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Structure of the rat subcutaneous connective tissue in relation to its sliding mechanism*

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Summary. Mammalian skin can extensively slide over most parts of the body. To study the mechanism of this mobility of the skin, the structure of the subcutaneous connective tissue was examined by light microscopy. The subcutaneous connective tissue was observed to be composed of multiple layers of thin collagen sheets containing elastic fibers. These piled-up collagen sheets were loosely interconnected with each other, while the outer and inner sheets were respectively anchored to the dermis and epimysium by elastic fibers. Collagen fibers in each sheet were variable in diameter and oriented in different directions to form a thin, loose meshwork under conditions without mechanical stretching. When a weak shear force was loaded between the skin and the underlying abdominal muscles, each collagen sheet slid considerably, resulting in a stretching of the elastic fibers which anchor these sheets. When a further shear force was loaded, collagen fibers in each sheet seemed to align in a more parallel manner to the direction of the tension. With the reduction or removal of the force, the arrangement of collagen fibers in each sheet was reversed and the collagen sheets returned to their original shapes and positions, probably with the stabilizing effect of elastic fibers. Blood vessels and nerves in the subcutaneous connective tissue ran in tortuous routes in planes parallel to the unloaded skin, which seemed very adaptable for the movement of collagen sheets. These findings indicate that the subcutaneous connective tissue is extensively mobile due to the presence of multilayered collagen sheets which are maintained by elastic fibers.

Introduction

The mammalian skin can be extensively mobile, as if "floating" on the underlying body tissues. When pushed or pulled, the skin of such animals as rats, cats and dogs easily moves. The back skin can be especially elevated from the trunk to some distance by pinching and pulling it, and the skin returns to its original position at the cessation of external forces. The skin in other regions is also mobile, although various factors such as anatomical site, age, and nutrition of the animal restrain the extent of its mobility. In our preliminary studies, we noticed that the mobility mainly originates from the thin connective tissue layer interposed between the skin (e.g., epidermis, dermis and skin muscle) and its underlying tissues (e.g., muscles, tendons and bones). This "sliding layer" is thought to reduce body damage by averting physical injuries to the body and help smooth and rapid muscle contractions beneath the skin by decreasing friction with it. Damage to the subcutaneous connective tissue can lead to adhesion between the skin and the underlying tissues, which may deteriorate muscle function and restrict the range of joint movements depending on the severity of adhesion. Thus, the subcutaneous connective tissue layer obviously plays important roles in movement. However, only a few and fragmental studies have been made on the fine structure of this layer (Imayama and Braverman, 1988; Sato *et al.*, 1997). In this study, the subcutaneous connective tissue of the rat was examined by light microscopy to clarify the structure and sliding mechanism. The routes of blood vessels and peripheral nerves from the underlying tissues to the skin were also observed to consider changes in the location of blood vessels and nerve adaptation to extensive sliding of the skin.

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Materials and Methods

Thirty-four 4-week-old Wistar rats of both sexes (80–150 g body weight) were used in this study. Young animals were used because the softness of their skin allows it to be cut more easily than that of adults, and there is only a little adipose tissue in the subcutaneous connective tissue. All rats were killed by the inhalation of ether.

The back skin

The back skin was pinched, elevated with fingers, and cut from the trunk. For the accurate observation of blood vessels, some rats were injected intravenously with 1 ml of India ink before sacrifice. In some other cases, very small amounts of diluted India ink (India ink : saline = 1 : 9) were injected into the connective tissue of the removed unfixed back skin under a dissecting microscope to observe its multilayer structure. The isolated subcutaneous connective tissue was also pulled using two pairs of forceps to simulate a loaded condition under a dissecting microscope. The tissue was additionally stained with 1% aniline blue in 0.1 M phosphate buffered saline (PBS, pH 7.4) for 30 min, rinsed in PBS, and observed by light microscopy. Some back skin samples were frozen, sectioned in transverse or parallel (horizontal) directions to the skin, stained, and observed in the same manner as described in the following section.

