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ORIGINAL ARTICLE

3D reconstruction of the crural and thoracolumbar fasciae

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Abstract
Purpose To create computerized three-dimensional models of the crural fascia and of the superficial layer of the thoracolumbar fascia.
Methods Serial sections of these two fasciae, stained with Azan-Mallory, van Gieson and anti-S100 antibody stains, were recorded. The resulting images were merged (Image Zone 5.0 software) and aligned (MatLab Image Processing Toolkit). Color thresholding was applied to identify the structures of interest. 3D models were obtained with Tcl/Tk scripts and Paraview 3.2.1 software. From these models, the morphometric features of these fasciae were evaluated with ImageJ.
Results In the crural fascia, collagen fibers represent less than 20% of the total volume, arranged in three distinct sub-layers (mean thickness, 115 μm), separated by a layer of loose connective tissue (mean thickness, 43 μm). Inside a single sub-layer, all the fibers are parallel, whereas the angle between the fibers of adjacent layers is about 78°. Elastic fibers are less than 1%. Nervous fibers are mostly concentrated in the middle layer. The superficial layer of the thoracolumbar fascia is also formed of three thinner sub-layers, but only the superficial one is similar to the crural fascia sub-layers, the intermediate one is similar to a flat tendon, and the deep one is formed of loose connective tissue. Only the superficial sub-layer has rich innervation and a few elastic fibers.
Discussion Computerized three-dimensional models provide a detailed representation of the fascial structure, for better understanding of the interactions among the different components. This is a fundamental step in understanding the mechanical behavior of the fasciae and their role in pathology.

Keywords Crural fascia · Thoracolumbar fascia · Connective tissue · 3D models · Collagen

Introduction

Classically, the deep fascia is classified as irregular, dense, connective tissue, only playing a role in enveloping muscles [8, 27, 37]. More recent studies [5, 15, 30, 31] provide evidence of a specific organization of the deep fasciae, and many surgeons, physiotherapists, osteopaths and other professionals working in alternative therapies are becoming interested in them. Nevertheless, the structure of the deep fasciae in the human body is not clearly understood. Some authors [6, 10, 20, 27] state that they are regular, dense, connective tissues similar to the aponeurosis, characterized by extremely ordinate, parallel bundles of anelastic collagen fibers. Others [8, 11] state that they are composed of intertwined bundles of collagen fibers. Recent research [28] shows that there are differences between the fasciae of the limbs and of the trunk. In particular, the deep fasciae of the trunk are usually formed of a single layer of undulated collagen fibers adhering to the underlying muscles, whereas the deep fasciae of the limbs are formed of two or three sub-layers of parallel collagen fiber bundles. In addition, according to various authors [4, 6, 17, 35], the

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superficial layer of the thoracolumbar fascia shows a bi-laminar nature, but the anatomical features of its sub-layers are not clear. Loukas et al. [17] call the superficial sub-layer the "anterior lamina of the posterior layer of the thoracolumbar fascia" and the deep sublayer "vertebral aponeurosis", describing the latter as a thin, loose, fibrous layer with a mean thickness of 3 mm. This is disputed by Barker and Briggs [4], who report that the thickness of the whole superficial layer of the thoracolumbar fascia is 0.53–0.56 mm.

Several studies [5, 29, 32–34, 36] have highlighted the presence of nerve elements inside the fasciae, but none has described the location of these nerve elements in relation with the various sub-layers of the fasciae. Also, the presence of elastic fibers inside the fasciae is not very clear: in particular, Stecco et al. [28] report that they are very scarce in the deep fascia of the lower limbs but numerous (approximately 15%) in the fasciae of the trunk. In addition, Cör et al. [7] report that the cross-sectional area of the endopelvic fascia contains 3.81–5.93% of elastic fibers.

We applied a 3D reconstruction technique for better understanding of how the sub-layers of the crural fascia and the superficial layer of the thoracolumbar fascia are arranged. This method also allowed us to define the structural conformation of these fasciae and the spatial disposition of the collagen, elastic and nerve fibers inside the samples.

