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学位論文題目	Study of Local Stiffness Measurement of Soft Biological Tissues Using Pipette Aspiration Method （ピペット吸引法を使った生体軟組織の局所剛さ測定に関する研究）
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論　文　内　容　要　旨

Chapter 1 Introduction

Numerous efforts have been made to obtain the mechanical properties of biological soft tissues, because it can provide an information to diagnosis of disease, as well as fundamental understanding of the tissues. For example, studies on the arterial wall mechanics have played an important role in solving clinical problems in the cardiovascular system such as atherosclerosis, and been helpful for the development of arterial prostheses. The arterial walls show heterogeneity, nonlinear behavior in the stress-strain relationship, and anisotropy, which are due to the complication of the arrangement and structure of the structural components. Besides, it has been known that the atherosclerotic lesions develop locally and the degree of the progress depends on the location. Therefore, we need to employ a simple and reliable method to clarify the local mechanical properties of the soft tissues. Moreover, it will be desired to measure under the conditions as close to *in vivo* as possible.

Conventional experiments generally adopted to test the mechanical properties of the blood vessel walls include a pressure-diameter test with whole tubular specimens and a tensile test with rectangular and ring specimens. The results to be obtained will show the entirely averaged mechanical properties of the specimen. In addition, these tests have their limitations in specimen preparation. It is difficult to apply such conventional tests to the stiffness measurement of the walls due to the difficulty in cutting a specimen out from the walls and mounting it in the experimental apparatus. As an experiment without specimen excision, an indentation test has been used by a few researchers, in which the relationship between force applied by an indentor and depth of indentation was measured. However, the mechanical properties obtained in this test are compressive properties and are affected by not only the properties of the top layer but also those of the bottom layer.

The objective of this thesis is to propose and develop a novel pipette aspiration technique for measuring the local stiffness of the soft biological tissues. In the proposed technique,. averaged tensile properties over a local region adjacent to the pipette tip are obtained without cutting the specimen out from its surroundings, even in the *in vivo* conditions. The numerical and experimental studies were carried on six subjects in the followings.

Chapter 2 Finite Element Analysis Applied to the Pipette Aspiration Technique for the Local Stiffness Measurement of Soft Tissues

The applicability of the pipette aspiration technique to the stiffness measurement of the soft tissues was discussed through numerical simulations, in which the initial slope of the pressure-deformation curve is identified to estimate the Young's modulus of the soft tissues. The tissues were assumed to be isotropic, macroscopically homogeneous, and incompressive. The numerical simulations by the linear finite element analysis were performed for the axisymmetric model to survey the effects of friction at the tissue-pipette contact boundary, pipette cross-sectional geometry, relative size of the specimen to the pipette, and the layered inhomogeneity of the tissue. The friction at the contact region had little effect on the measurement of the Young's modulus. The configuration of the pipette was shown to affect the measurement for small pipette wall thickness. The measurement also depended on the relative size of the specimen to the pipette for relatively small specimens. The extent of the region contributing to the measurement was roughly twice the inside radius of the pipette. Experimental verification for the isotropic, homogeneous artificial material showed that the present method is effective for the measurement of the soft materials.

Chapter 3 Intramural Distribution of Local Elastic Moduli in Bovine Aortas

Blood vessel walls generally show a large deformation and a nonlinear behavior in their stress-strain relationships. These mechanical properties might be closely related with components of the vessel wall and also with the wall structure. Blood vessel walls mainly consist of collagen fibers, elastic fibers, and smooth muscle cells. These components have different mechanical properties and distribute nonuniformly in the wall. Thus, intramural mechanical properties may vary depending on the radial position in the wall. Most experiments previously adopted to test mechanical properties of blood vessel wall were, however, based on the assumption that the wall is homogeneous. Results to be obtained from such experiments show entirely averaged mechanical properties of specimen. For better understanding of blood vessel wall mechanics, we need to employ a new reliable method to clarify the local mechanical properties easily.

In this chapter, the pipette aspiration technique was adopted to measure local elastic moduli of bovine aortic walls. The local elastic moduli in the wall of bovine thoracic aortas were measured in axial, circumferential, and radial directions. After approaching the tip of a glass pipette (I.D.=0.8 mm) to sections perpendicular to each direction, negative pressure was applied into the pipette to aspirate the specimen surface. Aspirated volume was optically measured while decreasing the pressure from 0 to -100 mmHg to calculate elastic moduli. The local elastic moduli of bovine aortas were found to decrease significantly from the inner to the outer walls. Histological observation showed that the bovine thoracic aortas did not have concentric layered structure which is typically shown in textbooks for aortas. These results suggested that the nonuniform distribution of the elastic moduli may have close correlation with the histological nonuniformity.

