Unveiling the Fourth Meningeal Layer: Implications for Brain Structure, Function, and Neurological Disease

Kamaljeet[a], Yavnika Minhas[a], Sourabh Kosey[a]*

[a]Department of Pharmacy Practice, ISF College of Pharmacy, Moga, Punjab-142001, India

***Address of Correspondence:**

Dr. Sourabh Kosey

Professor & Head

Department of Pharmacy Practice

ISF College of Pharmacy

GT road, Ghal Kalan, Moga, Punjab-142001, India

Orcid ID: 0000-0002-3102-0069

Email: sourabhkosey@gmail.com, sourabh@isfcp.org

Authors Details:

- **Kamaljeet**, E-mail ID: skamaljeet130@gmail.com, 2116957@isfcp.org
  Orcid ID: 0000-0002-1023-6880
- **Yavnika Minhas**, E-mail ID: yavnika2121@gmail.com, yavnika@isfcp.org
  Orcid ID: 0009-0006-4791-3976

ABSTRACT

The central nervous system (CNS), which consists of the brain and spinal cord, is protected by specialized connective tissue layers known as the meninges. These were traditionally regarded as passive barriers that offer mechanical protection and maintain the circulation of cerebrospinal fluid (CSF). Nonetheless, recent studies have found a fourth meningeal layer, a subarachnoid lymphatic-like membrane (SLYM) located in the subarachnoid space. The SLYM is a physical and immunological barrier that isolates CSF and mediates the local immunological surveillance. Increasing age of this membrane has been linked to neurodegenerative diseases like Alzheimer disease; and its mechanical and inflammatory characteristics indicate it has a role in traumatic

brain injury. This finding opposes classical neuroanatomical beliefs and points out to the active participation of meninges in the homeostasis and pathology of the brain. In case in vivo imaging methods have the ability to visualize the SLYM, it may become a new diagnostic biomarker and therapeutic target of CNS disorders. This review summarizes the architecture and role of the SLYM, its role in neurological disease, and has the potential to revolutionize how we understand the barriers in the brain and the immune system.

KEYWORDS: meningeal layers; subarachnoid lymphatic-like membrane; cerebrospinal fluid; traumatic brain injury; blood-brain barrier

1. INTRODUCTION

The brain, spinal cord, and numerous cranial nerves constitute the higher vertebrate central nervous system (CNS), which regulates and governs all body functions [1]. The CNS has developed in an exceptionally intricate manner to integrate various sensory inputs and generate the necessary outputs needed to transmit and receive the required intercellular messages across long distances. Critical developmental stages allow a neuron to obtain its specialized functions, including the generation and extension of action potentials, the transmission and reception of information by other neurons via neurotransmitters, the establishment and maintenance of more complex processes, and the movement of molecules and organelles through those processes [2]. In vertebrates, the somatic system has nerves that detect receptors on the skin, the spinal cord, and muscles, but the autonomic nervous system has nerves that control unconscious nervous activities including the heart rate and breathing rate. Neurons most likely offered initial control of opposing effectors in developing animals, which offered them multiple evolutionary advantages, such as the capability to regulate body temperature and avoid other constraints posed by a hydrostatic skeleton.

The meninges are connective tissue membranes that surround and protect the brain and spinal cord known as the meninges, consist of 3 tissue membranes. They consist of arachnoid, pia and dura mater respectively [3]. The outermost layer of the dura mater develops a sac containing the extra meningeal layers; this sac is also known as pachymeninges (patchy-thick) **(Figure 1)**. It appears as three folds: the first envelops the sella turcica and pituitary gland; the second folds the occipital lobe and cerebellum (tentorium cerebelli and falx cerebelli); the third folds the two cortical hemispheres (falx cerebri) [4]. It surrounds and supports the two venous sinuses. The two layers that make up the inner membranes together are called leptomeninges, which translates from Greek as "thin". Arachnoid, short for "spider," is the outermost layer, while pia mater is the innermost. The pia, which crosses the subarachnoid space and houses the cerebrospinal fluid (CSF) generated by choroid plexi, is connected to the arachnoid by arachnoid trabeculae [5].