The skin-muscle flap without sliding

As large an area as was possible of the abdominal wall of the whole thickness from the skin to the parietal peritoneum (skin-muscle flaps) was removed. Two flaps (1 × 5 cm; head to tail × right to left length) could be prepared from each rat and used for quantitative studies. For non-quantitative studies, skin-muscle flaps of other shapes and sizes were also used. Specimens were adhered to balsa wood (1–3 mm thick) which had been freshly coated with an adhesive (6% aqueous tragacanth gum). The skin-muscle flaps with balsa wood were placed on cork disks (18–22 mm in diameter and several mm thick) and supported with tragacanth gum in such a position so that specimens could be cut either in a plane rectangular (transverse section) or parallel (parallel section) to the skin. The skin-muscle flap, balsa wood, and cork disk were immersed together in liquid nitrogen and frozen. After trimming, the specimens attached to balsa wood were cryosectioned, because balsa wood did not affect the cutting. The frozen sections (10–20 μm) were stained with aldehyde fuchsin-Masson Goldner staining for light microscopy.

The skin-muscle flap loaded with shearing forces

In our preliminary experiment, the mobility of the skin was almost the same in all directions. For evaluation of the mobility of the subcutaneous connective tissue, the skin-muscle flaps were placed on tragacanth gum-coated balsa wood in such a manner that the upper end of the flap (1 cm wide) roughly met the upper end of the wood. Then only the skin layer (presumably with a part of the connective tissue) of the flap was tightly fixed to the wood by a clip. At the lower end of the flap, the remaining layers (part of the connective tissue, muscles and peritoneum) were clasped with a clip attached to a weight. The flap was kept vertical and loaded with a weight of 10, 20, 30, 40, or 60 g including the clip, and the distance between the upper end of the skin and the upper end of the muscle was measured (Fig. 1). The peripheries of the weight-loaded flap were then pinned to the attached wood under loaded conditions prior to removal of the weight. The pinned flap and wood were placed on a cork disk, supported by tragacanth gum, and frozen together in liquid nitrogen. The pins were removed after freezing, and transverse or parallel sections were cryosectioned (10–20 μm) along the direction of tension, stained, and observed with a light microscope.

Results

The back skin

In cross sections of the back skin, layers of the epidermis, the dermis and the skin muscle were observed by light microscopy, although the underlying connective tissue abruptly ended with an irregular edge on the inner surface of the skin (Fig. 2). Thus, the back skin was apparently removed at the layer which corresponded to the subcutaneous connective tissue of the skin-muscle flap (Fig. 3).

When observed with the naked eye, the inner surface of the unfixed back skin was covered with a part of the connective tissue. This connective tissue could be pulled along the skin even with a weak force, and semi-transparent thin layers easily moved extensively in all directions (Fig. 4a, b). Light microscopy of the unfixed aniline blue-stained materials revealed that each thin layer was a meshwork of collagen fibers. By applying a weak force, these sheets easily altered their relative positions to each other, while the fiber arrangement of the sheets remained essentially unchanged (Fig. 5a, b, c). At the dissecting microscopic level, the isolated connective tissue resembled a cotton ball, and stretching this with two pairs of forceps resulted in an increasingly parallel arrangement of the connective tissue fibers in the direction of force. The connective tissue could

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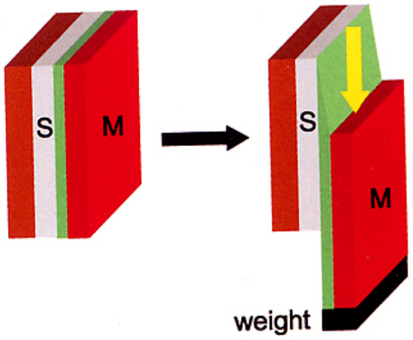

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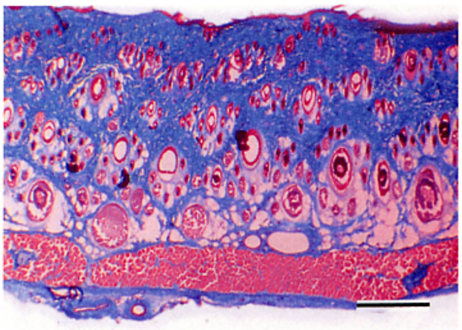

