The collagenic architecture of human dura mater

Laboratory investigation

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Object. Human dura mater is the most external meningeal sheet surrounding the CNS. It provides an efficient protection to intracranial structures and represents the most important site for CSF turnover. Its intrinsic architecture is made up of fibrous tissue including collagenic and elastic fibers that guarantee the maintenance of its biophysical features. The recent technical advances in the repair of dural defects have allowed for the creation of many synthetic and biological grafts. However, no detailed studies on the 3D microscopic disposition of collagenic fibers in dura mater are available. The authors report on the collagenic 3D architecture of normal dura mater highlighting the orientation, disposition in 3 dimensions, and shape of the collagen fibers with respect to the observed layer.

Methods. Thirty-two dura mater specimens were collected during cranial decompressive surgical procedures, fixed in 2.5% Karnovsky solution, and digested in 1 N NaOH solution. After a routine procedure, the specimens were observed using a scanning electron microscope.

Results. The authors distinguished the following 5 layers in the fibrous dura mater of varying thicknesses, orientation, and structures: bone surface, external median, vascular, internal median, and arachnoid layers.

Conclusions. The description of the ultrastructural 3D organization of the different layers of dura mater will give us more information for the creation of synthetic grafts that are as similar as possible to normal dura mater. This description will be also related to the study of the neoplastic invasion. (DOI: 10.3171/2010.12.JNS101732)

Key Words • dura mater • scanning electron microscope • Othani maceration protocol • collagen fiber

One of the earliest detailed morphological macroscopic descriptions of dura mater was by Vesalius in the 16th century in his De Humani Corporis Fabrica:

Dura cerebri comonstrat membranam adhuc illesam, neque aliqua ex parte pertusam, vulneratam us. Quamuis interim ipsius membrane vincula divulsimus, quae per capitis suturas ad membranam efformanda porriguntur. Atquae cum his fibris pariter vascula sunt effracta, quae per calvariae foraminula et sutures deducta, ipsi durae membranae, ac illi qua calvaria succingitur, communes censentur. [Dura mater is a continuous membrane, without any holes or openings. It is strictly adherent to the cranial structures. Its vessels are enclosed in the dura mater itself and can also enter the skull through bone foramina.]

These words, borrowed from one of the first scientific reports that would form the basis of later morphological studies, describe the dura mater and its structure very well. The dura mater, also called pachymeninx, forms a continuous collagenic sheet surrounding intracranial and spinal nervous structures. It provides efficient protection to the CNS against infections and traumatic injuries acting on the skull and spine.

Dura mater is firmly attached to the cranial vault and reaches the foramen magnum, where it divides into 2 layers. The external layer constitutes the periosteum of the spinal canal, and the internal layer creates the meningeal covering of the spinal cord and the first tract of spinal nerve roots.

Folds of dura mater form the falx cerebri and tentorium cerebelli, providing a macroscopic division of intracranial spaces into supratentorial (right and left) and infratentorial compartments. Some other lesser folds are also present, namely the sellar floor, olfactory tent, and falx cerebri.

Due to some arachnoid specialization called “Pachioni granulation” and venous bridging cortical vessels, the dura mater is highly involved in CSF resorption and, consequently, in the maintenance and balance of intracranial pressure.

Viewed through a light microscope, 3 different layers of dura mater have been described. The outer dural border layer, which is 2 μm thick, is the thinnest layer and is composed of fibroblasts with long cellular extensions and collagen and elastic fibers. The median layer, called the fibrous dura, is vascularized and its thickness varies depending on location (that is, the cranial or spinal region). The innermost layer, the dural border cell layer,
is 8 μm thick and is composed strictly of cells that adhere to arachnoid trabeculae.

Viewed from the outside, the bundles of collagen fibers are macroscopically oriented throughout the cranial convexity; due to the mechanical forces acting from inside and outside on the skull, the orientation follows the trajectories of the spongy bone. As a consequence, focal mechanical forces are distributed in a more extended area. This macroscopic disposition varies greatly depending on the location in the cranial region and the age and the development of the skull.

In neurosurgical practice, dural defects may be due to trauma, inflammatory or neoplastic processes, surgical procedures, or congenital abnormalities. In these circumstances, the need to close dural defects has prompted a quest for studying the microscopic structures and physical properties of the dura mater with the aim of making an “ideal” substitute.

