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Quantitative analysis of gliding between subcutaneous tissue and the vastus lateralis – Influence of the dense connective tissue of the myofascia

Kengo Kawanishi [a, b], Shintarou Kudo [a, c, *]

[a] Graduate School of Health Sciences, Morinomiya University of Medical Sciences, Japan
[b] Department of Rehabilitation, Kano General Hospital, Japan
[c] Department of Physical Therapy, Morinomiya University of Medical Sciences, Japan

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A B S T R A C T

Introduction: The thickness of connective tissue has been shown to be associated with pain (Stecco et al., 2014). However, the relationship between fascial thickness and gliding remains unclear. In addition, the influence between gliding and the motion rhythm and limb position isn't clear.
Method: A therapist moved patient's lower leg at one of two constant rhythms (40 or 60bpm). Gliding of both the vastus lateralis (VL) muscle and subcutaneous (SC) tissue were recorded during knee motion using ultrasonography. Particle image velocimetry analysis software was adapted to create the flow velocity from echo imaging. Gliding was calculated using a coefficient of correlation from each flow velocity. Myofascial thickness and SC were measured using Image-J. The ratios of the loose connective tissue (LCT) and dense connective tissue (DCT) thickness to the total myofascial thickness were calculated. Repeated-measures two-way ANOVA was used to compare the two motion rhythms and three positions, with stepwise multiple regression analysis to analyze the predictors that influenced the gliding coefficient at each rhythm.
Results: Repeated-measures two-way ANOVA showed that the effect of rhythm was statistically significant, but the effect of position was not. At a 40 bpm rhythm, stepwise multiple regression analysis selected SC thickness and DCT thickness as significant factors, while at a 60 bpm rhythm, SC thickness and DCT ratio were selected.
Conclusion: This study revealed that increased thickness of DCT of the myofascia and SC resulted in decreased gliding between the VL and SC, demonstrating that gliding is related to fascial thickness. Motion rhythm influences gliding between tissues.

1. Introduction

Many patients with a femoral trochanter fracture experience lateral femoral pain after treatment by open reduction or open reduction with internal fixation using a gamma nail. In these cases, femoral pain occurs not only in the proximal femoral part associated with the surgical wound, but also in the middle half of the thigh. Structures present in the region of the femoral pain include the vastus lateralis muscle (VL) and the ilio-tibial band (ITB) which is the thicker part of the myofascia of the lateral thigh. The gluteus maximus and the VL muscle attaching to the ITB contract under a load such as during gait. The deep fascia of the thigh is pulled in the proximal direction by contraction of the gluteus maximus (Schleips et al., 2012), and in the distal direction by contraction of the VL (Schleips et al., 2012). It is reported that a histological change of the connective tissue occurs as a result of surgery, causing increased firmness (Okita, 2014). Because a histological change of the connective tissue causes excessive muscular fasciae transmission, lateral femoral pain may result. In other words it is thought that the histological changes may be a cause of decreased gliding between tissues. However, no adequate method of quantifying gliding between tissues has been established. In particular there is no landmark for the lateral part of the thigh. Therefore we applied the

* Corresponding author. 1-26-16, Nankokita Suminoe ward, Osaka, 559-8611, Japan.
E-mail addresses: riverwest1004@yahoo.co.jp (K. Kawanishi), kudo@morinomiya-u.ac.jp (S. Kudo).