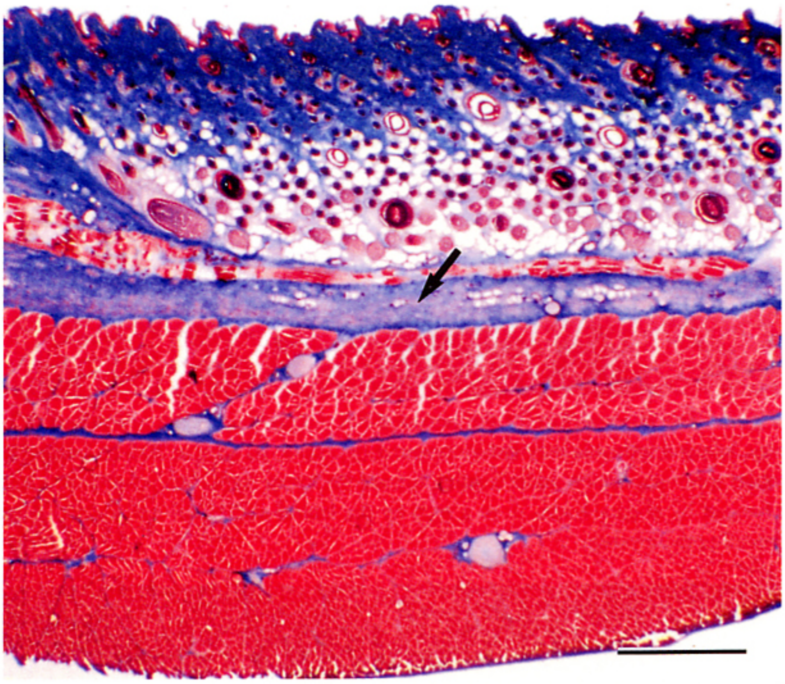

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Fig. 1. A schema showing measurements of the sliding distance (yellow arrow). Layers are, from left to right, balsa wood (brown), the skin (the epidermis and the dermis including the skin muscle, gray and indicated by S), the subcutaneous connective tissue (green) and the muscles (red and indicated by M).

Fig. 2. The transverse section of the back skin removed after sliding. The skin consists of the epidermis, the dermis and the skin muscle. The connective tissue under the skin muscle ends with an irregular edge. Bar = 0.5 mm

Fig. 3. A cross sectional view of the skin-muscle flap. The layers from top to bottom are the epidermis, the dermis, the skin muscle, the subcutaneous connective tissue (arrow), the muscles, and the peritoneum. Bar = 0.5 mm

be equally stretched in all directions along a plane parallel to the skin. Injection of diluted India ink into this layer resulted in the formation of minute drops at different depths of the connective tissue; the relative position of the drops could be changed. Drops could be overlapped and separated from each other without fusion, indicating that these drops were separated to one another by multiple layers of sheets.

The skin-muscle flap without sliding

In transverse sections, the skin-muscle flap from the abdominal wall was composed of the following layers by light microscopy: 1) the epidermis, 2) the dermis, 3) the skin muscle and its epimysium, 4) the subcutaneous connective tissue, 5) the epimysium and abdominal muscles, and 6) the peritoneum (Fig. 3). Thus, the dermis was usually underlined with the skin muscle. The thin subcutaneous connective tissue only contained a little adipose tissue since the animals used were young. Close observations revealed that both the epimysium of the skin muscle and that of abdominal muscles were composed of densely arranged collagen fibers studded with abundant thick elastic fibers extending into the subcutaneous connective tissue in various directions (Fig. 6a). In the subcutaneous layer between the two epimysia, collagen fibers tended to orient themselves parallel to the skin with undulations (Fig. 6a), and elastic fibers were thin and sparse as compared with those in the epimysia. Transversely cut profiles of blood vessels and nerves were occasionally found in this layer (Fig. 7a).