An “ideal” graft is one that can reproduce the biomechanical characteristics of host dura mater to avoid any inflammatory response or neurotoxicity. Moreover, the graft should be resorbed to form a newly developed dura with the same fiber architecture as in normal conditions. There should be no adhesion to surrounding tissues, and the graft should be resistant to tearing forces, should be watertight, free of prions and viruses, and easy to handle and apply.

For these reasons, beginning in the 20th century many natural and synthetic dural substitutes have been introduced in clinical practice, and over time, numerous experimental studies have been conducted on these products to examine their physiochemical properties and integration with normal dura mater. Autografts, allografts, xenografts, and nonabsorbable and absorbable polymer sheets have been used. Some complications have been reported, such as chronic infections, intense cellular reaction and thick encapsulation, hematoma or foreign body reaction, and Creutzfeldt-Jacob disease. Among all these substitutes, resorbable collagen implants seem to be best able to replicate the physiological composition of dura mater and to guarantee a natural scaffold for integration and replacement of the entire graft with normal dura mater.

Collagen has many properties; because it is a resorbable protein, its degradation can be controlled by cross-linking. Collagen is hemostatic and able to induce cellular growth with a final tissue reconstruction. The major problem of these types of substitutes is the risk of an immunological or severe inflammatory reaction: the collagen should be highly purified, not pyrogenic, and without telopeptides. Hence, various chemical or physical procedures have been used to inactivate viruses, bacteria, and prions. To date, many collagen-based dural substitutes have been synthesized and tested in experimental in vitro and in vivo studies, and data on the integration with normal tissue are available.

Despite the large number of these dural substitutes and the growing interest in finding “ideal” dural substitutes that can mimic the physiological structure of human dura mater and avoid complications, there have been few studies of the microscopic morphology, disposition, and 3D architectural distribution of the collagenic component in human dura mater. Our study aims to describe the microscopic 3D structural features and patterns of the collagen fibers as principal components of dura mater architecture.

Methods

All procedures were performed observing common guidelines in dealing with private personal data. Thirty-two specimens of dura mater that could not be reimplanted were collected during decompressive surgical procedures performed in patients with uncontrollable intracranial pressure or posttraumatic injuries.

The size of the specimens was constant (Fig. 1 lower; size = 1.25 cm²), and the shape was trapezoidal with squared angles to guarantee the correct orientation. The specimens were taken from frontal, temporal, parietal, and occipital (8 specimens of each) convexities and immediately immersed in Karnovsky solution (0.25% glutaraldehyde and 0.25% paraformaldehyde, in 0.1 M cacodylate buffer at pH 7.2). The collected specimens were then left in fixative solution for 2 weeks; the solution was changed after 1 week. After the fixation process, each specimen was cut with a razor blade, and from each specimen 3 more were obtained: one to observe the bone surface, another to study the thickness, and the last to examine the arachnoid surface (Fig. 1 lower [b]). All these specimens were then immersed in a solution of 1 N NaOH. The solution was changed daily to wash away the digested organic tissue.

All specimens were dehydrated in graded alcohol, critical point dried in an Emitech K850 CPD apparatus, mounted on aluminum stubs on adhesive film, and coated with 10 nm of gold in an Emitech K250 sputter-coater. Because of the size of some specimens, metallic bridges were needed to maintain a good conduction over the entire stub. The specimens were then observed using a Philips XL 30 FEG scanning electron microscope at 10 kV.

Three-Dimensional Micrometric Analysis

All the samples were analyzed using scanning electron microscopy 3D micrometric analysis software, and the data were recorded. The scanning electron microscopy stereo images were fed to proprietary surface reconstruction software, whose implementation details are discussed in detail elsewhere. The software reads a stereo-pair of scanning electron microscopy micrographs, selects a point in the first picture and identifies the same in the second picture, computes the height of all the key points thus identified in both micrographs, and connects the points obtained using a Delaunay triangulation to reconstruct the spatial shape of the original specimen.

All VRML (virtual reality modeling language) files were studied using a second proprietary software program (MicroMetric version 1.1.3, 2004, M. Raspanti) that yields mathematically correct 3D measurements. The obtained quantitative data were evaluated using SPSS statistical software.