Chapter 4 Intramural Distributions of Local Elastic Moduli in Porcine and Bovine Aortas and Their Relationships to Histology

The pipette aspiration method applying to porcine aortas to measure intramural distributions of local elastic moduli and histological image analysis were presented. Histological image analysis using color classification based on Mahalanobis' generalized distance was performed to quantitatively assess the correlation between elastic moduli and histology. In porcine aortas, the local elastic moduli were independent of the radial position of the wall. The porcine aortas had a typical layered structure. The area fraction values of the three structural components were almost uniform in the wall. These results were then compared with those in bovine aortas obtained in Chapter 3. In contrast, the elastic moduli of bovine aortas decreased significantly from the inner to the outer side in a wall. This mechanical nonuniformity corresponded with the decrease in the area fractions of collagen and elastic fibers from the inner to the outer side. The intramural mechanical properties in aortic walls were closely related to histological structure.

Chapter 5 Change in Local Elastic Moduli of Cholesterol-Fed Rabbit Aortas

Atherosclerosis is generally characterized by the formation of atheroma on the intima, which occurs primarily at bends, branching, and bifurcations of the arterial walls. It has been defined that atherosclerosis is a vascular disease accompanying accumulations of lipids and fibrous thickening of the intima, followed by the formation of atheroma, calcification and ulcer. The fatty streak region is a precursor of advanced lesions and fibrous plaques found in clinically significant atherosclerosis and characterized by an accumulation of cholesterol ester within foam cells.

For many years, atherosclerosis research has been dedicated to changes in mechanical properties of the arterial walls during the process of the development of atherosclerosis. However controversy still clouds the relevance of mechanical properties of arterial wall to the development of atherosclerosis. Some researches reported the increased elastic modulus with the development of atherosclerosis, while others reported the contrary data. One of the reasons for this inconsistency is that mechanical properties of atherosclerotic arteries have been evaluated mainly with global elastic moduli measured from conventional pressure-diameter test and tensile test of the whole vessel segment. From the viewpoint of local tissue degeneration, it is necessary to clarify the mechanical properties of atherosclerotic lesion themselves.

In this chapter, the practical study on atherosclerotic legions using the pipette aspiration method was described. The local mechanical properties of cholesterol-fed rabbit aortas were measured. The aortas were excised from New Zealand White rabbits fed diet containing 0.5% cholesterol and 0.5% olive oil for 33-41 weeks and fed normal diet. A part of the inner surface of small specimen of aortas was aspirated into the pipette. The elastic moduli obtained for aortic arch, thoracic and abdominal aortas from normal diet-fed rabbits did not show statistically significant differences among them and the average value was approximately 49 kPa. The elastic modulus of the fatty streaks of cholesterol-fed rabbit aorta, being approximately 37 kPa, was significantly smaller than that of the normal ones with the progression of atherosclerosis. It was confirmed that the pipette aspiration method would be useful in understanding the process of atherogenesis.

Chapter 6 Measurement of Anisotropic Properties of Soft Biological Tissues Using a Rectangular Cross-Sectional Pipette

Soft biological tissues are generally anisotropic. This anisotropy might closely correlate to heterogeneity in the tissue structure. Since the blood vessel walls are heterogeneous and the smooth muscle cells mainly orient circumferentially and longitudinally as noted previously, it can generally be expected that the elastic moduli in these directions will be larger than that in the radial direction. Canine ligamentum nuchae can be assumed to be unidirectionally reinforced material in which elastic fibers and collagen fibers align with its axial direction.

In Chapter 2, the pipette aspiration method using pipettes with circular cross-section has been developed to measure local elastic properties of soft biological tissues, in which linear numerical simulations were performed for the circular pipettes using an axisymmetric model that was assumed to be isotropic. However, for anisotropic materials, it is difficult to identify the accurate value of elastic moduli using the circular pipettes due to many independent material constants and the interference among material constants.

In this chapter, the use of rectangular cross-sectional pipettes to clarify the local anisotropic properties of soft biological tissues was described. Numerical simulations by using finite element analysis showed that if the aspect ratio of the rectangular cross section is more than 4, the rectangular cross-sectional pipettes can eliminate the effect of the modulus parallel to the major axis of the pipette cross section. The pipette aspiration method with the rectangular cross-sectional pipettes should be effective for clarifying the anisotropy of biological tissues. Experimental verification was performed for canine ligamentum nuchae, in which the elastic modulus in the longitudinal direction, *i.e.*, fiber direction, could be measured.

