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Histological observation for Needle-Tissue Interactions

Yoshiyuki NAKAGAWA, Yoshihiko KOSEKI

Abstract— **We histologically investigated tissue fractures and deformations caused by ex vivo needle insertions. The tissue was formalin-fixed while the needle remained in the tissue. Following removal of the needle, the tissue was microtomed, stained, and observed microscopically. This method enabled observations of cellular and tissular conditions where deformations caused by needle insertions were approximately preserved. For this study, our novel method presents preliminary findings related with tissue fractures and the orientation of needle blade relative to muscle fibers. When the needle blade was perpendicular to the muscle fiber, transfiber fractures and relatively large longitudinal deformations occurred. When the needle blade was parallel to the muscle fiber, interfiber fractures and relatively small longitudinal deformations occurred. This made a significant difference in the resistance force of the needle insertions.**

I. Introduction

Hypodermic needles have been routinely used for blood sampling, dialysis, drug administration, and many other vascular access applications. This is because of the hypodermic needle's minimal invasiveness, low manufacturing costs, rapid and easy access to subcutaneous tissue (mainly veins) of patients [1]. However, most patients have suffered pain and stress associated with mechanical damage caused by needle insertion.

Egekvist studied the perception of the pain highly depends on mechanical injury of the tissue [2]. Kataoka [3] and Okamura [4] studied a force model of needle insertion, and proposed that the resistance force could be classified into tissue deformation, tissue friction, and tissue cutting. Many researchers also have studied the major mechanical factors of needle insertion, such as needle diameter [5], shape of needle [6], the natural bending action of a needle [7], ruptures caused by needle insertion [8].

In order to identify contributions of the major mechanical factors to mechanical injuries of tissue, and to produce a numerically precise model of needle insertion, many researchers have utilized novel methods to observe needle insertion. Misra [7] observed the interaction within gel for the naturally bending action of the needle and proved that microscopic and macroscopic observation of needle interactions within the gel influenced the model of needle insertion. Shergold [9] investigated the penetration mechanism of a soft solid, examining its dependence upon punch tip geometry. This was done by observing the penetration crack on human skin and silicon rubber and pointed out that the skin is more complex due to the fact that skin is layered, orthotropic and heterogeneous.

In order to clarify intra-tissular fractures and deformations of biological material caused by needle-tissue interactions, we histologically observed the tissue around the needle trajectory. While the needle remained in the tissue, the tissue was formalin-fixed to preserve the tissue deformation caused by needle insertion. Following removal of the needle, the tissue was microtomed, and then multiple cross-sections in an arbitrary direction were intermittently obtained. Appropriate staining could enhance the biological features of tissue fractures occuring around needle trajectory. In addition, appropriate magnification enabled observation of the cellular and tissular conditions where deformations caused by needle insertion were approximately preserved.

In this paper, we firstly introduced our method to histologically observe a needle insertion into ex vivo muscle tissue. Through this observation method, we preliminary discuss how the fracture mechanism in anisotropic tissue, like muscle tissue is different in cases where the orientation of the needle blade relative to the muscle fiber is different. We also discuss how the resistance force of the needle insertion is different in these cases. The contributions of this paper are observations of needle-tissue interactions inside that biological material and findings of different fracture mechanisms in anisotropic biological material.

II. Materials and Methods

A needle was inserted into muscle tissue ex vivo when the blade of the needle is parallel and perpendicular to the fiber of the muscle, respectively. The orientation of the blade to the fiber was defined as shown in Fig. 3 (a)-perpendicular puncture and Fig. 4 (a)-parallel puncture. The resistance force on the needle was measured during insertion. Multi-directional slices of the muscle tissue were histologically prepared to observe spatial deformations inside the tissue.

Fig. 1 shows the experimental setup for needle insertion. The outer diameter of the needle was 1.25mm (18G) and the bevel angle of the needle tip was 16 degree. The needle used was similar to commercially available hypodermic needles except that it had no hollow to avoid complexities caused by the hollow. The tissue was skinless, commercially available fresh chicken breast trimmed into approximately 15×15×20mm.