In sections parallel to the skin, the dense arrangement of elastic fibers in the epimysium was more evident than in

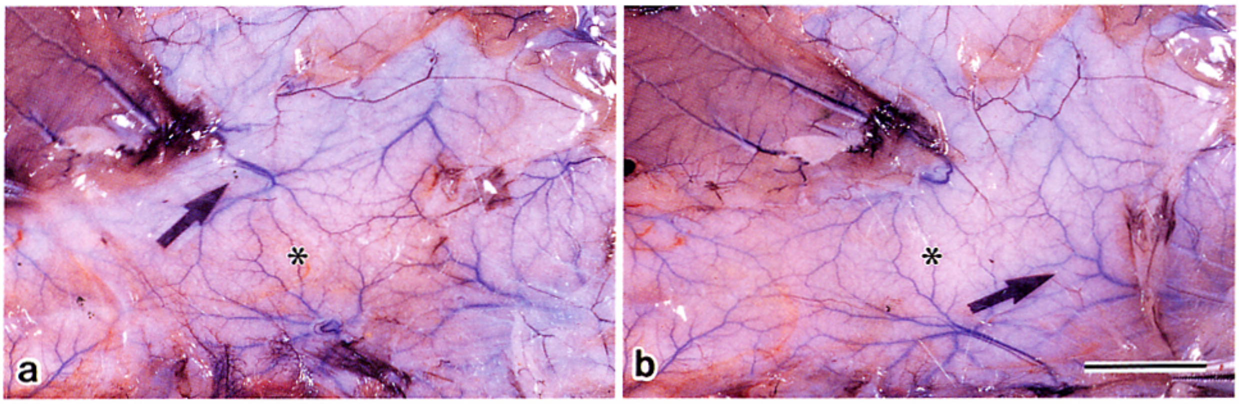

Fig. 4. The inner surface of the removed but unfixed back skin covered with thin layers of connective tissue. Indian ink was injected intravenously before preparation. Bar = 10 mm. **a:** Before loading tension an to the connective tissue. **b:** The same specimen as in **a** under loading toward the lower right. The position of a marker (arrow) placed on the sliding layer is changed by about 18 mm from **a** to **b** by the lateral sliding of thin sheets. Asterisks in **a** and **b** indicate the same blood vessel in the mysium.

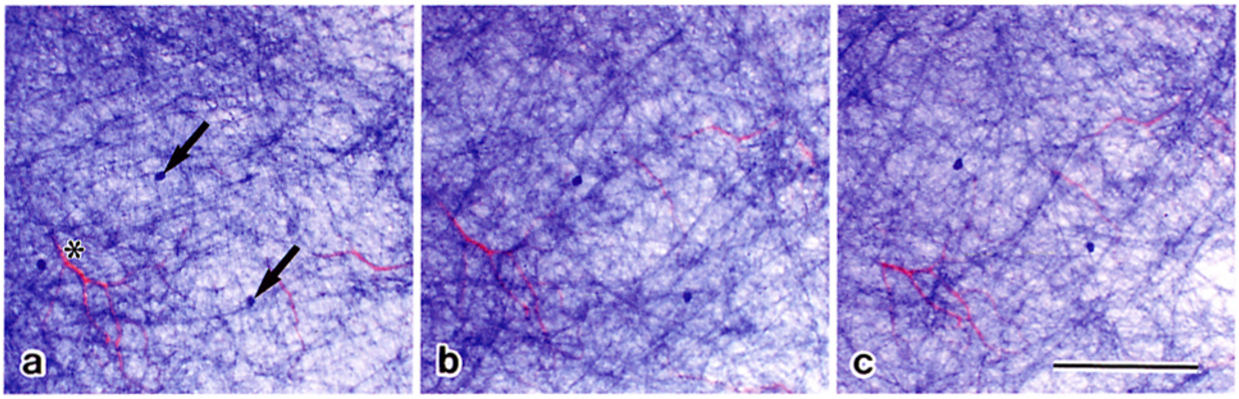

Fig. 5. Light micrographs of the unfixed subcutaneous connective tissue. With shearing forces, fibers in collagen sheets considerably change their relative positions. Fields are selected in a way that the blood vessel (asterisk) and mast cells (arrows) come to almost the same positions in **a**, **b** and **c**. Aniline blue staining. Bar = 0.5 mm