Materials and methods

Samples

The study was performed on four samples (1 × 1 cm) of deep fasciae, obtained from two subjects (males, 39 and 67 years old). The cadavers were neither embalmed nor frozen prior to examination, and did not present evidence of traumatic lesions or pathologies in the regions examined. Two of the specimens were collected from the crural fascia in the posterior-proximal region of the leg, where the deep fascia is easily separable from both the superficial fascia and the muscular plane (Fig. 1); the other two specimens were collected from the thoracolumbar fascia, at 7 cm from the spinous process of the third lumbar vertebra, on the right side.

The specimens were accurately oriented, mounted on cardboard to avoid deformation artefacts, stored in neutral 10% formalin and then embedded in paraffin wax.

Histological and histochemical techniques

From each sample, 7 µm-thick serial sections, oriented parallel to the plane of the deep fascia, were obtained. For each series, the even sections (50 slides) were stained with Azan-Mallory to study the arrangement of the collagen fiber bundles, and the odd sections (50 slides) were stained in two different manners. One series of the crural fascia and one of the thoracolumbar fascia were stained according to the van Gieson method to visualize the elastic fibers; the other two series received immunohistochemical staining with a rabbit anti-human S100 antibody (Dako, Milan, Italy), according to a previously described method [29]. To visualize the whole thickness of the deep fascia and the relationship between its layers directly, 7 µm-thick vertical sections were also obtained from the remaining tissue samples. They were stained according to the van Gieson and Azan-Mallory methods.

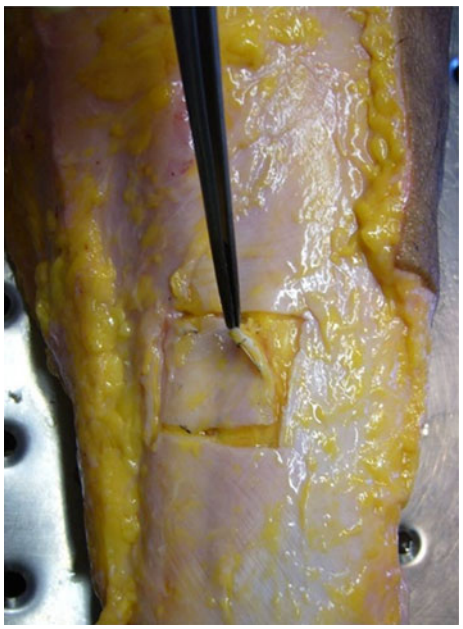

Fig. 1 Sampling mode of crural fascia from posterior-proximal region of leg

Image acquisition

Images of the obtained preparations were recorded in full color (24-bit), at a primary magnification of 25×, with a digital camera (DFC 480, Leica Microsystems, Wetzlar, Germany) connected to a DM4500-B light microscope (Leica Microsystems) and saved as TIFF files. The chosen magnification allowed observation of the structures of interest at a good level of detail, but prevented panoramic views of the whole sections. Thus, each section was systematically sampled and the image of each field was acquired. As shown in Fig. 2, the images of each field were subsequently merged with Image Zone 5.0 software (Hewlett-Packard, Milan, Italy) to obtain a high-resolution single image of the whole surface.