It is a well-known fact that meninges and CSF protect the central nervous system. To a great extent, this is due to the ability of the meninges to firmly fix the CNS to the surrounding bones, restricting movement and enhancing stability [6]. The periosteal layer, the outer layer of the dura, is securely affixed to the head's skull. Twenty-one pairs of denticulate ligaments, in

addition to the periosteal layer, join the pia, arachnoid, dura mater, and vertebral bones in the spinal cord. Additionally, meninges contain CSF, which enables the central nervous system to "float" and protects it from traumatic experiences.

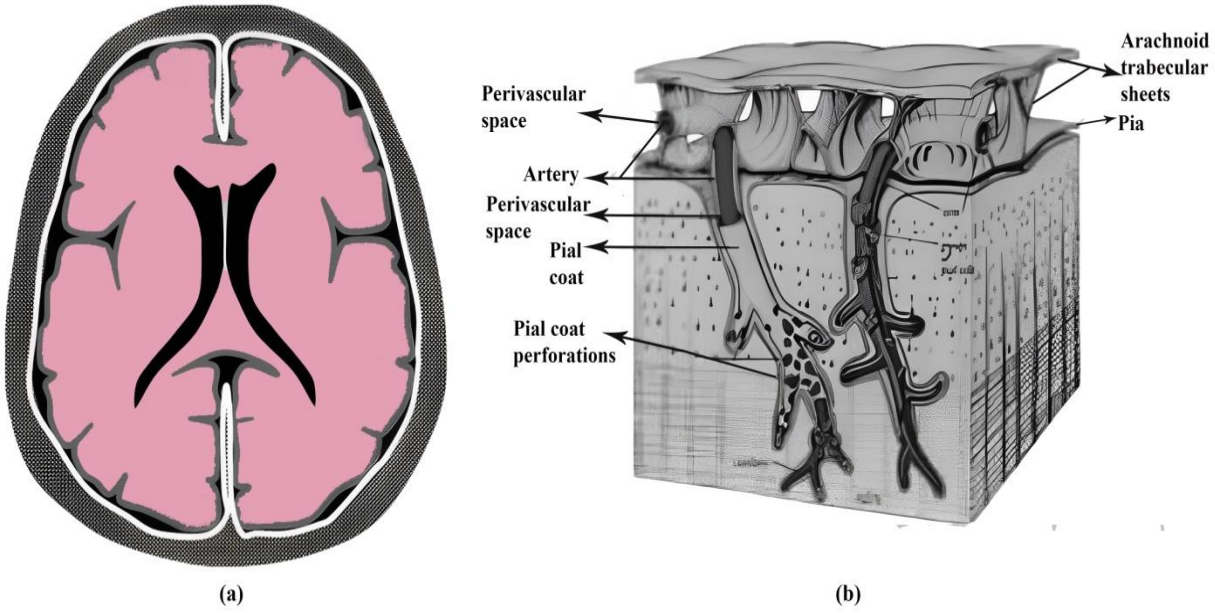

Figure 1. (a) The falx in the interhemispheric fissure, the tentorium in-between the cerebellum, the vermis, and the occipital lobes, and the inner table of the skull are regions in the diagram where dura-arachnoid augmentation could be present. Pure dural augmentation cannot fill sulci or basilar cisterns without pial or subarachnoid involvement. (b) A schematic illustration of the brain leptomeninges, showing the subarachnoid space enclosed by the arachnoid mater and pia mater. The pia mater closely adheres to the brain surface and extends along the outer surfaces of arteries and veins within the subarachnoid space, entering the brain only along the perivascular regions of penetrating vessels. The Figures were created by using Adobe Photoshop.

1.1. Literature Search Strategy

This narrative review was conducted using literature retrieved from major scientific databases including PubMed, Scopus, and Google Scholar. Keywords used in the search included

"meninges", "subarachnoid lymphatic-like membrane", "SLYM", "meningeal immunity", and "cerebrospinal fluid dynamics". Articles published between 2015 and 2025 were primarily considered, with particular emphasis on recent studies describing the discovery, structure, and functional significance of the SLYM. Relevant review articles and experimental studies were evaluated to provide a comprehensive overview of current knowledge regarding meningeal biology and its implications for neurological disease.