The resistance force was measured by a load cell (LMC-6970, Nissho Electric Works Co., Ltd.) attached to the back end of the needle. The load cell had a 50N rated load, ±0.5% nonlinearity, ±0.05% sensitivity, and 50Hz sampling. The load cell was mounted on a linear stage (NLT-0610X-B, Sigma Koki Co., Ltd.). The needle was firstly positioned 5mm above the surface of the tissue and driven down 15mm at a constant velocity of 5 mm/sec.

Yoshiyuki NAKAGAWA is with Togo Medikit Co.,ltd., Hyuga, Japan (e-mail: yoshiyuki.nakagawa@togomedikit.co.jp).

Yoshihiko KOSEKI is with Human Technology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan (phone: +81-29-861-7845; e-mail: koseki-y@aist.go.jp).

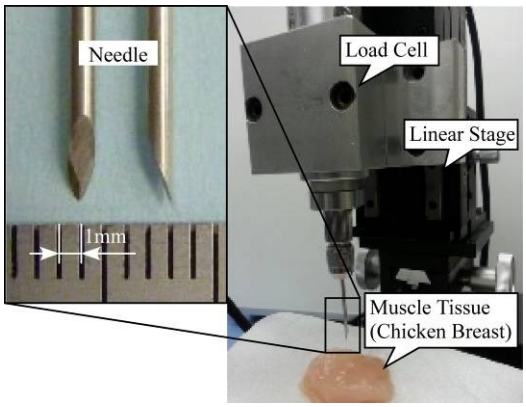

Figure 1 Experimental setup for needle insertion into muscle tissue: a beveled 18G needle was moved into a chicken breast by a linear stage, a resistance of needle insertion was measured with a load cell.

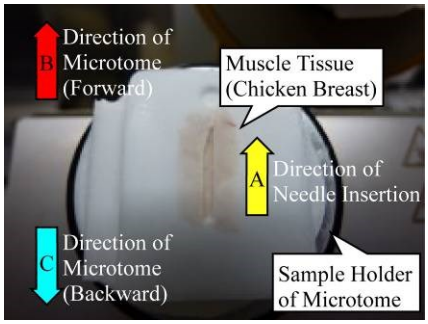

Figure 2 Setup for microtome: a muscle tissue was sliced with frozen sectioning in forward direction to that of needle insertion.

The tissue was fixed with 10% neutral buffered formalin immediately after puncturing, while the needle remained in the tissue. Following 24 hour fixation, the needle was removed, and the tissue was dewatered with saccharose.

As shown in Fig. 2, the tissue was rapidly frozen in liquid nitrogen, and microtomed into approximately 10 micrometers thick slices with cryomicrotome (HM 550 Cryostats, Thermo Fisher Scientific Inc.). Three samples were prepared for each condition and microtomed in three orthogonal directions, respectively, in order to provide 3-dimensional understanding of tissue deformation. 4 slices, (S1±, S2±, S3±, and S4±) as shown in Fig. 3 (a) and Fig. 4 (a), were obtained finally for each condition. According to preliminary tests, no significant differences were found when the tissue was microtomed forward (Arrow B in Fig. 2) and backward (Arrow C in Fig. 2) against the direction of the needle insertion (Arrow A in Fig. 2). Since freezing and cutting artifacts (ice cracks, nicks and folds) were unavoidable, several samples and slices were prepared for each condition until slices, whose artifacts were sufficiently few to observe tissue fracture and deformation were obtained.