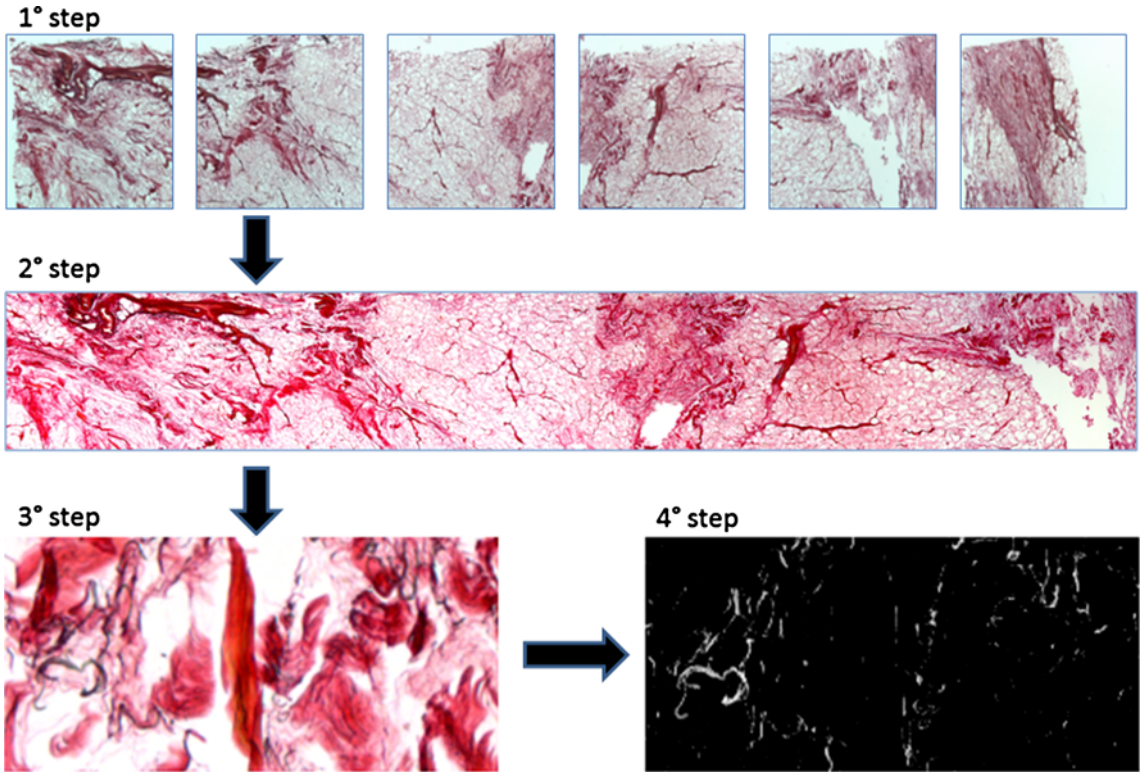

Fig. 2 *Step 1* acquisition of histological images. *Step 2* images of each field were merged to obtain a single high-resolution image of whole surface of section. *Step 3* color thresholding to identify structure of interest. *Step 4* creation of a binary image stack

Image registration

Before the images were used to create 3D models, a registration procedure was carried out. 'Registration' is the process of finding the spatial transformation which maps points from one image to corresponding points in another image [24]. Many criteria have been used as the basis for aligning two images, such as landmark-based, segmentation-based or intensity-based criteria [18]. In the present study, an interactive, landmark-based approach was applied, in which salient micro-anatomical features (landmarks) selected by the operator were used as reference points for registering each image in the set with the preceding one (Fig. 2). Starting from the central image of a set, the procedure was applied to aligning the sections above and below the central one in sequence. This involved rigid transformations (such as rotations and translations) as well as non-rigid operations [2, 19], aimed at correcting misalignments due to stretching or distortions of the sections due to tissue handling and cutting. A specific computer-assisted routine based on MatLab Image Processing Toolkit (The MathWorks Inc., Natick, MA, USA) was developed by the authors to support the image registration procedure.

At the end of this processing step, eight image stacks were obtained, each composed of 50 images of 1,280 × 400 pixels, with a resolution of 184 pixel/mm. Thus, as the distance between consecutive images was 14 μm, each stack corresponded to a tissue volume of about 1 cm × 1 cm × 0.7 mm.

Image thresholding

Color thresholding was applied to the image stacks to identify the structures of interest (Fig. 2). According to this procedure, pixel colors were represented as HSI (hue, saturation, intensity) values [25]. In general, for stains used in biological samples, "hue" identifies the location of a particular stain, "saturation" corresponds to the amount of staining, and "intensity" indicates the overall density of the stained specimen. Thus, collagen bundles, elastic fibers and nerve fibers can be discriminated by setting appropriate thresholds for the hue and saturation components [13, 14]. Following an interactive approach, the collagen bundles were further classified according to their orientation. As a result, three binary image stacks were obtained for each available sample of crural and thoracolumbar fasciae (corresponding to two different orientations of the collagen bundles and the elastic fibers or nerve fibers, respectively) (Fig. 2). All image processing was performed with the public domain image analysis software ImageJ [1] (see also http://rsb.info.nih.gov/ij/).