2. Origin and Structure of the Fetal Meninges

Early symptoms of an embryo with an established meningeal layer may be observed in a chick on HH15 (embryonic day, or E2), a mouse between E9 and E10, and a human at a Carnegie stage 15, or the fourth gestation week [7]. The portion of an influx of cranial neural crest cells that are migrating rostrally and originating from the diencephalic neural crest is this initial layer of meningeal cells in the forebrain. The pia and arachnoid layers of the leptomeninges, the first layer of meningeal cells, essentially make up these structures [8]. Throughout the blood arteries of the perineural vascular plexus in embryos at the glial limitans, or the border of the brain, is a loose network of cells referred to as the leptomeninges. Mouse embryos Leptomeninges first develop at E13, and in human embryos at stage 17 to 18. The dura, the outermost layer of the meninges lies between the leptomeninges and the calvarial mesenchyme that will later become the calvarial bones. Studies showing that the transcription factor Foxc1 is required for meningeal assembly creation in the forebrain have provided insight into meningeal assembly. An amorphous Foxc1 mutation Although Foxc1's precise function in meningeal development is yet understood, its less severe meningeal phenotype indicates that it controls meningeal cell migration [9]. In Foxc1 hypomorphs, in particular the more dorsal regions, the meninges are absent; the dural and arachnoid layers partially encircle the forebrain in the Foxc1hith/lacz hybrid (Foxc1hithlacz/lacz). This implies that Foxc1 plays a crucial role in meningeal development, in any case at least in the forebrain where meningeal cell migration can happen in a ventral-dorsal wave.

2.1. Developmental and Age-Related Changes of Meninges and SLYM

The development of the meninges begins early during embryogenesis, primarily originating from neural crest cells and mesodermal contributions. In humans, primitive meningeal layers can be

identified around the fourth gestational week, with progressive differentiation into dura mater, arachnoid mater, and pia mater occurring during subsequent stages of fetal development [10]. The leptomeninges (arachnoid and pia) develop earlier, while the dura mater forms later as a more fibrous protective layer. Emerging evidence suggests that the SLYM develops as part of the leptomeningeal system during the maturation of the subarachnoid space. Although precise developmental timelines remain incompletely defined, studies indicate that SLYM formation coincides with cerebrospinal fluid compartmentalization and meningeal maturation.

Aging-related changes in meningeal structures, including potential alterations in SLYM integrity, have been proposed. Structural thinning, reduced barrier function, and altered immune cell distribution may occur with advancing age, potentially impacting cerebrospinal fluid dynamics and immune surveillance [11]. These changes may contribute to age-associated neurological disorders; however, direct evidence in humans remains limited and warrants further investigation.

3. Meninges and Other Barriers

To protect homeostasis and preserve the CNS, the meninges are a vital three-layered tissue that covers the brain and spinal cord and which consists of dura mater, arachnoid mater, and pia mater [12]. These layers function both as physical protection and assist in segregating essential physiological operations like immunological monitoring and CSF transport. The dura mater is attached to the inside of the skull and forms the outermost layer of the skull with its thickness and durability. The dura mater consists of two sublayers that form its structure: the meningeal layer closer to the brain tissue while the periosteal layer lies next to the cranial bone [13]. Dural venous sinuses develop frequently through the layers that drain venous blood from the brain **(Figure 2.)**.

The arachnoid mater achieves its name from its web-like features because it exists under the dura. This tissue covers the cerebral cortex sulci to separate the dural tissue from the pial tissue. This space is the subarachnoid space between the arachnoid mater and the pia mater and it gets filled with CSF due to this place [14]. Arachnoid trabeculae maintain the integrity of both membranes by crossing the area to create structural stability. The inside-most meningeal layer named pia mater follows precisely the brain's shape by inserting into sulci and fissures. CSF

passes through this tissue layer because it is semi-permeable while the tissue is very thin and contains an abundant blood supply [15]. The tissue spaces of Virchow-Robin gaps embedded in pia mater allow blood vessels to access brain parenchyma while enabling fluid passage and immune cells to move throughout the tissue. The development of the meninx started as a single meninx in fish before evolving into the three-layered structure seen in birds and mammals. Thus the human meninx represents greater evolutionary complexity. The growing necessity for better CNS protection from physical and viral attacks alongside enhanced control of CNS environment explains this developmental pattern [16].