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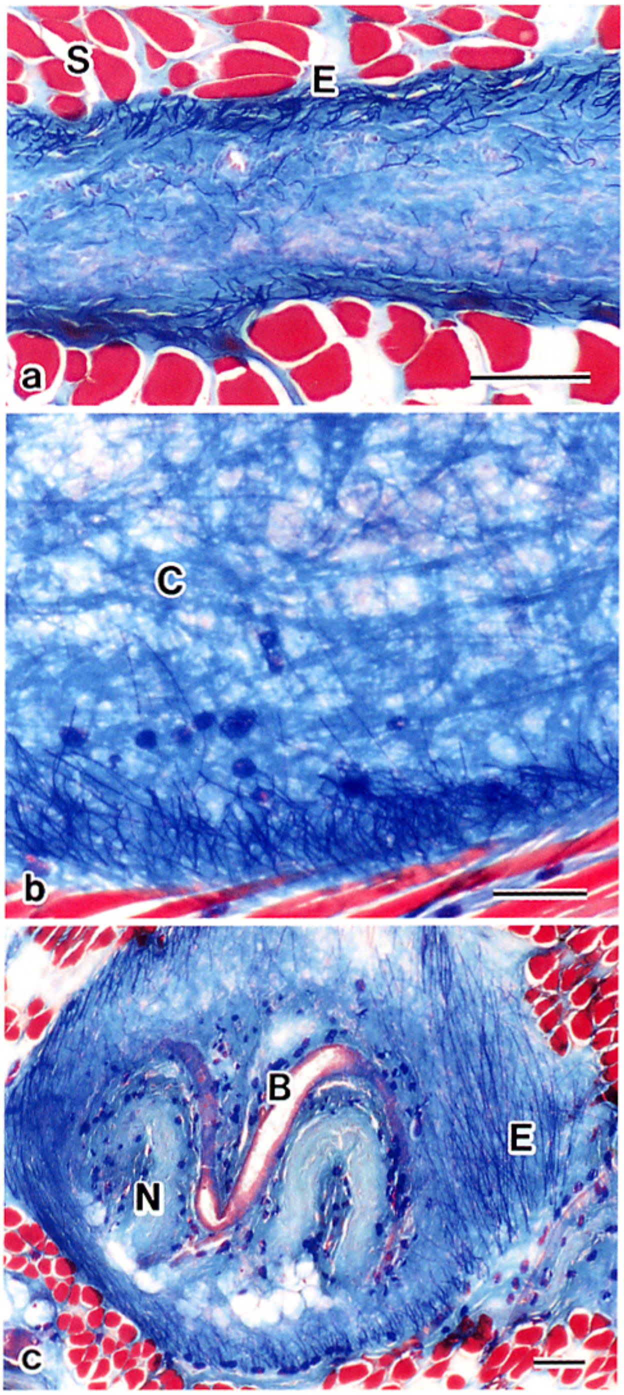
Fig. 6. Skin-muscle flaps without sliding. **a:** Transverse section. Thick elastic fibers (E) originating from the mysium extend into the connective tissue in various directions. Collagen fibers in the connective tissue tend to run parallel to the skin with undulations. S: skin muscle. **b:** Section parallel to the skin. Numerous elastic fibers are seen in the mysium of the skin muscle. Collagen fibers (C) in the connective tissue run in different directions. **c:** Parallel section of a slightly domed skin-muscle flap. The blood vessel (B) and the nerve (N) extend very tortuously. Note the straight extending elastic fibers (E). Bars = 100 μm

Fig. 7. Skin-muscle flaps with sliding (left-right direction in figure). Bars = 100 μm. **a:** Transverse section. Elastic and collagen fibers apparently oriented parallel to the skin. Loaded with 40 g. Arrow: transversely sectioned nerve. **b:** Parallel section. Collagen fibers (C) and elastic fibers in the connective tissue and the mysium are aligned parallel to the direction of force. Loaded with 30 g. MC: mast cells. **c:** Higher magnification of the lower left portion of **b**. Collagen fibers (C) run parallel.