3D reconstruction and rendering

3D models of the tissue samples were obtained with the help of Tcl/Tk scripts, with algorithms implemented in the Visualization Toolkit library (VTK 4.0, Kitware Inc., New York, NY, USA). Briefly, the procedure was separately applied to the binary image stack of each tissue component and involved the 'marching cubes' algorithm [16], to extract the 3D surfaces of the object as a combination of vertices and polygons connecting them, followed by smoothing techniques [9] to refine the resulting geometric mesh. Results were then stored in a data file.

After processing all the tissue components, the 3D model of the tissue was obtained by displaying all components together. This was achieved with Paraview 3.2.1 software (Kitware Inc.) and allowed interactive exploration of the model in a 3D space. The user can not only confer on each surface a specific color and degree of opacity, to distinguish the different tissue components clearly, but also freely define the viewing angle and the degree of magnification.

Morphometry

ImageJ was used to evaluate a series of morphometrical features of the deep fasciae. The volume fraction of each tissue component was estimated as the ratio between the number of voxels belonging to each structure in the 3D model and the total number of voxels in the reconstructed tissue. To measure the angle between collagen bundles, the 3D model was oriented according to the *x*–*y* plane and the parameter was interactively measured in ten different locations. A similar procedure was applied to measure the thickness of the collagen layers in the *x*–*z* plane.

For the sake of comparison, the same parameters were also evaluated on real tissue sections with standard stereological techniques [26].

Results

Crural fascia

In the 3D reconstruction, the crural fascia appears as a lamina of connective tissue with a mean thickness of 650 μm, formed of three, or at some points two, layers of parallel collagen fiber bundles separated from the adjacent one by a thin layer of loose connective tissue (Fig. 3). The mean thickness of the layers, measured at five points in the *x*–*z* plane for each layer, is 115 μm, with a standard deviation of 21 μm. In each layer, collagen fiber bundles are arranged parallel in one direction, whereas the orientation changes from layer to layer.

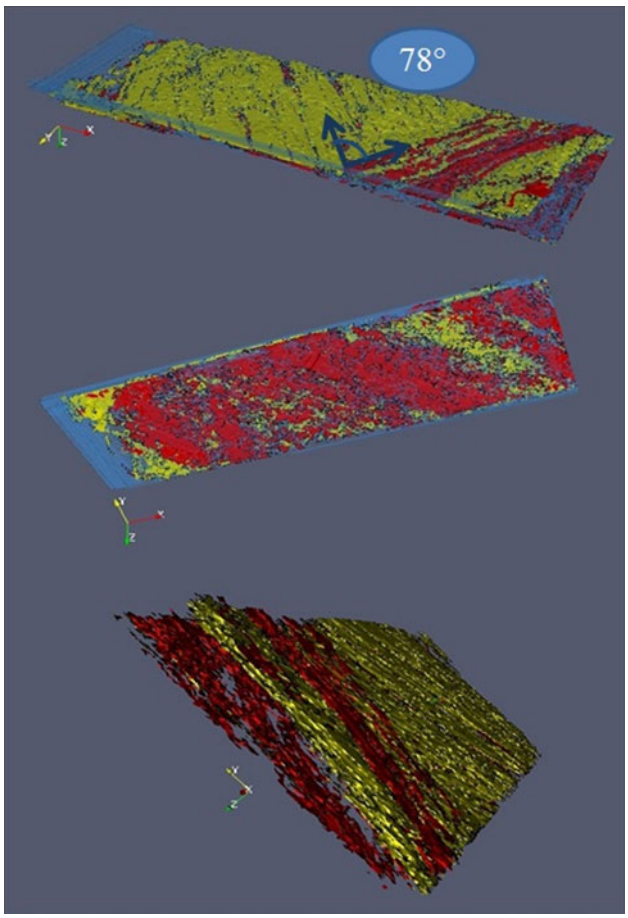

Fig. 3 3D reconstruction of collagen fibers of crural fascia. Note the arrangement of layers and different orientation of collagen fibers between contiguous layers

Voxel Counter analysis of ImageJ results shows that the two directions have a comparable volume fraction of almost 8%, whereas the remaining tissue components are predominant, making up 80% of the volume. The angle between the orientations of the fibers of two adjacent layers, evaluated in the *x*–*y* plane with the Angle Tool of ImageJ, has a mean value of 78°. The standard deviation is less than 4.3°.

