}=\{0.5c\lambda_2^{2}[a_2(\lambda_2^{2}-1)+a_3(\lambda_1^{2}-1)]*\exp((1/4)[a_1(\lambda_1^{2}-1)^{2}+a_2(\lambda_2^{2}-1)^{2}+2a_3(\lambda_1^{2}-1)(\lambda_2^{2}-1)]\}$

The second model was the modified Mooney-Rivlin model which shows anisotropic behavior of the tissues and can also be implemented into many standard FE packages. The strain energy density

Function of this model is given by:

(10) $W=C_1(I_1-3)+D_1[\exp(D_2(I_1-3))-1]+(k_1/2k_2)[\exp[k_2(I_4-1)^{2}-1]]$

where $I_1=\lambda_1^{2}+\lambda_2^{2}+(\lambda_1\lambda_2)^{-2}$ and $I_4=C_{ij}(n_c)_i(n_c)_i$, n_c is the circumferential direction of the tissue and c_1, D_1, D_2, k_1 and k_2 are the model parameters (Holzapfel et al., 2004; Bathe, 1996; Bathe, 2007).

According to the strain energy function, Cauchy stresses in the two axes are as follows:

(11) $\sigma_{11}=(2(\lambda_1^{2}-\lambda_3^{2})(C_1+D_1*D_2*\exp(\lambda_1^{2}+\lambda_2^{2}+\lambda_3^{2}-3)))\ (2*k_1*\lambda_1^{2}(\lambda_1^{2}-1)\exp(k2(\lambda_1 2-1)^{2}))$

(12) $\sigma_{22}=(2(\lambda_2^{2}-\lambda_3^{2})(C_1+D_1*D_2*\exp(\lambda_1^{2}+\lambda_2^{2}+\lambda_3^{2}-3)))$

Experimental data then were fitted to Cauchy stress of each model by using genetic algorithms and the parameters of constitutive models were obtained for each data set.

Then the anisotropy is calculated by using the Fung model parameters as follows:

(13) $\text{Anisotropy}=\min[(a_1+a_3)/(a_2+a_3),\ (a_2+a_3)/(a_1+a_3)]$

Statistical analysis: The results of the experimental tests were presented as average ± SD. The two-tailed paired t-test was used to compare collagen fibre orientation along the axes. Significance was set at $p<0.05$.

Results

After the specimens are placed in the biaxial device, a minimal force has to be applied to obtain a perfectly planar position. So preload of 0.01 N was applied along both axes to record meaningful measurements. Since it was not possible to protect the samples from dehydration, some specimens did not provide any acceptable data for us to report. It should be carefully noted that in the calculation of stresses from force data, the average thicknesses for every sample are used. Naturally, this assumption may lead to additional error sources in the estimation of the constitutive