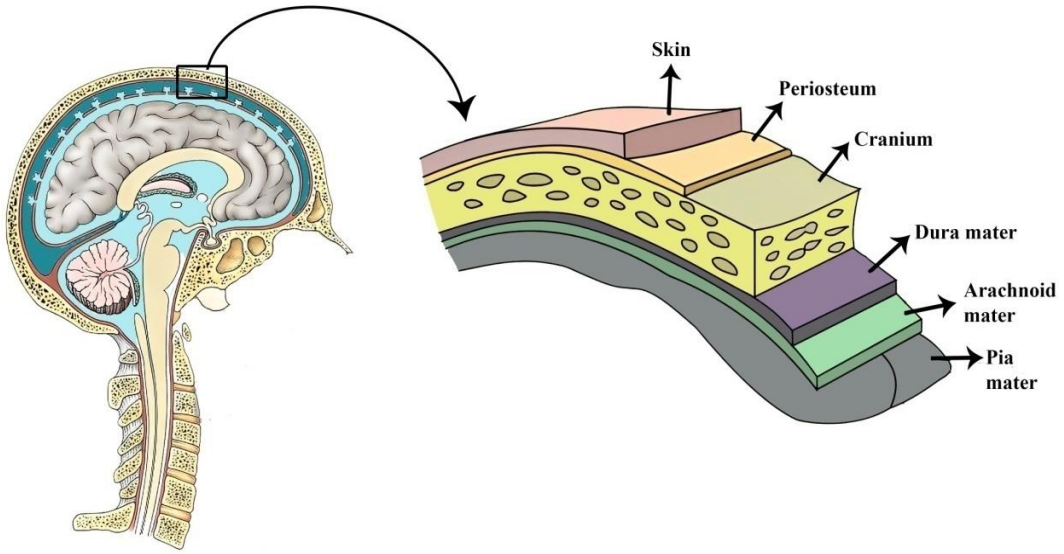

Figure 2. Overview of the meninges and their connection to the skull and brain. The Figures were created by using Adobe Photoshop.

Notably, these meningeal barriers control exchange of substances between bloodstream and brain tissue via their association with brain barriers such as blood-brain barrier (BBB) and blood-CSF barrier [17]. The subarachnoid space contains two fundamental functions: CSF storage and metabolic waste clearance operations and buoyancy-supported weight reduction of the brain. These multifunctional meninges actively regulate immunity, support disease processes, and maintain central nervous system stability. A comparative overview of the structural and functional characteristics of the meningeal layers, including the recently proposed SLYM, is presented in ***Table 1***. They serve purposes greater than providing fundamental protection.

Table 1. Comparison of Meningeal Layers

Layer	Location	Structure	Function
Dura mater	Outer layer	Thick fibrous tissue	Mechanical protection
Arachnoid mater	Middle layer	Web-like membrane	Maintains CSF space
Pia mater	Inner layer	Thin vascular membrane	Covers brain surface
SLYM	Subarachnoid space	Thin mesothelial membrane	CSF compartmentalization and immune surveillance

4. Role of Meninges

4.1 Role of meninges in corticogenesis

The functionality and existence of the meninges are essential in the proper development of the CNS. It is still unknown how the meninges and meningeal cells involve themselves in the process in a molecular sense [18]. The anterior neural folds form the neural crest which produces connective tissue and pericytes. At the same time, the mesodermal components of the forebrain meninges provide the endothelial walls of blood vessels that pass through the neuroepithelium. The apoptosis of the entire neuroepithelium of the forebrain takes place and is killed when the posterior diencephalic and mesencephalic neural folds are deleted. [19]. The cells of the neural crest in the neural folds can be replaced by paraxial mesoderm, which results in the primitive leptomeninges required for encephalon formation. In later development, the meninges surrounding the cerebellum degenerate resulting in subarachnoid space neuronal ectopia and gliosis, cerebellar hypoplasia and the loss of the granular cells [20].