Reconstruction of the elastic fibers shows that they are more densely packed in the loose connective tissue, but are very scarce among the collagen fiber bundles (Fig. 4). In addition, the percentage of the elastic component in each slide shows only a slight increase in the elastic component from the superficial to the deep surfaces (Fig. 5). Their volume fraction ranges from 0.3 to 1.5%, but this value probably underestimates the true presence of elastic fibers inside the crural fascia, because it does not consider fibrils with a diameter less than 7 μm, which are under the resolution threshold of this method of analysis.

Immunohistochemical study of the nerve fibers shows rich innervation of the crural fascia (mean 1.2%), mainly in

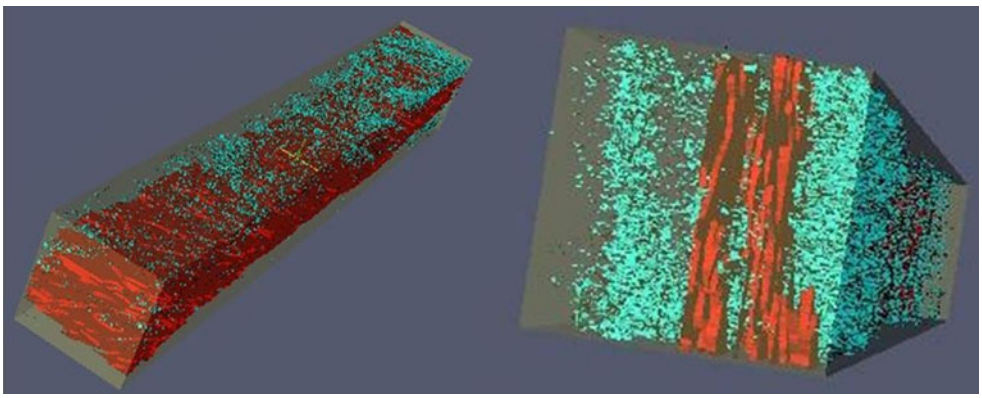

Fig. 4 3D reconstruction of distribution of elastic fibers inside the crural fascia. Collagen fibers form two clearly visible layers in the middle

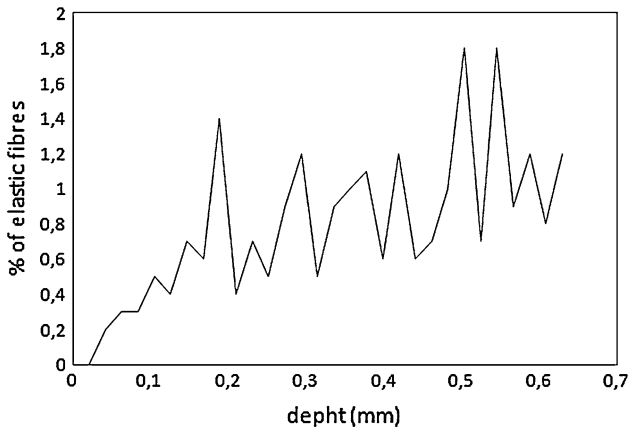

Fig. 5 Distribution of elastic fibers in each slide of serial sections of crural fascia. Volume fraction in the range of 0.3–1.5%, showing slight increase from superficial to deep surfaces

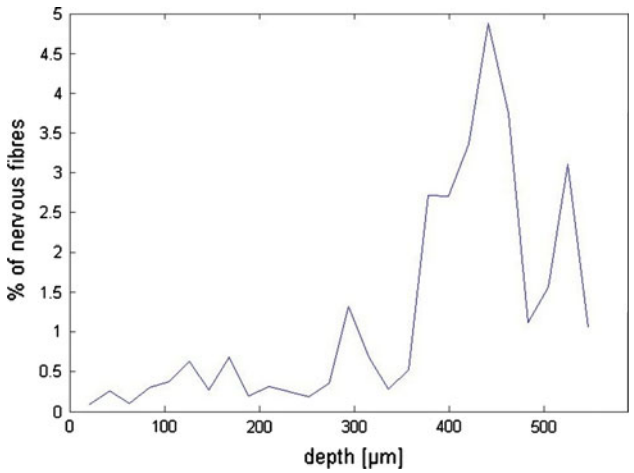

Fig. 6 Distribution of nerve fibers in each slide of serial sections of crural fascia: note the maximum presence in the middle slides