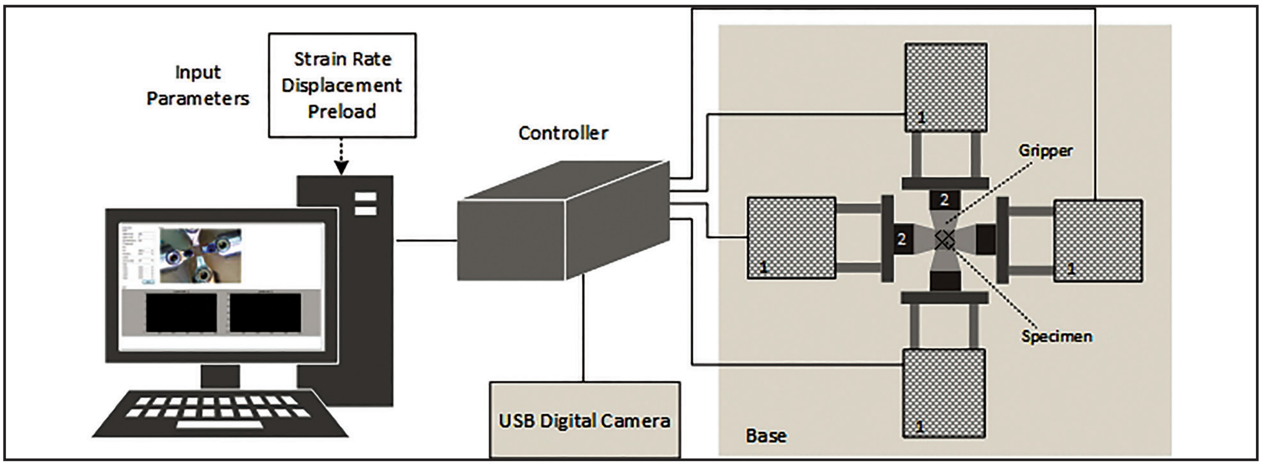

Figure 1. The biaxial test device.

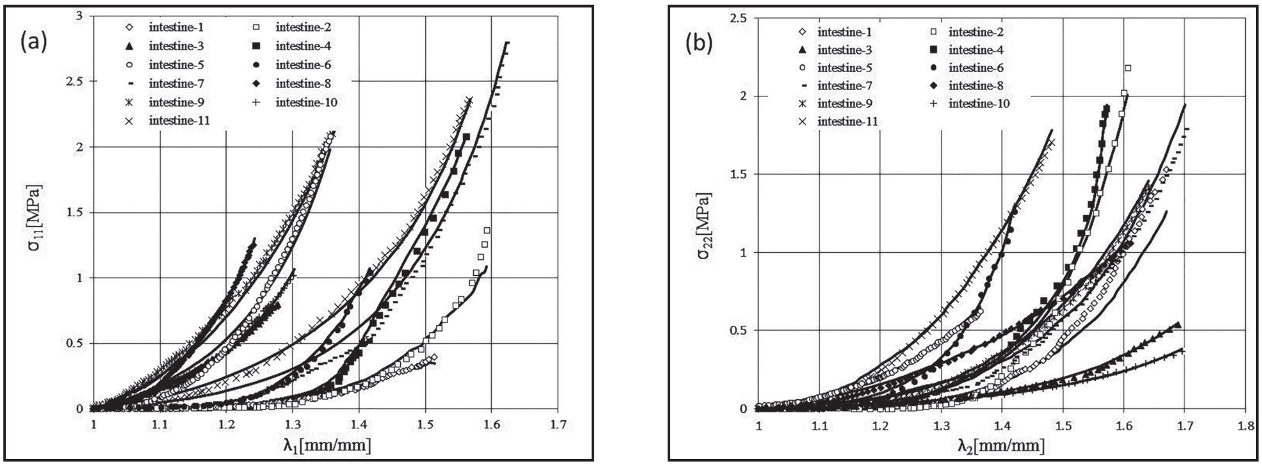

Figure 2. Stress-stretch data for sheep intestine fitted to the modified Mooney-Rivlin model. The left hand plots (a) show the stresses and stretches in the circumferential (11) direction while the right hand plots (b) show the stresses and stretches in the longitudinal (22) direction. Fitted modified Mooney-Rivlin models (solid lines) were superimposed over the raw data.