Secondary abnormalities in the dentate gyrus can be developed meningeal loss in the hippocampus. Due to their privileged location within the parenchyma and their close association with blood vessels, the meninges are able to provide short-range signals to neural cells during the maturation of brain structures [21]. Since it may bind several trophic and morphogenetic components, the extracellular matrix that makes up the basal membrane is only employed during corticogenesis. Meninges' most common extracellular matrix structures are collagens, of which

kinds I, III, and IV are the most commonly seen. Non-collagen proteins such as fibronectin, laminin, tenascin, and collagen are produced by meningeal cells [22]. The radial processes provide neurons with a moveable framework throughout cortical and cerebellar development, ensuring cellular layering. When there is a hereditary ablation of the proteins governing extracellular matrix attachment, or of laminin components α1, α2, and α5, it leads to the disruption of cortical and cerebellar histogenesis, loss of pial integrity, and separation of the radial endplates. One possible explanation is that laminins are involved in connexins' functional localization [23]. As clusters of neurons invaded the marginal zone and retracted radial glial endfeet, midline fusion of the brain hemispheres and gliosis, as in congenital muscular dystrophy, deletion of focal adhesion kinase in the meninges of the brain targeted resulted in to altered histogenesis of the cortex, resembling type II cobblestone lissencephaly [24].

4.2 Role of meninges in CNS homeostasis

There are numerous extracellular matrix syntheses, such as heparan sulphate-derived proteoglycans, collagens IV, XV, and XVII, laminin, and fibronectin that are found on the surface of the brain and surrounding the blood vessels [25]. It was believed that this material formed a clear interface that provided a physical and functional distinction between the brain parenchyma (glia and neurons) and the extra parenchymal tissues (meninges and arteries). Other significant trophic factors derived from the meninges include retinoic acid, CXCL12, FGF-2, and insulin-like growth factor-II. FGF2 and EGF, heparin-binding molecules that could interact with the heparan Sulphate chains of the heparan Sulphate proteoglycan that is abundantly present in meninges, are found in numerous growth factors and cytokines [26]. Interestingly, it has been found out that meningeal cells are very sensitive to major mitogenic factors like EGF, FGF-2 and BDNF. Gap junction proteins are another sign that the meninges are somehow connected to the brain tissue. The cells that have been found to be Cx43, Cx30 and Cx26 include meningeal sheaths of blood vessels and stroma of the choroid plexus along a network of cells within the meninges and their projections into the brain [27]. These proteins are found to support anatomical and functional connections between ependymocytes and astrocytes, meningeal cells and meningeal perivascular cells.

4.3 Maintenance of the pial basement membrane by the meninges

A basement membrane (BM) that is rich in extracellular matrix components covers the pial meningeal layer. Pial BM attaches the endfeet of radial processes which have a neuronal origin in the ventricular zone (VZ), and it is also a physical obstacle to migratory neurons [28]. Radial processes provide neurons with a moveable framework throughout cortical and cerebellar development, facilitating proper cellular layering. Pial BM integrity is lost, radial endfeet separation occurs, and cortical and cerebellar histogenesis is disrupted when genes encoding for cell-ECM connection is ablated [29]. Increased neural progenitor cell mortality and decreased cortical neuron formation result from premature radial endfeet separation.

Similar to the case of Foxc1 mutants, as well as Zic1/3 double mutants, BM defects in the neocortex have also been observed to be in correlation with cellular issues in the meninges. The radial glial scaffold and cortical organization are severely disrupted in Foxc1-null mutants because of a gradual breakdown in the pial BM beginning in mid-corticogenesis. Zinc-finger transcription factors certain cell types in the growing forebrain and meninges express Zic1 and Zic3 [30]. Meningeal genes such as Cxcl12 and Foxc1, as well as meningeal-derived laminin, express less in Zic1/3 mutants. These mutants exhibit separation of the radial glial endfeet and a disruption of the pial BM. This shows that initial meningeal abnormalities may disturb the pial BM and ultimately result in problems in cerebral cortex formation [31].

4.4 Recent Discovery of Fourth Meningeal Layer

A study on the meningeal architecture along with its brain health significance underwent a revolution due to the identification of a fourth meningeal layer. The subarachnoid lymphatic-like membrane (SLYM) is a newly identified layer of the single-layered, which occurs between the arachnoid mater and the pia mater [8]. Research scientists identified the SLYM through two-photon imaging of genetically modified mice expressing fluorescent markers. The continuous thin membrane expressed both podoplanin (PDPN) as the lymphatic marker and CRABP2 as the meningeal marker thus demonstrating its mesothelial character [32]. Testing with immunostaining revealed the presence of SLYM in human tissues which confirms its significance for human health and validates the involvement of this tissue throughout biological history.