The tissue was stained with hematoxylin-eosin and was observed using an optical microscope (OLYMPUS, CK2, ×40). A high-resolution and large-sized image including the whole needle trajectory (approximately, 10×15mm, 10k×15k pixels, and 1 micrometer resolution) were produced by merging small-sized images using an image stitching procedure (PhotomergeTM, Adobe, Photoshop CS6). This image was compared with a low-resolution (1200dpi) image directly obtained by an optical scanner (CanoScan LiDE 210, Canon Inc), and it was confirmed that the artifact of image stitching was smaller than that of micotomy.

III. Results and Discussions

A. Observation of tissue section

Fig. 3 and Fig. 4 show the tissue slices when the blade of the needle were perpendicular and parallel to the fiber of the muscle, respectively. Fig. 3 (a) and Fig. 4 (a) approximately show position and orientation of slices (S1±, S2±, S3±, and S4±, plus and minus represent perpendicular and parallel punctures, respectively) relative to the needle's blade. Fig. 3 (b) and Fig. 4 (b) show slices (S1±) which were perpendicular to the blade and included the long axis of the needle. Fig. 3 (c) and Fig. 4 (c) show slices (S2±) which were parallel to the blade and included the long axis of the needle. Fig. 3 (d), (e), Fig. 4 (d), and (e) show slices (S3± and S4±), which were perpendicular to the long axis of the needle and sectioned the blade of the needle (S3±) and the body of the needle (S4±).

When the needle blade was perpendicular to the muscle fiber, the fracture mechanism was deduced as shown in Fig.3 (f). The tip of the blade sheared the muscle fiber, and caused transfiber fractures as shown in Fig. 5 (b). The bevel of the needle might push the tissue up to above the surface of the bevel, but it was not clearly observed. The shear friction deformed the fibers in the longitudinal direction of the needle insertion as indicated by the Area A and the Arrows B.

When the needle blade was parallel to the muscle fiber, the fracture mechanism was deduced as shown in Fig. 4 (f). The tip of the blade split the muscle fibers, and caused interfiber fractures as shown in Fig. 5 (c). The bevel of the needle and the side of needle expanded the fracture surface and wedged the surrounding tissue as indicated by the Area C and the Lines D. With this process, some fibers could be stretched and cause tensile fractures as shown in Fig. 5 (a), but it was not clearly observed. The shear friction deformed the fibers in the longitudinal direction of the needle insertion, but the longitudinal deformation occurred in parallel punctures, appearing to occur less than perpendicular punctures. This result was a hypothesis based on the few observations collected in this study.

B. Needle resistance force

The resistance forces of needle insertion were measured under conditions of perpendicular puncture and parallel puncture. 15 trials were done for each condition. Fig. 6 shows the averages (bar) and standard deviations (error bars) of maximum resistance forces during perpendicular punctures (Fig. 3) and parallel punctures (Fig. 4). As a result, the parallel punctures were statistically significantly smaller than perpendicular punctures ($p < 0.01$ by two-sample t-test at a significance level of 1%).

As pointed out in [3] and [4], the resistance force of needle insertion is composed of fracture (cutting), deformation, and shear friction. In the case of perpendicular punctures, transfiber fractures and relatively large longitudinal deformations were observed, and in the case of parallel punctures, interfiber fractures and relatively small longitudinal deformations were observed. These results are supported by the general fact that transfiber fractures require more energy than interfiber fractures.