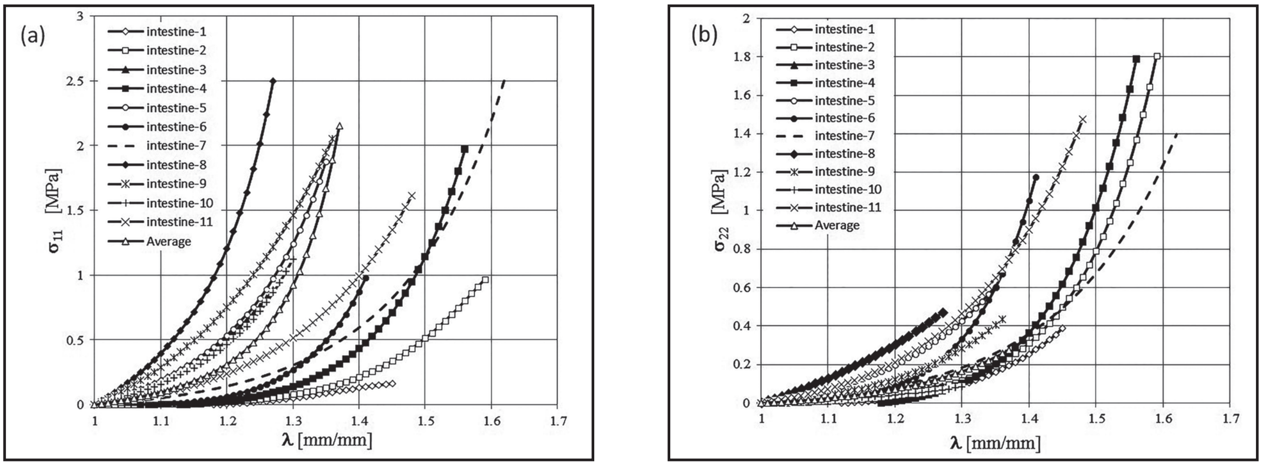

Figure 3. Stress-stretch curves obtained from the modified Mooney-Rivlin constants in circumferential (a) and axial (b) direc-tions ($\lambda 1 = \lambda 2 = \lambda$).

parameters. Average thickness of specimens was considered 0.7 mm. The plausible specimens exhibited stress-stretch curves which were derived from the Eqs. (1) and (2) which state that the experimental data were well-fitted to the Fung and modified Mooney-Rivlin

Table 1. Parameters for the two constitutive models.

Fung						
	c(MPa)	a1	a2	a3	Anisotropy	rms(MPa)
intestine-1	0.085	0.686	1.67	0.054	0.43	0.084
intestine-2	0.061	1.36	2.095	0.032	0.65	0.069
intestine-3	0.429	2.543	0.269	0.03	0.12	0.047
intestine-4	0.083	1.88	1.94	0.053	0.96	0.116
intestine-5	0.828	1.94	0.605	0.028	0.32	0.093
intestine-6	0.085	2.63	3.31	0.014	0.79	0.049
intestine-7	0.432	0.915	0.524	0.03	0.59	0.093
intestine-8	0.453	2.467	0.639	0.43	0.37	0.170
intestine-9	1.549	1.19	0.25	0.0076	0.22	0.082
intestine-10	0.644	1.897	0.126	0.027	0.08	0.019
intestine-11	0.812	0.815	0.804	0.05	0.98	0.112
Average	0.295	2.175	0.396	0.313	0.29	0.020
Modified Mooney-Rivlin						
	c1 (MPa)	D1 (MPa)	D2	k1 (MPa)	k2	rms(MPa)
intestine-1	-0.096	0.116	0.61	-0.099	1.3	0.066
intestine-2	-0.01	0.019	1.099	-0.01	1.02	0.088
intestine-3	-0.001	0.0595	0.6	0.33	0.52	0.015
intestine-4	-0.090	0.0422	0.99	0.014	0.3	0.049
intestine-5	0.001	0.2	0.64	0.22	0.97	0.079
intestine-6	-0.05	0.035	1.33	-0.016	1.2	0.03
intestine-7	-0.002	0.093	0.62	0.042	0.43	0.094
intestine-8	0.002	0.964	0.28	0.48	2.02	0.044
intestine-9	-0.07	0.241	0.54	0.49	0.07	0.032
intestine-10	0.008	0.042	0.57	0.27	1.06	0.017
intestine-11	-0.06	0.348	0.54	0.02	0.21	0.047
Average	0.0048	0.049	0.786	0.13	1.92	0.015

constitutive equations. According to calculated RMS error (Table1), the modified Mooney-Rivlin model approximately provides the best qualitative fit to the data. Figure 2 shows the stretch-stress curves obtained from the biaxial mechanical testing of all specimens in the circumferential (2-a) and in the axial (2-b) directions that were fitted to the constitutive modified Mooney-Rivlin model. It cannot be stated that the circumferential direction is consistently the stiffer (or less stiff) direction. But on average, the specimens act stiffer in the circumferential direction rather than the axial direction. Simultaneous loading in these two orthogonal directions also allowed us to conclude that the mechanical response in one direction is influenced by the characteristics in the other direction.