The concept of the SLYM gained significant attention following the landmark study by Møllgård et al. (2023), which demonstrated that a mesothelial membrane divides the subarachnoid space into functionally distinct compartments [33]. This discovery challenges the traditional view that the subarachnoid space is a single continuous cavity. The SLYM appears to act as a selective barrier that regulates the movement of CSF, immune cells, and macromolecules. By compartmentalizing the subarachnoid space, the membrane may contribute to immune surveillance and controlled CSF circulation, thereby playing a role in maintaining central nervous system homeostasis [34].

The subarachnoid area contains the SLYM as a barrier that regulates CSF flow and supports local immune functions [35]. The CNS immune functions of SLYM become evident through its leukocyte densities that match those found in the dura mater. The study results oppose popular beliefs about brain immunity by showing that the brain retains immune privilege contrary to existing knowledge about its status [36]. Research examining meningeal immune functions continues to grow **(Figure 3)**. Traditionally believed to shield the brain from the systemic immune response, the BBB partially confers immunological privilege. Many diseases, including multiple sclerosis, delirium, severe systemic inflammation, and brain injury, have been linked to increased blood-brain barrier permeability. Meninges around the brain's edges have been proven crucial for CNS immunity. Meningeal immunity and psychopathology are related because the loss of certain immune cells in the meninges and skull directly affects anxiety-like behavior [37]. Moreover, it has been demonstrated that brain injury may enhance the bone marrow's ability to generate immune cells, creating an immune cell reserve close to the brain.

Current evidence suggests that SLYM develops as part of the leptomeningeal system during early meningeal formation. While detailed embryological characterization remains limited, available studies indicate that the membrane arises during the maturation of the subarachnoid space as mesothelial cells organize into a thin barrier structure. Age-related structural alterations have also been proposed. Experimental studies suggest that thinning or disruption of SLYM may occur during aging, potentially influencing cerebrospinal fluid circulation and immune regulation. However, these age-related changes remain an area of ongoing investigation and require confirmation in human studies [38].