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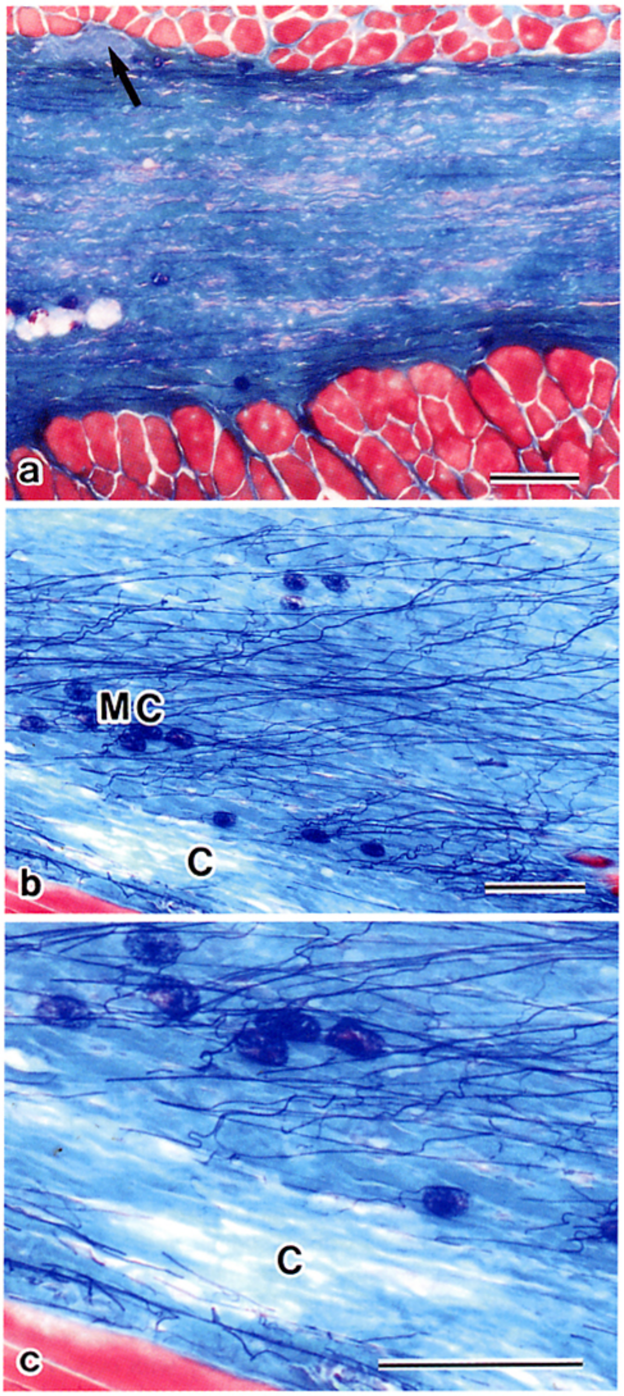

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Fig. 6 and **7.** Legends on the opposite page.

transverse sections. Elastic fibers usually ran linearly and in parallel (Imayama and Braverman, 1988) even under the conditions without sliding (Fig. 6c). Collagen fibers of varying diameters were oriented at random along the plane of the collagen sheet (Fig. 6b). Thin blood vessels and peripheral nerves were also extended and ran in tortuous routes in the plane parallel to the skin (Fig. 6c). Mast cells were frequently observed near the mysium and blood vessels.

The skin-muscle flap loaded with shearing forces

The extent of muscle sliding was not proportional to the strength of the force, and there were also some specimen differences. However, later in the experiment, when the same specimens were increasingly loaded with various weights, it was found that the muscle slid considerably (often 10–15 mm) with the smallest weight we used (10 g) and further loading ranging from 20 to 60 g resulted in a gradual and slight increase in sliding (an increase in sliding distance at most of several mm).