Detailed information on average model coefficients and anisotropy values are provided in Table 1. By using these material constants, stretch-stress curves were extracted and plotted in Figure 3. The mean biaxial stretch-stress curve for intestine was also obtained from the average modified Mooney-Rivlin

constants mentioned in Table 1 and plotted in Figure 3 as well.

Discussion

This study provides a complete set of experimental planar biaxial data for sheep intestine fitted to the Fung-type anisotropic model and the modified Mooney-Rivlin constitutive model to describe its mechanical characteristics.

The nonlinear thick-walled tubular theory (modified Mooney-Rivlin model) which could be used for FE analysis developed in this article, seems to be a realistic starting point in the simulation of intestine. These constitutive models demonstrated mechanical anisotropy and nonlinearity of the tissues and the data (Table 1) present some degree of anisotropy for intestine wall. Stiffer behavior in the longitudinal direction was mentioned in previous research from uniaxial tensile tests as well (Chong et al.,2005). Inflation tests on porcine small bowel (Hoeg et al.,2000) also showed stiffer characteristics in the circumferential direction. Indeed, it cannot be stated that the longitudinal direction is consistently the stiffer (or less stiff) direction. But on average, the specimens act stiffer in the circumferential direction rather than the axial direction. Overall variability in the direction of relative stiffness is in agreement with previous findings (Bellini et al., 2011; Terry et al., 2010). In Fung model the elevated values of parameter a1 than a2 (Tables 1) may be imparted by a preferred collagen fibre orientation along the circumferential axis, as certified for the extracellular matrix component of intestine tissue (Sokolis et all,2012).

All of the models presented in this study are nonlinear and anisotropic; in particular, the modified Mooney-Rivlin model is an anisotropic model which can be used for numerical analysis. Although the two models considered in this paper are phenomenological, they may be helpful as a research reference, offering good descriptive capability.

Conclusion: In this study the mechanical properties of sheep intestine have been determined by using biaxial mechanical device. The obtained stretch-stress curves from the experimental data were fitted to the four-parameter Fung-type model as well as the modified Mooney-Rivlin model, and the anisotropy values of the samples were calculated by the constitutive parameters. The samples showed some degree of the anisotropy. The curves, on average, also showed stiffer behavior in the circumferential direction than the longitudinal direction. To conclude, this paper can be the best reference for the mechanical investigations of sheep intestine tissue.

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References

1. Bathe, K.J. (1996) Finite Element Procedures, Englewood Cliffs. NJ: Prentice-Hall.
2. Bathe, K.J. (2007) Theory and Modeling Guide, Watertown, MA: ADINA and ADINA-F, ADINA R&D, Inc, Vol. I and II.
3. Bellini, C., Glass, P., Sitti, M., Di Martino, E.S. (2011) Biaxial mechanical modeling of the small intestine. J Mech Behav Biomed Mater. p.1727-1740.
4. Chong, C., Hung, H., Steinhart, A., Trexler, J.(2005) Design of a Biaxial Test Device for Compliant Tissue. Worcester Polytechnic Institute. 4: 3-44.
5. Egorov, V., Schastlivtsev, I., Prut, E., Baranov, A., Turusov, R.(2002) Mechanical properties of the human gastrointesti-nal tract. J Biomech. 35: 1417-1425.
6. Frøkjaer, J.B., Andersen, S.D., Drewes, A.M., Gregersen, H.(2006) Ultrasound-determined geometric and biomechanical properties of the human duodenum. Dig Dis Sci. 51: 1662-1669.
7. Fung, Y.C., Fronek, K., Patitucci, P. (1979) Pseudoelasticity of arteries and the choice of its mathematical expression. Am J Physiol. 237: 620-631.
8. Fung, Y.C. (1991) What are the residual stresses doing in our blood vessels?. Ann Biomed

Eng. 19: 237-249.