the middle slides (Fig. 6). However, reconstruction of the nerve fibers is not satisfactory, because the software cannot recreate an accurate depiction of the true nerve morphology. This is mainly due to the mean diameter of the intrafascial nerve fibers which, being only about 12.1 µm, with a standard deviation of 6.1 µm, is again under the resolution threshold of the method of analysis.

Superficial layer of thoracolumbar fascia

Our results show a mean thickness of the superficial layer of the thoracolumbar fascia of 680 µm (±15 µm). It is composed of three thinner sub-layers having different morphologic features (Fig. 7): the superficial sub-layer has a mean thickness of 75 µm and is formed of parallel, undulating collagen fibers mixed with a few elastic fibers. The intermediate sub-layer has a mean thickness of 152 µm, and is formed of packed, straight collagen fiber bundles, all disposed along the same direction, without elastic fibers. Lastly, a layer of loose connective tissue, with a mean thickness of 450 µm, separates the thoracolumbar fascia from the underlying muscular planes. The collagen volume proportions in the three sub-layers are 9.12, 9.11 and 5.67%, respectively. The angle between the collagen fibers of the superficial and intermediate sub-layers is 78°. S100 immunohistochemical study and 3D nerve reconstruction show some small nerves (mean diameter 15 µm), which flow through the superficial sub-layer and into the loose connective tissue among the fibrous bundles at a depth of about 140 µm (Fig. 8). However, no nervous fibers were visible along the intermediate and deep sub-layers. Three-dimensional reconstruction of the elastic fibers was not performed, because they were too scarce in the superficial slides and absent in all others during microscopic analysis.

Discussion

The three-dimensional reconstruction method allowed simple, immediate understanding of the structure of the