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Particle Image Velocimetry assay and tried to obtain quantified results. Hyaluronan has been postulated to play a prominent role in the gliding function between fascia and muscle and between the different fascial sublayers (Stecco et al., 2011). Many hyaluronic acids (HA) are distributed in loose connective tissue (LCT) (Stecco et al., 2011), which is an important reservoir of water and ions for surrounding tissues (Stecco et al., 2011). It is believed that the viscous increase in LCT causes a reduction in gliding between the layers of collagen fibers of the deep fasciae (Stecco et al., 2014). This viscosity depends on speed of movement. It is reported that, at a fast speed, gliding increases based on a principle similar to hydroplaning. Therefore, it is necessary to investigate the influence of motion rhythms to quantify gliding (Roman et al., 2013). In addition, it has been suggested that this increase in viscosity causes pain (Stecco et al., 2014). It is reported that the thickness of connective tissue, including LCT, is associated with low back pain and neck pain (Stecco et al., 2014). However, the relationship between fascial thickness and gliding remains unclear, and requires investigation. The hardness of the VL or the ITB in this region often increases, and this increase coincides with the appearance of lateral femoral pain. From this, it is predicted that the change of tissue hardness of the lateral part of the thigh may affect gliding. Compared to the normal one-leg standing condition, ITB stiffness was significantly increased in the contralateral pelvic dropped position (i.e. hip adduction) (Tateuchi et al., 2016). In other words differences in the hip joint in relation to limb position change the tissue hardness of the lateral part of the thigh. Therefore, it is necessary to examine gliding from the viewpoint of differences of joint position to investigate whether tissue hardness has any influence on gliding. This study investigated the influence of motion rhythm and limb position. In addition, we decided to examine the association between gliding-related coefficient and connective tissue thickness.

2. Methods

Twenty-four limbs of 12 normal volunteers (nine men, three women: aged 27.1 ± 3.0) participated in this study. Exclusion criteria included the presence of disease of any joint in the lower extremity/spinal joints and neurological disease. Three limbs of 3 volunteers that matched the exclusion criteria (ankle ligament injury, medial meniscus injury, and medial collateral ligament injury) were excluded and Twenty-one limbs of 12 normal volunteers participated in this study.

All subjects provided informed consent and the protocol was approved by the Ethics Committee of Morinomiya University of Medical Sciences (authorization number:2017-015).

Sonography was performed using a Toshiba Medical Aplio500 and 12 MHz linear probe (PLT1204ST). The gain, dynamic range and frame rate were kept constant throughout all measurements and did not change between participants. In addition, a focus prescribed ITB. A 12 MHz transducer was fixed on the lateral side of the thigh using our original fixation device (Fig. 1). Accuracy of the original fixation device was verified using a three-dimensional analyzer (Vicon MX; Vicon, Inc., Oxford, UK) with an infrared camera (Vicon Vero) and digital video camera (Vicon View). As a result, the accuracy of the original fixation device was good. Subjects were positioned relaxed and lying on their side (hip adduction/hip intermediate/hip abduction) (Fig. 2). One physical therapist moved the patient's shank between 10 and 100 degrees of knee flexion at one of two constant rhythms (40 or 60 bpm) with the guidance of a digital metronome. Gliding of both the VL and its superficial subcutaneous tissue occurred during this motion task.

Particle image velocimetry (PIV) is a quantitative flow visualization tool developed to measure fluid velocities over a wide range of lengths and time scales. The present analytical method employed the successive abandonment method to determine relative movement between adjacent frames in sequences of US images (flow PIV fluid measurement software: Library Co. Ltd., Tokyo, Japan). Based on an ultrasonogram moving image, two regions of interest (ROIs) were placed on the VL and its superficial SC (settings of the PIV were: tracking pixels 15 × 21; two frame intervals) (Fig. 3). Flow velocity was arranged according to time-series data (Fig. 4). The gliding was used to calculate a coefficient of correlation from each time-series dataset (Fig. 5). This gliding-related coefficient was calculated using the mean of three trials. In cases with a high gliding coefficient, the results showed that among tissues that moved together, gliding appeared to be decreased. In addition, it showed that these tissues each became independent when the coefficient of correlation was low and gliding appeared to be increased. To investigate the velocity change in the two ROIs according to the change in rhythm, flow velocity was arranged into a time-series dataset for each rhythm. The peak value and the mean velocity were calculated from each time-series dataset, using the mean of three repeats. The reliability of gliding among tissues was examined using intraclass correlation coefficients (ICC), and the standard error of the mean (SEM) was calculated. We used Image J software (National Institutes of Health, Bethesda, MD, USA) for imaging analysis. The measurement image was converted into a static image based on the change that it caused in the hip joint middle position. Total myofascial thickness, being the total thickness of LCT which was defined as the low-echo layer in the myofascia, and DCT thickness which was defined as the high-echo layer in the myofascia, were measured using Image J. The ratios of the LCT and DCT thickness to the total myofascial thickness were then calculated to give the LCT thickness ratio and DCT thickness ratio,