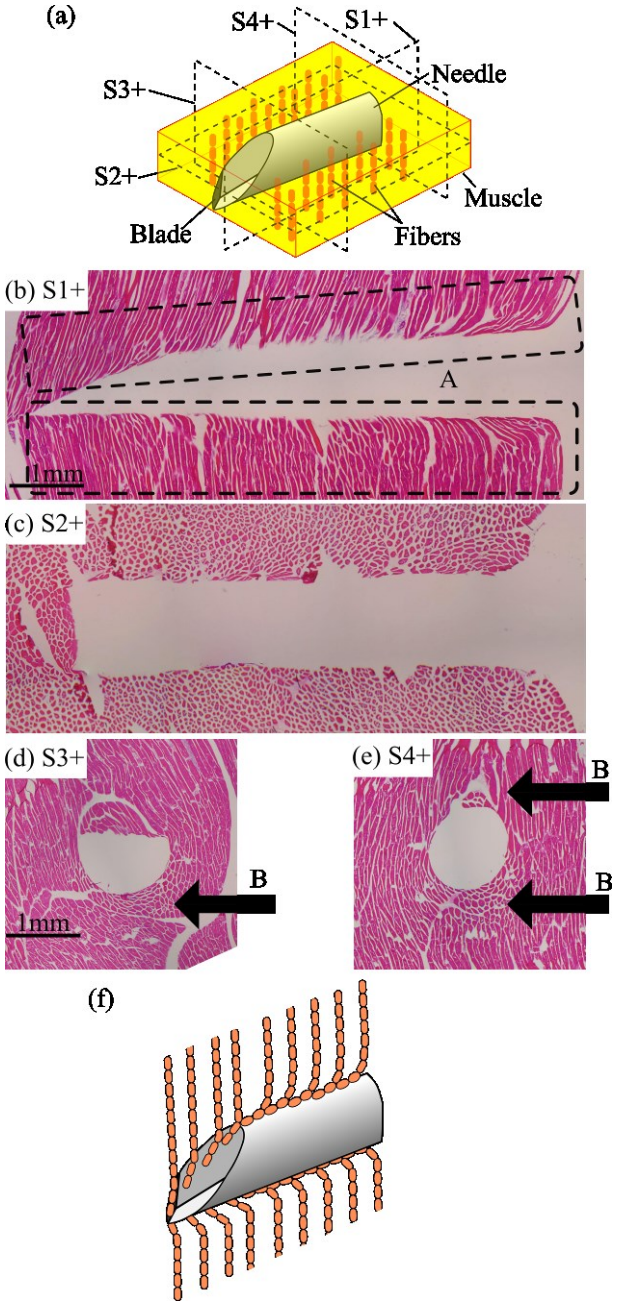

Figure 3 Setup, result, and discussion of perpendicular puncture: (a) approximately shows positions and orientations of slices relative to the needle's blade. (b) shows slice which were perpendicular to the blade and included the long axis of the needle. (c) shows slice which were parallel to the blade and included the long axis of the needle. (d) shows slice which was perpendicular to the long axis of the needle and sectioned the blade of the needle. (e) shows slices, which was perpendicular to the long axis of the needle and sectioned the body of the needle. (f) shows deduced fracture and deformation of perpendicular puncture: When a needle was inserted to a muscle keeping the needle blade perpendicular to the muscle fiber, the tip of the blade sheared the muscle fiber, and caused transfiber fractures as shown in Fig. 5 (b). The bevel of the needle might push the tissue up to above the surface of the bevel, but it was not clearly observed. The shear friction deformed the fibers in the longitudinal direction of the needle insertion as indicated by the Area A and the Arrows B.