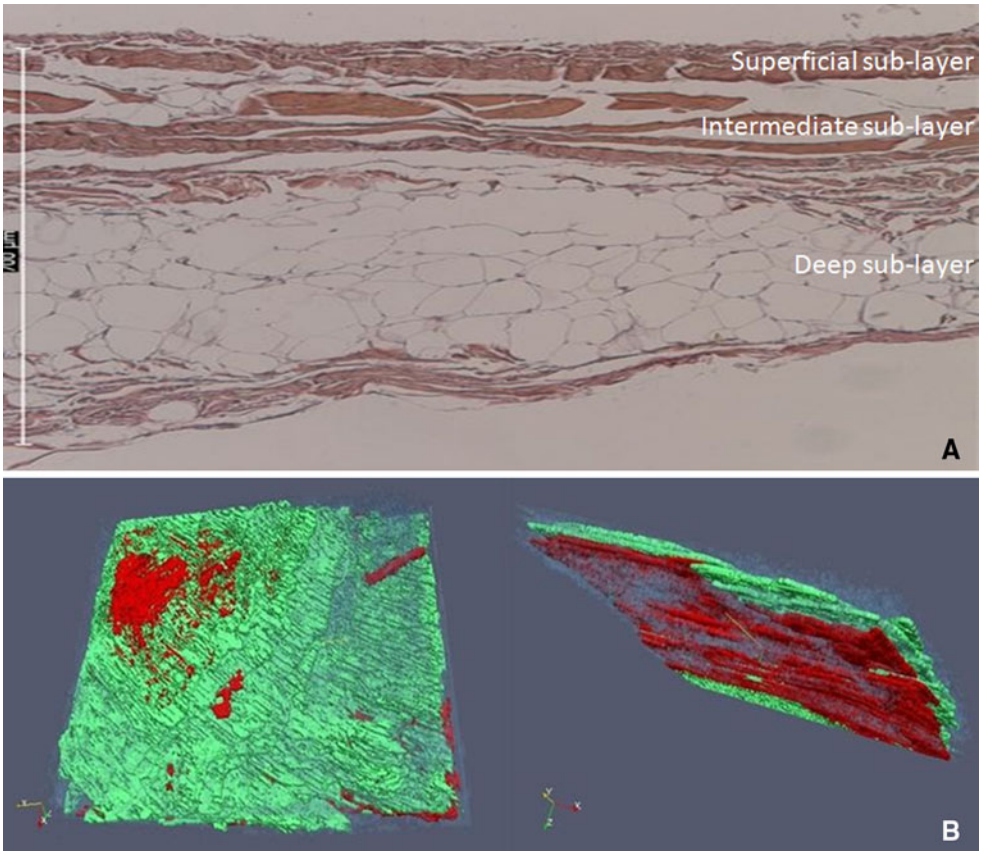

Fig. 7 **a** Histological view of the superficial layer of the thoracolumbar fascia, distinguishing three sub-layers. **b** 3D reconstruction of collagen fibers of superficial layer of the thoracolumbar fascia: a close packed layer of collagen fibers, a layer with the same characteristics as crural fascia and loose connective tissue, is evident

fasciae in question, the relationships among their constitutive elements, and also easier comparisons among the various fasciae of the body. In particular, the present study confirms that the deep fasciae only seem to be irregular, dense connective tissues, as normally stated in anatomical textbooks; in fact, they may be viewed as multilayered structures, formed of two or three sub-layers of collagen fibers with different spatial orientations.

The development of a 3D model is also useful as a measuring tool, thanks to the high resolution and accuracy guaranteed by computerized methods, and the digital nature of the model allowed analysis of tissues from any point of view and enlargement. In addition, 3D allows quantitative determination of the elements forming the tissues and their spatial dispositions and relationships. This is essential in studying the mechanical properties of the deep fasciae and their adaptations to various loads [23, 31]. Unfortunately, the method of image acquisition and processing requires quite small samples of fascia. This limitation means that the results cannot be generalized; though the match between the two sample sites and with literature data [4, 6, 10, 17, 20, 28, 30–33] indicates that at least the deep fasciae of the limbs and the aponeurotic fasciae of the trunk correspond to the described structures.

Our results show that the mean thickness of the superficial layer of the thoracolumbar fascia is 680 μm, a value comparable with that found by Barker and Briggs [4], but completely different from that of Loukas et al. [17]. This is probably because the latter measured that thickness at the midline, where it adheres to the fascia covering the erector spinae muscles, whereas our samples were taken at 7 cm from the midline.

The existence of various sub-layers inside the thoracolumbar fascia has been previously described, but differs according to author. For example, Loukas et al. [17] believe that only two sub-layers exist: one superficial, formed of collagen fibers oriented from craniolateral to caudomedial, and one deep, also called the vertebral aponeurosis, formed of a thin, loose, fibrous layer consisting of longitudinal and transverse collagen fibers blended together. Instead, Bogduk and Macintosh [6] describe specific reinforcements of the thoracolumbar fascia, called "posterior accessory ligaments". Our reconstructions clearly show a superficial sub-layer with characteristics similar to