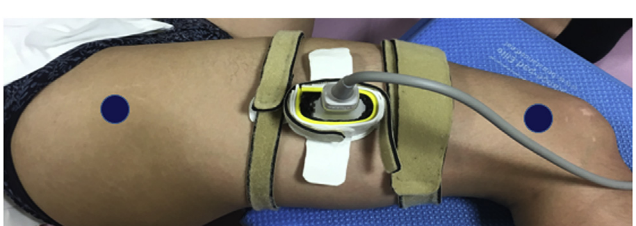

Fig. 1. Original fixation device. A 12 MHz transducer was fixed on the lateral side of the thigh using our original fixation device.

Fig. 2. Hip joint position. (a) Hip adduction (b) Hip intermediate (c) Hip abduction.

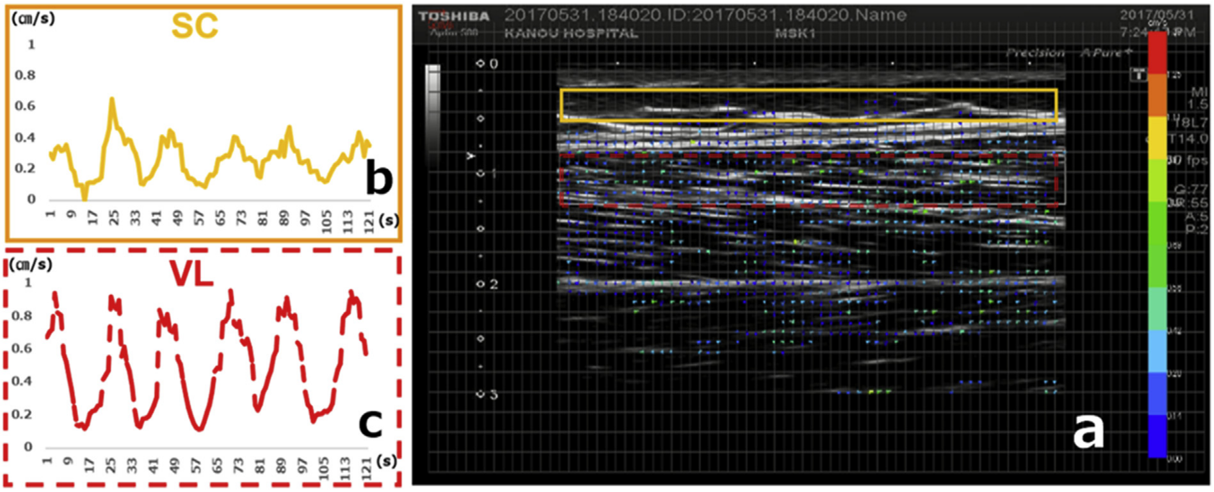

Fig. 3. Flow PIV fluid measurement software. (a) Based on an ultrasonogram moving image, two ROIs were placed on the VL and its superficial subcutaneous tissue (b) Time series data of SC (c) Time series data of the VL
Abbreviation: ROIs, regions of interest. VL: Vastus lateralis. SC: Subcutaneous tissue.

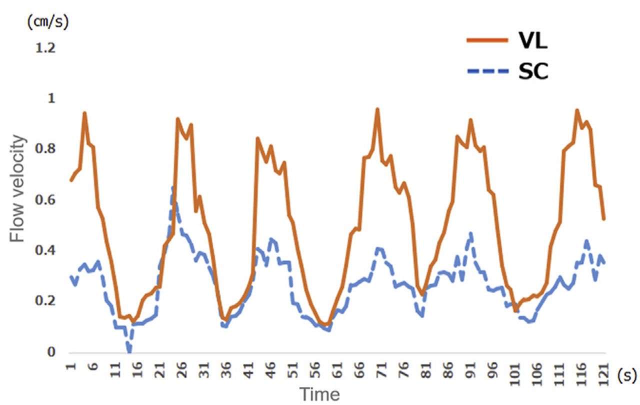

Fig. 4. Time series data on the flow velocity of the vastus lateralis muscle and subcutaneous tissue.