Digital Image Directional Analysis

We conducted a computerized colorimetric investi-
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gation of the specimens, and we evaluated the orientation of
the collagen fibers. The quantification was performed
automatically by obtaining a colored map that showed the
distribution of fibers along different orientations. Speci-
mens were analyzed at ×4000 to allow a precise recogni-
tion of single fibers and to easily follow their course along
the investigated layers.

Results

Scanning electron microscopic observation of the
thickness of dura mater allowed us to distinguish 5 dif-
ferent layers as follows (Fig. 1 upper): one facing the inner
Table of the cranial bone (the bone surface layer (α), the
external median layer (β), the vascular layer (γ), the inter-
nal median layer (δ), and the layer facing the arachnoid
membrane (ε). To provide a detailed description of dura mater col-
lagenic composition, we investigated all the areas repre-
ated in Fig. 1 lower (a). The specimens were kept as
represented and their orientation was maintained (Fig. 1
lower [b]).

At low magnifications, the layers were distinguish-
able on the basis of the reciprocal orientation and shape
of their collagen fibers, and at high magnification by the
presence of certain specialized structures. They are de-
scribed in detail below.

Bone Surface Layer

At low magnification (×300) at the level of the sur-
face facing the bone, the dura mater appears to be flat,
without any significant corrugation even if the imprints
left by meningeal arteries and veins are sometimes visible
(Fig. 2A asterisk). In some cases, we can also observe the
“organic collagenic skeleton” of larger vessels lying on
the surface of the dura mater.

The thickness of the bone surface layer can be seen in
a transverse section and measures approximately 20 μm
(Fig. 2B). At a higher magnification, the collagenic com-
position of this layer appears to be made up of 2 different
components. The upper component is characterized by a
thin net of interlaced single collagen fibers without any
particular orientation (Fig. 2C), and the lower compo
nt is formed by collagen fibers organized in larger bundles
that run parallel to each other and that maintain more or
less the same orientation (Fig. 2D). When observed in
detail, these fibers reveal their characteristic 67-nm-long
stripes.

In the transverse and oblique sections of this layer, it
is possible to observe the relationships with the underly-
ing external median layer. The shape of collagen bundles
lying between the external median layer and the bone
surface layer is convoluted, tortuous, and without any
constant orientation (Fig. 2E arrow). These bundles dif-
fer from those in the bone surface layer and from those in
the external median layer. They most likely represent the
extracellular matrix structures that provide connection
between these 2 layers.

The collagen fibers of the bone surface layer may
have some specialized organization. Focal aggrega-
tions of disorganized collagen fibers are clearly visible
among regularly directed underlying fibers (Fig. 3A). At

Fig. 1. Upper: Transverse section of the dura mater seen by scan-
ing electron microscopy. At low magnification, it is possible to distin-
guish 5 different layers depending on the orientation with respect to the
bone. The most external is in direct contact with the bone and is called
the bone surface layer (α). The fibrous dura recognized by the anato-
mists as the median layer comprises the external median layer (β), the
vascular layer (γ), and the internal median layer (δ). The most internal
layer in direct contact with dural border cells and facing the arachnoid
mater is called the arachnoid layer (ε). Lower: The scheme is divided
into 2 parts. The first (a) represents each layer described in the upper
panel and is examined in the study as follows: the bone surface layer
(α), the external median layer (β), the vascular layer (γ), the internal me-
dian layer (δ), and the arachnoid layer (ε). The bone of the calvaria (χ),
the arachnoid membrane (ψ), and the brain cortex (k) are also shown.
The second part (b) represents a single specimen divided into 3 lesser
specimens for the study: the first (1) is the specimen used to study the
bone surface layer, the second (2) to study the arachnoid layer, and the
third (3) to study the thickness of the dura mater.

high magnification, these fibers reveal a characteristic
180°-angled shape (Fig. 3B). Collagen bundles also line
some lacunae that represent the loci usually occupied by
fibroblasts (Fig. 3C). At high magnification, these lacunae
appear to be partially covered by a thin sheet of inter-
laced, angled collagen fibers (Fig. 3D and E). At the bot-
tom of these lacunae, a well-organized collagenic sheet
of larger bundles is visible. Digital image analysis has dem-
onstrated that almost all of these fibers are oriented in one
direction (details below). Thin collagen fibers can also be
seen extending between the walls of the lacunae.