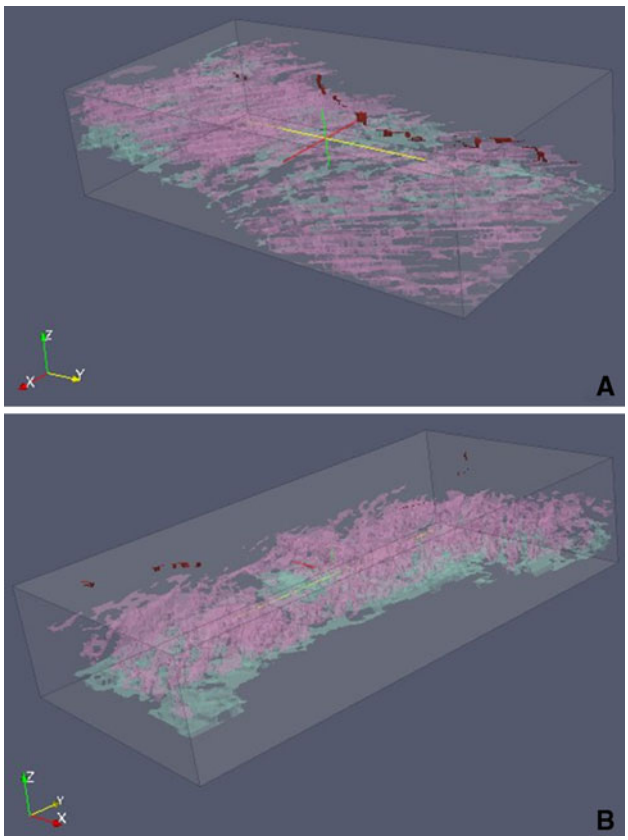

Fig. 8 Two 3D reconstructions of nerve inside the superficial layer of the thoracolumbar fascia. Note how nerves flow in the superficial loose connective tissue

the sub-layers of the crural fascia or the pectoralis fascia, as described by Stecco et al. [28], an intermediate sub-layer, similar to a flat tendon, and a deep sub-layer, formed mainly of loose connective tissue. We therefore propose that the superficial sub-layer be considered the continuation of the deep fascia of the latissimus dorsi muscle, and the intermediate sub-layer the tendon of that muscle; in other words, it could appropriately be called the vertebral aponeurosis, as suggested by Loukas et al. [17].

Lastly, the deep sub-layer should not be considered a true fascial layer, since it allows gliding to occur between the latissimus dorsi and the deeper muscular layer. The angle between the two adjacent sub-layers of the thoracolumbar fascia has been measured by many authors, each reporting different results. For example, Gracovestky et al. [12] state that the collagen fibers of the superficial layer have a 20–30° angle superolaterally (above the horizontal) and those of the deep layer a 20–30° angle below the horizontal. Similarly, according to Barker and Briggs [4], the superficial fibers have an angle of 16–22° (but may reach 35° superolaterally) and the deep ones 68–70°, and McGill and Norman [22] described an angle of 40° above the horizontal. Our 3D model clearly shows the layered arrangement of the fibers, so that this angle could be measured precisely. For confirmation of whether this angle is really 78°, we must increase the number of samples and take them from different levels.

The value of the orientation angle of the fibers limits the extension of the fascia, and thus its mechanical proprieties. In addition, as the fascia is formed of different layers, each with its collagen fibers specifically oriented, the mechanical response of the fascia to stretching is characterized by strong anisotropy, which means that differences are found if the fascia is loaded along the direction of the collagen fibers or along another direction. Knowledge of fiber orientation would provide better understanding of the pathology of fascial tissues. The angle of 78° between the collagen fibers of two adjacent layers does allow a certain mechanical adaptability of the deep fasciae, although the percentage of elastic fibers is low. This finding may explain why the crural fascia can adapt to volume variations during muscular contractions [3] but, over a certain threshold, it stiffens, and internal volume increase leads to an increase in the compartment pressure, e.g., in compartment syndromes. We also hypothesize that in chronic compartment syndromes [21], normal adaptation of the fascia is reduced, due to alterations in the loose connective tissue, which usually allows a certain independence to exist between two adjacent fibrous sub-layers, so that the fascia cannot adapt to muscle volume variations during exercise. Trauma or surgery may also alter the arrangement of the collagen fibers or the independence of the sub-layers produced by the loose connective tissue, although specific research is necessary to demonstrate this hypothesis. Several manual therapies are also used to work on fascial tissues, but each one applies different methods: various types of stretching, light or heavy massage, sudden or progressive compressions, etc. Only deeper understanding of the fascial structure and its biomechanical behavior will definitively allow us to understand what type of action is best to restore fascial functions, permitting them to respond appropriately to different movements.

Conclusions

This study demonstrates that the deep fasciae are only apparently an irregular, dense connective tissue: they are in fact formed of three sub-layers of connective tissue. In each sub-layer, the collagen fibers are all parallel to each other, although their orientation changes in the adjacent sub-layer, forming an angle of 78°. There are few elastic fibers. This specific structure confers upon the deep fasciae a particular biomechanical behavior, which must be analyzed in more depth for better understanding of fascial

pathologies and the roles which various manual therapies may play in the fasciae.

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Conflict of interest The authors declare that they have no conflict of interest.

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