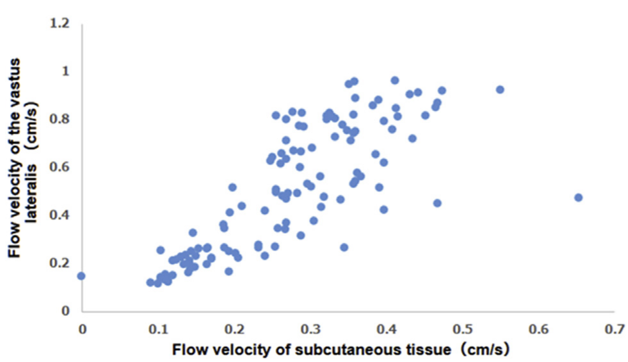

Fig. 5. Coefficient of correlation. Gliding was used to calculate a coefficient of correlation from each time series dataset.

respectively (Fig. 6).

Next we analyzed the reliability of the gliding-related evaluation using intraclass correlation coefficients. Repeated-measures two-way ANOVA was used to compare the two motion rhythms (40 bpm/60 bpm) and three positions (hip adduction/hip intermediate/hip abduction). Changes of flow velocity with the differing motion rhythms (flow velocity peak value/flow velocity average value) were analyzed using the Wilcoxon signed-rank test. This was used to analyze the association between each item and the gliding coefficient of the tissue measurement using Pearson's correlation coefficient. Stepwise multiple linear regression analysis was also performed to analyze the predictors that influenced the gliding coefficient at a rhythm of 40 or 60 bpm. All data were analyzed using SPSS Statistics version 24.0 (IBM Corp., Armonk, NY, USA). Significant differences were set at a level of $P < 0.05$.

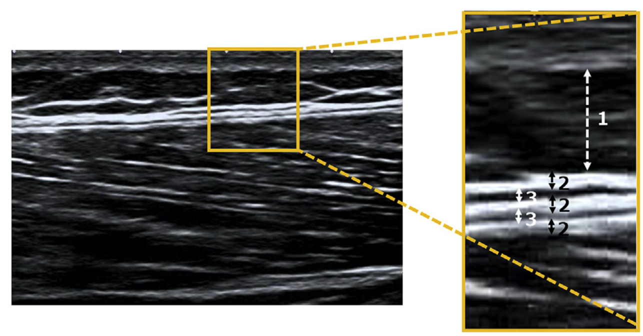

Fig. 6. Tissue measurement of the lateral part of the thigh
1. Subcutaneous tissue thickness. 2. Dense connective tissue thickness. 3. Loose connective tissue thickness. 4.
2
+
3: Total connective tissue thickness. 5.
2/4: Dense connective tissues ratio. 6.
3/4: Loose connective tissues ratio.

3. Results

3.1. <Intraclass correlation coefficients (ICC)>

The ICC of gliding among the different tissues was 0.93 and the SEM was 0.02.

3.2. <Two-way analysis of variance (ANOVA) with replication (Fig. 7)>

The gliding coefficient (r) among tissues at 40 bpm was

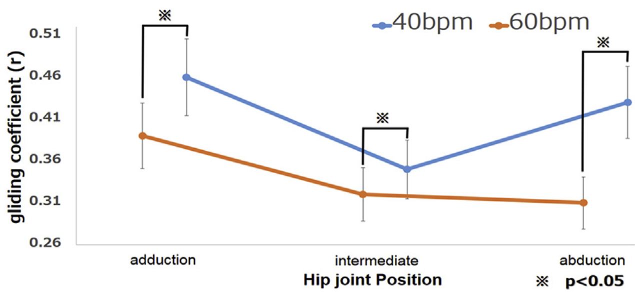

Fig. 7. Two-way ANOVA with replication.