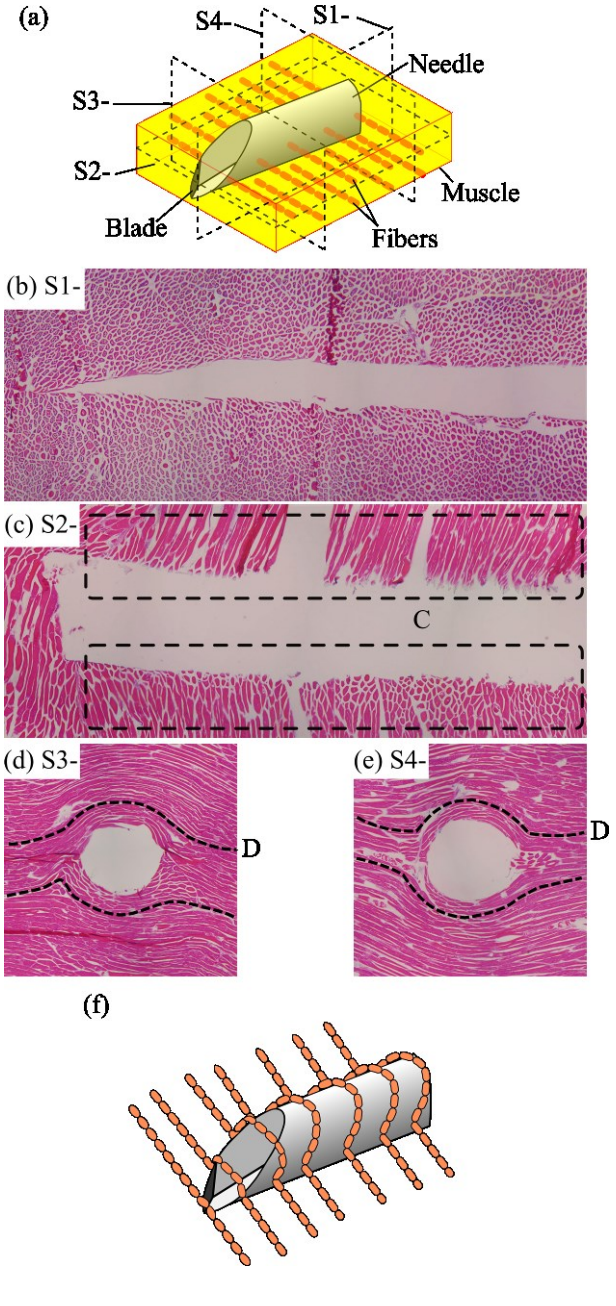

Figure 4 Setup, result, and discussion of parallel puncture: (a) approximately show position and orientation of slice relative to the needle's blade. (b) shows slice which were perpendicular to the blade and included the long axis of the needle. (c) shows slice which were parallel to the blade and included the long axis of the needle. (d) shows slice which was perpendicular to the long axis of the needle and sectioned the blade of the needle. (e) shows slices, which was perpendicular to the long axis of the needle and sectioned the body of the needle. (f) shows deduced fracture mechanism of parallel puncture: When a needle was inserted to a muscle keeping the needle blade parallel to the muscle fiber, the tip of the blade split the muscle fibers, and caused interfiber fractures as shown in Fig. 5 (c). The bevel of the needle and the side of needle expanded the fracture surface and wedged the surrounding tissue as indicated by the Area C and the Lines D. With this process, some fibers could be stretched and cause tensile fractures as shown in Fig. 5 (a), but it was not clearly observed. The shear friction deformed the fibers in the longitudinal direction of the needle insertion, but the longitudinal deformation occurred in parallel punctures, appearing to occur less than perpendicular punctures.

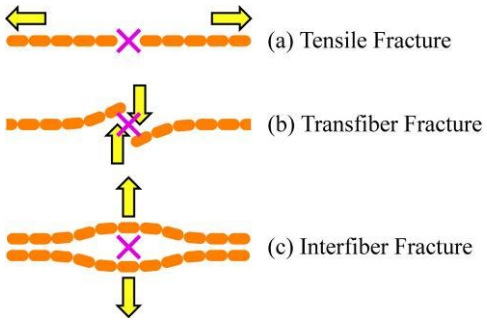

Figure 5 Major fractures occur in fibrous materials. (a) tensile fracture: the fiber stretched in longitudinal direction, (b) transfiber fracture: the fiber sheared in transversal direction, (c) interfiber fracture: the fibers split in transversal direction.

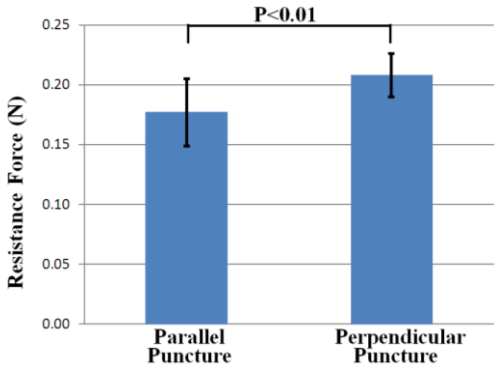

Figure 6 Maximum of resistance force of perpendicular puncture and parallel puncture (error bars extend one standard deviation in each direction): Resistance force of parallel puncture was statistically significantly smaller than that of perpendicular puncture.