External Median Layer

The most external subdivision of the so-called “fi-
brous dura” (Fig. 4A) median layer contains collagen
bundles that seem to be oriented in one direction (β),
which is different from that of the layer above. We cannot be more precise about the orientation with respect to the cranial vault axes because the direction varies according to tensile forces of bone growth and depends on convexity curvature. What remains constant is the reciprocal distribution of the orientation of collagen fibers. The only exception is represented by the external median layer: in fact, in some specimens we cannot distinguish this layer from the vascular layer and we can recognize only a single thicker layer enclosing collagen fibers with almost the same orientation.

Vascular Layer

The vascular layer is the median layer of “fibrous dura” (γ), which lies between the external median and in-
Internal Median Layers (Fig. 4D). Even at low magnification, many holes are visible. The holes are more concentrated than those found in the external median layer and are clearly visible between collagen bundles. At high magnification, it is possible to see that the holes are vascular channels with a whorl-like arrangement of collagen fibrils constituting the wall (Fig. 4E). Between them, disorganized collagen fibers and thin interlaced single fibers are visible, constituting the extravascular interstitial compartment. However, the distribution and orientation of these collagen fibers reflect that of the upper external median layer.

Internal Median Layer

The internal median layer includes collagen fibers organized in bundles transversely oriented with respect to those in the upper layers (Fig. 4F). These bundles run parallel to each other. It is sometimes possible to observe a clear division between the vascular layer and the internal median layer caused by the shrinkage of collagen fibers due to tensile forces acting during the dehydration procedure. These artifacts clearly demonstrate the presence of thin and weak collagenic sheets loosely connecting them.

No significant specialized organization is observed in this layer with the exception of occasional vascular wall sections that refer to the inner dural capillary plexus.

Arachnoid Layer

The arachnoid layer is directly in contact with dural neurothelial border cells. At low magnification, the surface appears to be irregular with deep invaginations and furrows (Fig. 5A and B). At high magnification, disorganized collagen bundles with a tortuous shape are visible, forming collagenic structures without any constant orientation. Some little holes are visible among them, identifiable as capillaries belonging to the well-known plexus (Fig. 5C). At high magnification, the collagen bundles reveal their specific shape as formed by spiral collagen fibers stuck as fibers in a cord (Fig. 5D).

Digital Analysis

Digital image analysis demonstrated that, in the bone surface layer, almost all the fibers are oriented in one direction (Fig. 6A–C); however, the fibers in the arachnoid layer are oriented in many different directions (Fig. 6B and D).

Discussion

For a long time, the structure of the dura mater has been described in 2 different ways. Neurosurgeons view it as a double fibrous layer of collagen fibers adherent to the entire internal skull surface, whereas anatomists, using microscopic investigations, distinguish 3 different layers.28,29

As a matter of fact, during surgical procedures it is possible to see and distinguish, while gently opening the dura mater, its 2 layers, but when observed under a light microscope 3 layers are clearly evident. These are called outer dural cells, fibrous dura, and dural border cells facing the arachnoid membrane.

Despite its apparently simple structure, dura mater has multiple biomechanical and physical properties that guarantee the protection of intracranial structures, CSF turnover, and drainage of venous blood from the brain.
This, along with the increasing need to repair dural defects due to surgical procedures or traumatic injuries, has always driven the scientific interest in reproducing the same morphological characteristics in an “ideal” substitute that could mimic the physical and biological features of dura mater itself. As yet, however, no detailed report is available regarding the 3D architecture, disposition and orientation of collagen fibers, and the number of layers in human cranial dura mater.

We have analyzed specimens from surgical decompression procedures performed in patients affected by brain swelling or traumatic brain injuries. We have analyzed fresh human specimens to avoid any bias caused by artifacts that are commonly observed in cadaver specimens due to conservation, and we focused our attention on the major components of dura mater, that is, collagen fibers, with a goal of describing its 3D disposition and architectural organization. Therefore, we decided to use the 1 N NaOH maceration method and observation with scanning electron microscopy to obtain 3D data regarding collagen fiber shape, disposition, and orientation without interference of other organic (cellular or vascular nervous structure) components. The 1 N NaOH scanning electron microscopy investigation technique is a commonly used method to study the collagenic component of many tissues and organs in normal and pathological conditions.