0.46 ± 0.17 in the adduction position, 0.35 ± 0.14 in the intermediate position, and 0.43 ± 0.18 in the abduction position, while at 60 bpm, the gliding coefficient (r) among tissues was 0.39 ± 0.19 in the adduction position, 0.32 ± 0.15 in the intermediate position, and 0.31 ± 0.16 in the abduction position. The result of repeated-measures two-way ANOVA showed that the effect of rhythm was statistically significant ($F_{(1,120)} = 6.53$, $P < 0.05$); however the effect of position was not statistically significant.

3.3. <Wilcoxon signed-rank test>

At 40 bpm, the subcutaneous tissue flow velocity peak value was 0.52 ± 0.21 cm/s, mean subcutaneous tissue flow velocity was 0.21 ± 0.10 cm/s, the VL muscle flow velocity peak value was 1.12 ± 0.08 cm/s, and the mean flow velocity value was 0.71 ± 0.09 cm/s. Meanwhile at 60 bpm, the subcutaneous tissue flow velocity peak value was 0.50 ± 0.21 cm/s, mean subcutaneous tissue flow velocity was 0.25 ± 0.13 cm/s, the VL muscle flow velocity peak value was 1.14 ± 0.08 cm/s, and the mean value was 0.79 ± 0.07 cm/s. Analysis using the Wilcoxon signed-rank test showed that the subcutaneous tissue flow velocity average value and the mean VL muscle flow velocity value at 60 bpm were both significantly increased.

3.4. <A coefficient of correlation and multiple regression analysis (Table 1)>

At a gliding coefficient of 40 bpm, a meaningful correlation was observed between DCT thickness (r = 0.631), total connective tissues (r = 0.531) and SC thickness (r = 0.673). In addition, at a gliding coefficient of 60 bpm, a meaningful correlation was observed between the DCT ratio (r = 0.542), DCT thickness (r = 0.534), and SC thickness (r = 0.722). At a 40 bpm rhythm, stepwise multiple regression analysis selected SC thickness and DCT thickness ($R = 0.768$, $R^2 = 0.590$, $P < 005$). At a 60 bpm rhythm, stepwise multiple regression analysis selected SC thickness and DCT ratio ($R = 0.816$, $R^2 = 0.665$, $P < 005$).

4. Discussion

A prominent role of hyaluronan in the gliding function between fascia and muscle and between the different fascia sublayers has been postulated (Stecco et al., 2014). A lot of HA are distributed throughout the LCT (Stecco et al., 2014). It has been reported that the thickness of connective tissue, including LCT, is associated with low back pain and neck pain (Stecco et al., 2014). Multiple regression analysis showed that there was a high correlation between the gliding coefficient and DCT thickness, SC thickness and DCT ratio. LCT showed a relatively decreased association, and it was thought that gliding decreased. These findings demonstrate that gliding is related to fascial thickness. It will be necessary to consider whether gliding is related to pain in future.

SC is classified into four types: a basic two-layer structure with the superficial fascia, a two-layer structure with no obvious superficial fascia, all SC consisting of only the solid structure, and an anchoring structure (Nakajima et al., 2004). The SC of the lateral part of the thigh was reported to comprise a two-layer structure with no obvious superficial fascia (Nakajima et al., 2004). The deep part of this SC structure is known as a lubrication dispofascial system and is extremely mobile (Nakajima et al., 2004). Consequently, it is thought that the SC structure is the factor which is important in gliding between tissues. Such a structure (skin ligament or striated fascia) to connect perpendicularly exists between SC and deep fasciae in the lubrication dispofascial system (Nakajima et al., 2004; Ishida et al., 2015; Stecco, 2015). It is predicted that tension of the skin ligament and striated fascia increases because SC thickness increases. This tension is connected with the VL muscle through the deep fasciae, and it was thought that gliding decreased here.