In transverse sections, elastic fibers ran in parallel to the direction of force. Collagen fibers were aligned more orderly in parallel to the skin (Fig. 7a) compared with unloaded controls. In sections parallel to the skin, elastic and collagen fibers were oriented generally following the direction of tension (Fig. 7b, c).

Discussion

Most specimens were prepared by cryosection in the present study because the staining of elastic fibers seemed more intense than with paraffin sections, and possible artifacts which may occur during specimen preparation were probably held to a minimum using our method as compared with previous ones (Crissman *et al.*, 1980; Wasano and Yamamoto, 1983; Ushiki, 1992a).

The elastic fibers of the subcutaneous connective tissue have been observed by Imayama and Braverman (1988) under natural conditions without sliding. In their scanning electron microscopic studies, elastic fibers were arranged in multiple layers (5 to more than 15 layers in 2- to 6-week-old rats) and interconnections between different layers were rarely seen. Their findings agree closely with ours. They also suggested that collagen fibers appeared arranged in layers by transmission electron microscopy, and correlated the extensive mobility with the multiple layer structure of the connective tissue.

The present study actually examined the subcutaneous connective tissue both functionally and structurally as it slides in parallel to the skin by the application of shearing forces. We observed not only elastic fibers but also collagen components. Our study clearly demonstrated that the subcutaneous connective tissue can extensively move laterally, and that this tissue is characterized by multiple layers of numerous collagen sheets. Mobility seems helpful to reduce tissue damage by averting physical injury. Watanabe and Nishizono (1994) suggested that the slippery cushion of the elastic fiber network protects the serous cells in the hepatic capsule. Similarly, the extensive lateral mobility of the subcutaneous tissue probably protects the skin or the underlying tissues (or both) by preventing physical injuries.

Elastic fibers usually run straight even under conditions without sliding (Imayama and Braverman, 1988) and are irregularly connected to collagen fibers (Sato *et al.*, 1997). This finding suggests that collagen sheets are maintained at their proper positions by a balance between tensions of elastic fibers, resulting in the uniform distribution of stress forces in tissues (Ushiki, 1992b, 2002). The accumulated collagen sheets and scarcity of fibers binding to adjacent collagen sheets (Imayama and Braverman, 1988) are helpful for the extensive mobility of collagen sheets. In addition, changes in the arrangement of collagen fibers also probably contribute to sliding when a strong shear force is applied to the subcutaneous tissue; this may be partially explained in that the fibers of a cotton ball become increasingly parallel to the direction of force when stretched from both ends.

It is considered that the extensive mobility of the subcutaneous connective tissue is attributed to: 1) initial considerable sliding among collagen sheets and, for further loading, 2) subsequent gradual sliding through alterations of the arrangement of the collagen fibers in each sheet. These positional changes of the collagen sheets and altered arrangements of the collagen fibers are possibly reversed by the elastic fibers as suggested in visceral pleura (Lemos *et al.*, 1997) and, in part, by the stability of collagen fibers. Tortuous and redundant routes of the blood vessels and nerves in the plane parallel to the skin probably allow these structures to adapt to changes in the relative positions of the skin and the underlying muscles.

In some unique structures such as the joint, the tendon synovial sheath and the serous membrane, a great deal of movement is possible between two surfaces separated by a narrow gap; the gap prevents the direct access of blood and lymphatic vessels between the two separate surfaces. On the other hand, in the subcutaneous connective tissue, no gaps exist and the blood and lymphatic vessels and nerves can traverse the connective tissue from the underlying tissues to the skin. Therefore, the subcutaneous connective tissue is a sophisticated multilayer structure which provides considerable mobility without restricting nutritional and nerve supplies. The extensive mobility of this tissue also allows the relatively independent movement of the skin and the muscles (Imayama and Braverman, 1988), which is important for normal muscle functions and unrestrained joint movements.

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