In terms of clinical application, this technique is quite similar to that recommended by the WHO (1992) (1 N NaOH for 1 hour at 20°C) for prion inactivation for allografts. Other techniques can be used for this purpose, such as sodium hypochlorite 2% for 1 hour at 20°C or autoclaving at 132°C for 1 hour, but NaOH maceration is chosen because it is the only technique that really preserves the collagen structure of the allograft. At the end of this treatment, the goal is to obtain a collagenic allograft that has a very low risk of conventional and unconventional agent transmission without losing its 3D collagenic architecture. As previously stated, dura mater has always been considered to be composed of 2 or 3 layers. Using this technique, we were able to observe 5 different layers of dura mater, distinguishable mainly based on the distribution and reciprocal direction of collagen fibers.

However, it is not possible to precisely define the orientation of the fibers with respect to the cranial vault axis for 2 main reasons. First, the vault axis varies according to tensile forces of bone growth and its curvature. What remains constant is the reciprocal distribution of the orientation of collagen fibers in each layer. Second, even if the orientation of collagen fibers varies depending on the layer observed, visualization of these fibers, with the exception of those in the bone surface and arachnoid layers, also depends on how the specimens were cut. For these reasons, only the reciprocal directions of the fibers can be taken into account.
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Following our analysis, the outermost layer that is in direct contact with the internal surface of the skull has been named the bone surface layer. The median layer, previously identified by anatomists as fibrous dura, has been divided into 3 layers: the external, vascular, and internal layers. The innermost layer directly in contact with dural border cells has been named the arachnoid layer.

It is widely known that physical characteristics such as tensile strength and resistance to tearing forces strictly depend on the orientation of collagen fibers. Such fibers provide the dura mater with all its mechanical features and form the basis of its multiple characteristics. For these reasons, we particularly focused our attention on this aspect using scanning electron microscopy analysis software that is able to analyze the distribution of the entire length of the collagen fibers in each layer.

The bone surface layer is very regular even if the contact with bone would suggest a more disorganized shape. This flat layer probably represents the basal membrane of a limiting fibroblast sheet that guarantees the attachment to bone tissue. Computerized colorimetric examination of this layer revealed a single common direction of collagen fibers.

Collagen fibers in the median layer are variously arranged in 3 dimensions forming 3 different layers: the external, vascular, and internal median layers. The change in the direction of the collagen fibers is mostly visible between the vascular and internal median layers. Therefore, this site is subject to the highest stretching forces representing a locus minoris resistentiae that allows neurosurgeons to distinguish dura mater into 2 layers. In the vascular layer, it is possible to see how the collagen fibers that make up the dura mater also provide the organic scaffold to cellular components (fibroblasts, vessels, and nerve endings). Fibroblasts are enclosed in collagenic lacunae well visible from the bone surface layer and in median layers. Connection with dural border cells and the passage to the arachnoid layer is evident by the presence of the loosely composed arachnoid layer. This layer is characterized by large bundles of collagen causing a corrugated layer already visible at low magnification. Computerized colorimetric analysis demonstrates a wide distribution of the fibers in all directions without any prevalent orientation.

Conclusions

This study focuses on the structural and ultrastructural features of the dura mater, highlighting its intrinsic collagenic structure. The 1 N NaOH maceration method applied to scanning electron microscopy analysis has been shown to be a good investigative technique to evaluate features of the dura mater in depth. Knowledge of the disposition of its collagen fibers in 3 dimensions and their distribution along all its layers could be useful in evaluating dura mater allografts and in creating new allografts with morphological features that are more similar to the dura mater itself.

Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author contributions to the study and manuscript preparation include the following. Conception and design: Protasoni, Sangiorgi. Acquisition of data: Protasoni, Sangiorgi, Cividini, Reguzzoni, Raspani. Analysis and interpretation of data: Protasoni, Sangiorgi, Reguzzoni, Raspani, Tomei, Cividini. Drafting the article: Protasoni, Sangiorgi. Critically revising the article: Protasoni, Dell'Orbo, Raspani, Balbi, Sangiorgi. Reviewed final version of the manuscript and approved it for submission: Protasoni. Administrative/technical/ material support: Culuvaris, Raspani. Study supervision: Tomei, Dell’Orbo, Raspani, Balbi, Reguzzoni.

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