One result of this study is increased understanding that exercise rhythm influences gliding between tissues. As the speed of the rhythm increased, the average flow velocity of the SC and the VL muscle significantly increased in concert. HA in the LCT are thought to participate in gliding between tissues. Solutions of high molecular weight hyaluronan can be highly viscous with non-Newtonian flow properties (Cowman and Matsuoka, 2005). These properties may affect the movement of HA-containing fluid layers within and beneath the deep fascia (Stecco, 2015). In steady shear conditions, the solution viscosity is highest when the rate of shear is low, and molecules can reorient and relax to the undisturbed shape as rapidly as they move (Stecco, 2015). However with increasing shear rate, the molecules cannot relax fast enough, and the viscosity drops (Cowman et al., 2015). We applied a three-dimensional mathematical model to explore the relationship between the three manual therapy motions (constant sliding, perpendicular vibration, and tangential oscillation) and the flow characteristics of HA below the fascial layer (Roman et al., 2013). The results showed that high vibration (60 Hz) raised fluid pressure (Roman et al., 2013). This pressure can "lift" the upper fascial layer in the same way that water between a tire and the road surface can lift a moving

Table 1
Multiple regression analysis (stepwise method).

Dependent variable	Independent variables	β	P value	Adjusted R2
gliding coefficient (40bpm)	Subcutaneous tissue thickness	0.51	p < 0.01	0.59
	Dense connective tissue thickness	0.41	p < 0.05	
Dependent variable	**Independent variables**	**β**	**P value**	**Adjusted R2**
gliding coefficient (60bpm)	Subcutaneous tissue thickness	0.63	p < 0.01	0.67
	Dense connective tissue ratio	0.39	p < 0.01	

automobile, causing the tire to lose traction with the road surface (Roman et al., 2013). Fluid pressure accords with a theory that an automobile works faster when it is larger. The fluid pressure increases in this way because the rhythm becomes faster, and it is thought that gliding between tissues increases. Therefore, the rhythm of 60 bpm was regarded as the value that had the lowest gliding coefficient.

The gliding coefficient did not show any significant difference with changes in limb position. The difference in joint limb position influences the tension of the deep fascia of the thigh, thus ITB stiffness was significantly increased in the contralateral pelvic dropped position (i.e. hip adduction) (Tateuchi et al., 2016). The strain on the ITB compresses the VL muscle. Therefore, we postulated that gliding would decrease. However, when a normal adult in this study was tested, it appeared that the VL muscle and the ITB had enough extensibility. Therefore, the strain of the ITB did not influence gliding between tissues.

This study has some limitations. The technique of particle image velocimetry used in this study cannot perform inspection during actual movement *in vivo*. The effect on the 60 bpm more over and 40 bpm or less rhythm is not clear for gliding between subcutaneous tissue and vastus lateralis muscle. In addition, it is predicted that the state of subcutaneous tissue, fascia, and muscle will be different in a patient compared with a normal adult. Therefore, examination is necessary even when patients are similar.

5. Conclusion

Through multiple regression analysis, the following factors showing associations independently of the gliding coefficient were identified: SC thickness, DCT thickness, and DCT ratio. Increased thickness of the SC led to increased tension of the skin ligaments. The traction force generated by the skin ligaments is transmitted to the VL through its myofascia. Therefore, increased thickness of the DCT of the myofascia as well as the SC resulted in decreased gliding between the VL and subcutaneous tissue. These findings demonstrate that gliding is related to fascial thickness.

One result of this study is increased understanding that exercise rhythm influences gliding between tissues. HA in LCT are thought to participate in gliding between tissues. The gliding coefficient did not show any significant difference with changes in limb position. When a normal adult in this study was tested, it was thought that the VL muscle and the ITB had enough extensibility. Therefore, the strain on the ITB did not influence gliding between tissues.

6. Clinical relevance

- Evaluation of non-invasive tissue gliding is clinically significant.
- There is a possibility of gliding improvement by intervening in the SC and DCT.
- Increasing the speed of exercise is effective for improving gliding.

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CRediT authorship contribution statement

Kengo Kawanishi: Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Visualization. **Shintarou Kudo:** Conceptualization, Methodology, Software, Validation, Resources, Data curation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

None.

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