Chapter 7 Measurement of Nonlinear Elastic Properties of Porcine Aortic Walls under Biaxial Stretching

Under physiological conditions, rabbit descending thoracic aortas are stretched by about 35% in the axial direction, and stretched by about 70% in the circumferential direction from the state at 0 mmHg due to the mean blood pressure of 100 mmHg (=13.3 kPa). And also, cyclic strains of about 10% and 0.8% are added to the circumferential and longitudinal directions due to the pulse pressure for the canine descending thoracic aorta, respectively. Since the blood vessel walls generally exhibit large deformation under physiological conditions and nonlinear behavior in the stress-strain relationship, it is very important to predict their nonlinear mechanical behavior accurately, in designing vascular prosthetics and in understanding disease processes such as atherosclerosis.

As discussed in the previous chapters, the elastic modulus obtained by the pipette aspiration method was an initial value of the modulus, *i.e.*, Young's modulus on the assumption of linear deformation. In this chapter, a pipette aspiration technique under biaxial stretching was proposed for the measurement of nonlinear elastic properties of soft biological tissues. To validate this method, an inner surface of porcine aortas was aspirated with a glass pipette while stretching the specimen in axial and circumferential directions. Pressure-diameter test is also performed to compare the elastic modulus obtained from the present method. The elastic modulus obtained from the pipette aspiration test agreed well with incremental elastic modulus obtained from a pressure-diameter test of the same specimen in the lower range of circumferential stretch ratio than 1.3. This range corresponds to mean stretch ratio of porcine aortas under physiological conditions. In addition, the use of a rectangular cross-sectional pipettewas also discussed to clarify directional difference.

Chapter 8 Conclusions and Recommendations

The pipette aspiration method developed in this thesis would be much more effective tool for the determination of local mechanical properties of soft biological tissues. By understanding the local mechanics of the tissues, prediction of disease such as atherosclerosis may become feasible.

The present work has provided some fundamental understanding of the soft biological tissues, as well as valuable insight of the cause of a disease. Further study would be, however, required since it is expected that more promising applications become available even in the *in vivo* conditions. Some future research directions were indicated, such as in miniaturizing and integrating the pipette aspiration system by using the micro machining technology.

審査結果の要旨

近年、バイオメカニクスの観点から生体軟組織の局所的な力学特性を測定する研究が盛んに行われている。例えば、動脈壁の局所力学特性は心臓血管系の基本的な病態生理の理解や、動脈硬化症のメカニズムの解明において重要である。しかし、引張試験などの従来法では局所の力学特性を求めることは非常に困難であり、簡便で信頼性の高い新たな測定法が望まれている。本論文は、生体軟組織の局所力学特性の新たな測定法としてピペット吸引法を提案したものであり、全編8章よりなる。

第1章は序論であり、本研究の背景及び目的を述べている。

第2章では、ピペット吸引法の有効性を確認するため有限要素法による数値計算を行い、測定時の諸条件が測定結果に与える影響を詳細に検討し多くの知見を得ている。

第3章では、本法をウシ大動脈壁に適用し壁内の局所弾性率分布を求めている。弾性率は内壁側から外壁側に向かって有意に低下することを見出している。また組織像との比較から、力学特性の不均一性は組織像の不均質性に密接に関連していることを示唆している。このような詳細な壁内弾性率分布はこれまでに報告がなく、貴重な知見である。

第4章では、壁内弾性率分布測定のブタ大動脈への適用事例について述べている。ブタ大動脈では弾性率は壁内でほぼ一定であり、組織像の画像解析から壁構成要素の面積割合も壁内でほぼ一定であることを示している。一方、ウシ大動脈では壁を構成する各要素の面積割合が一定ではないことを明らかにしている。以上の結果は血管壁のミクロな力学環境を理解する上で重要な成果である。

第5章では、コレステロール食を負荷したウサギ大動脈壁において、動脈硬化の進展に伴う壁の局所力学特性の変化を測定している。病変部の弾性率は正常部に比べて有意に低下することを明らかにしている。これは動脈硬化症のメカニズムの解明の上で有用な成果である。

第6章では、異方性弾性率の測定法として、矩形断面を有するピペットを提案しその有効性を検討している。イヌ項靭帯を対象に実験を行い、靭帯軸方向の高い弾性率を選択的に測定できることを実証している。これは極めて独創的な成果である。

第7章では、大変形時の弾性率を求めるため、予め二軸方向に引張を加えた場合のピペット吸引法について述べている。得られた弾性率は内圧ー外径試験で求めた増分弾性係数と生理的ひずみ状態までよく一致することを示している。これは実用上有用な成果である。

第8章は結論であり、本研究で得られた成果を総括している。

以上要するに本論文は、生体軟組織の局所剛さの新たな測定法としてピペット吸引法を提案し、数値計算を用いてその有効性を検証すると共に、実際の生体材料への適用事例を通じて軟組織の局所力学特性を明らかにしたもので、機械工学ならびに生体工学の発展に寄与するところが少なくない。

よって、本論文は博士（工学）の学位論文として合格と認める。