9. Liao, D., Zhao, J., Gregersen, H. (2010) 3D mechanical properties of the partially obstructed guinea pig small intestine. J Biomech. 43: 2079-2086.
10. Munday, J.S, Brennan, M.M, Jaber, A.M, kiupel, M. (2006) Ovine intestinal adenocarcinomas: histologic and phenotypic comparison with human colon cancer. J Comp Med (Memphis). 56: 136-41.
11. Holzapfel, G.A., Eberlein, R., Wriggers, P., Weizsacker, H. (1996) Large strain analysis of soft biological membranes: Formulation and finite element analysis. Comput Method Appl M. 132: 45-61.
12. Holzapfel, G.A., Gasser, T.C., Ogden, R.W. (2000) A new constitutive framework for arterial wall mechanics and a comparative study of material models. J Elasticity. 61: 1-48.
13. Holzapfel, G.A., Gasser, T.C., Ogden, R.W. (2004) Comparison of a multi-layer structural model for arterial walls with a Fung-type model, and issues of material stability. J Biomech ENG-T Asme. 126: 264-275.
14. Holzapfel, G.A. (2005) Similarities between soft biological tissues and rubber like materials. In: Constitutive Models for Rubber IV, A.A. Balkema Publishers. P.-E., Keri, L. (eds.). Balkema, Leiden. The Netherlands. p. 607-617.
15. Holzapfel, G.A., Sommer, G., Regitnig, P. (2004) Anisotropic mechanical properties of tissue components in human ath-erosclerotic plaques. J Biomech ENG-T Asme. 126: 657-665.
16. Humphrey, J.D., Strumpf, R.K., Yin, F.C.P. (1990) Determination of a constitutive relation for passive myocardium: A New Functional Form. J Biomech ENG-T Asme. 112: 333-339.
17. Humphrey, J.D., Strumpf, R.K., Yin, F.C.P. (1990) Determination of a constitutive relation for passive myocardium: II. Parameter Estimation. J Biomech ENG-T Asme. 112: 340-346. [19] Slatkin.(1999) Modeling and experiments for a class of robotic endoscopes. Ph.D. Thesis California Institute of Technology.
18. Sun, W., Sacks, M.S., Scott, M.J. (2003) Numerical simulations of the planar biaxial mechanical behavior of biological materials. Bioengineering Conference, Florida. p. 1-2.
19. Terry, B.S., Lyle, A.B., Schoen, J.A., Rentschler, M.E. (2010) Preliminary mechanical characterization of the small bowel for in vivo mobility. ASME International Mechanical Engineering Congress and Exposition, Vancouver, British Columbia, Canada.
20. Sokolis, D.P. (2012) Multiaxial mechanical behaviour of the passive ureteral wall: experimental study and mathematical characterisation. Computer methods in biomechanics and biomedical engineering. 15: 1145-1156.

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اندازه گیری رفتار مکانیکی روده کوچک گوسفند با استفاده از آزمون‌های تجربی

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چکیده

زمینه مطالعه: اطلاعات جامعی در مورد خواص مکانیکی روده گوسفند وجود ندارد. **هدف:** برای این منظور تعدادی آزمون‌های کشش دومحوری انجام شد و مدل بنیادی برای توصیف خواص مکانیکی بافت روده گوسفند استخراج شد. **روش کار:** یازده نمونه تازه گوسفندی تهیه شد و آزمون‌های مسطح دومحوری بر نمونه‌ها با اعمال همزمان نیرو در دو جهت طولی و پیرامونی انجام شد. سپس داده‌های اندازه‌گیری شده با مدل ناهمسانگرد چهار پارامتر فانگ و مدل مونی–ریولین بهینه شده برازش شدند. **نتایج:** داده‌ها درجه‌ای از ناهمسانگردی را نشان دادند. جهت سختی بافت قابل پیش بینی نبود. تعدادی از نمونه‌ها در جهت پیرامونی سخت تر بودند و بعضی‌ها در جهت طولی. میانگین نتایج نشان داد که جهت پیرامونی سخت تر می باشد. **نتیجه گیری نهایی:** می‌توان نتیجه گرفت که روده گوسفند به عنوان بافت ناهمسانگرد غیر خطی رفتار می کند که با مدل مونی–ریولین بهتر برازش می شود.

واژه‌های کلیدی: مدل بنیادی ناهمسانگرد، مدل فانگ، بافت غیر خطی، مدل مونی–ریولین بهینه شده، تابع انرژی کرنشی

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