IV. Conclusions and Future Works

This paper presented histological observations of intra-tissular fractures and deformations of ex vivo muscle tissue caused when a needle was inserted parallel and perpendicular to the muscle fiber. The observations revealed that when the blade is perpendicular to the muscle fiber, transfiber fractures and relatively large longitudinal deformations occurred and when the blade is parallel to the muscle fiber, interfiber fractures and relatively small longitudinal deformations occurred. In addition, the resistance forces of needle insertion were measured in both conditions. The resistance force when the blade is perpendicular to the muscle fiber was larger than that when the blade is parallel to the muscle fiber. This finding is supported by the previous observations.

The histological observations presented in this paper could be a powerful method to establish and prove the model of needle insertion into biological materials. This method enabled observations of cellular and tissular conditions where the deformations caused by needle insertion were approximately preserved. This method also enables a three-dimensional understanding of tissue fracture and deformation by selecting multiple slices and multiple orientations. Although it is not sufficiently investigated in this paper, appropriate staining could enhance the biological feature of tissue fracture around needle trajectory.

The authors are expecting that histological observations will be used for quantitative evaluations of damage cause by needle insertions. Histological observations will be also used for numerical modeling of fracture modeling for needle insertion simulation.

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References

[1] N. Abolhassani, M. Moallem, "Needle insertion into soft tissue: a survey", Medical Engineering & Physics, Vol.29, Issue 4, pp. 413–431, 2007

[2] H. Egekvist, P. Bjerring, L. Arendt-Nielsen, "Pain and mechanical injury of human skin following needle insertions", European Journal of Pain, Vol.3, Issue1, pp.41-49, 1999.

[3] H. Kataoka, T. Washio, M. Audette, K. Mizuhara, A Model for Relations Between Needle Deflection, Force, and Thickness on Needle Penetration, Medical Image Computing and Computer-Assisted Intervention - MICCAI 2001, Lecture Notes in Computer Science, Vol. 2208, pp. 966-974, 2001

[4] A.M. Okamura, C. Simone, M.D. O'leary, "Force Modeling for Needle Insertion into Soft Tissue", IEEE Trans. on Biomedical Engineering, Vol.51, pp.1707–1716, 2004.

[5] H.S. Gill, and M.R Prausnitz, "Does needle size matter?" J. Diabetes Science Technology, Vol.1, pp.725–729, 2007

[6] T.K. Podder1, D.P. Clark, J. Sherman, D. Fuller, E.M. Messing, D.J. Rubens, J.G. Strang, Y.D. Zhang, W. O'Dell, W.S. Ng, Y. Yu, " Effects of Tip Geometry of Surgical Needles: An Assessment of Force and Deflection", Third European Medical and Biological Engineering Conference EMBEC'05, Prague, Czech Republic, pp.1641–1644, 2005.

[7] S. Misra, K. Reed, B. Schafer, K. Ramesh, A.M. Okamura, "Observations and models for needle-tissue interactions", IEEE International Conference on Robotics and Automation (ICRA), pp.2687-2692, 2009.

[8] M. Mahvash, P.E. Dupont, "Mechanics of dynamic needle insertion into a biological material", IEEE Trans Biomedical Engineering, Vol.57, No.4, pp.934–943, 2010

[9] O.A. Shergold, N.A. Fleck, "Experimental investigation into the deep penetration of soft solids by sharp and blunt punches, with application to the piercing of skin", Trans ASME, Journal of Biomechanical Engineering, Vol.127, pp.838-848, 2005