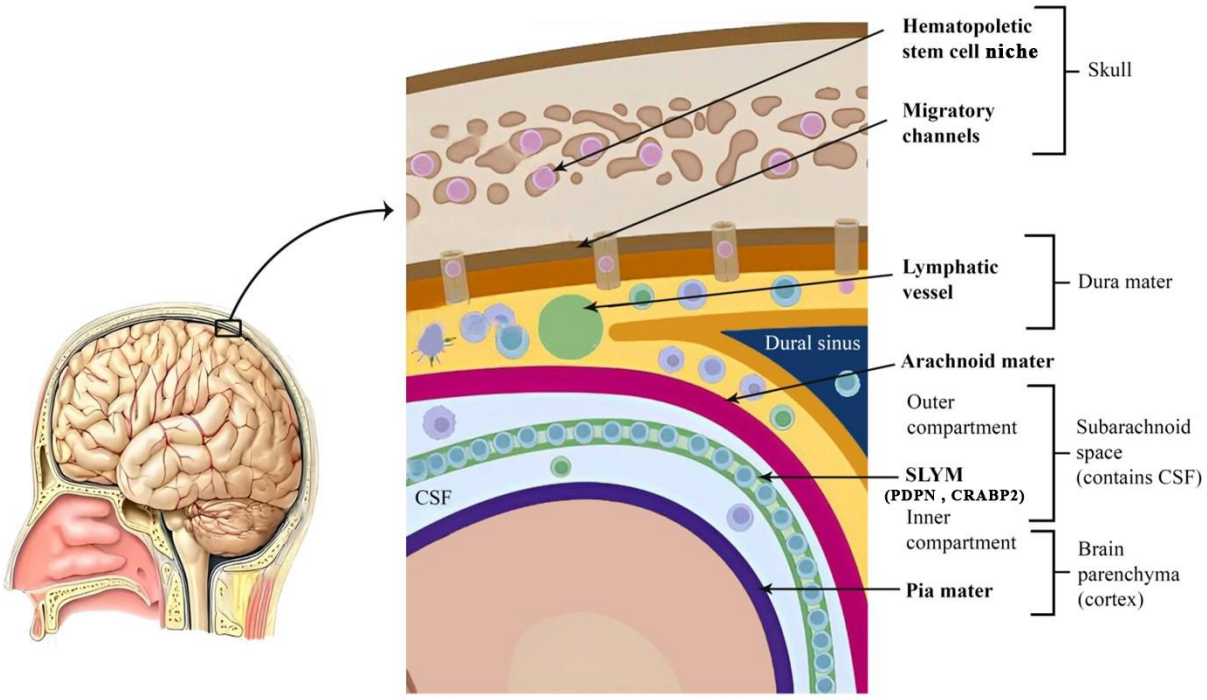

Figure 3. In relation to SLYM. The subarachnoid area is divided into functional spaces by a recently found membrane. The Figures were created by using Adobe Photoshop.

The mechanical protective functions of SLYM are indicated by its properties to absorb shock forces and minimize mechanical shear between brain tissue and the skull [39]. Some studies have suggested that age-related alterations in SLYM may influence cerebrospinal fluid dynamics and immune responses, processes that are also implicated in neurodegenerative diseases such as Alzheimer's disease. However, current evidence remains preliminary and further research is needed to establish a direct causal relationship. The innovative character of SLYM leaves various unanswered questions. Scientists need to prove how SLYM connects to spinal meninges and where it stands within the glymphatic system while providing full definitions of its human immune system functions [40]. Future research must validate the present findings by further examining how SLYM affects illnesses present in the central nervous system. The discovery of SLYM facilitates promising opportunities to develop new therapeutic approaches which specifically focus on treating this layer for neuroinflammatory and neurodegenerative diseases [41].

The SLYM may also interact with other central nervous system barrier systems, including the BBB and the glymphatic system. The BBB regulates the entry of substances from the bloodstream into the brain, while the glymphatic pathway facilitates the clearance of metabolic waste through cerebrospinal fluid circulation. By compartmentalizing the subarachnoid space, SLYM may contribute to the regulation of CSF flow and immune cell trafficking, thereby indirectly influencing glymphatic clearance mechanisms.

Although the structural characteristics of SLYM have been convincingly demonstrated in animal models, particularly in mice, direct functional evidence in humans remains limited. The delicate nature of the membrane and its thin structure make it difficult to visualize using conventional imaging or routine histological techniques. Consequently, some researchers suggest that the presence and functional significance of SLYM in humans require further validation through advanced imaging approaches and larger histological studies.

5. Conclusions and Future Directions

The meninges, once regarded just as a protective layer for the brain, are now acknowledged as dynamic and multifunctional entities crucial for the development, homeostasis, and immunity of the CNS. The paper focuses on the classical role of the dura mater, arachnoid mater, pia mater, and also introduces the recently discovered subarachnoid lymphatic-like membrane as a fourth meningeal layer. The SLYM signifies a transformative advancement in neuroanatomy and neuroimmunology, providing novel perspectives on cerebrospinal fluid compartmentalization and central nervous system immune control. The distinctive structural and functional characteristics such as immune cell retention, fluid barrier establishment, and potential implications in aging and disease indicate that it may be pivotal in the pathophysiology of neurodegenerative disorders, including Alzheimer's disease, as well as traumatic brain injuries. Comprehending this novel meningeal element presents intriguing opportunities for next research. Specifically, in vivo imaging methods and molecular analysis of SLYM in humans may uncover novel biomarkers or treatment targets for central nervous system illnesses. Additionally, pharmacological regulation of meningeal-derived signals and stem cell function may present interesting approaches for neuroregeneration. The incorporation of SLYM into the existing meningeal model enhances our understanding of brain structure and function, prompting the

scientific community to reassess conventional neurobiological concepts and investigate novel avenues in brain disorder research and treatment.

From a therapeutic perspective, targeting the SLYM presents both opportunities and challenges. Potential strategies may include modulation of its barrier function, immune activity, or cerebrospinal fluid dynamics using pharmacological or nanotechnology-based approaches. However, significant technical barriers exist, including the difficulty of accessing this delicate structure in vivo, lack of specific molecular targets, and limitations in current imaging techniques. Future research should focus on confirming the presence and functional significance of SLYM in humans through advanced imaging techniques and histological studies. Understanding how this membrane interacts with cerebrospinal fluid circulation, the glymphatic system, and immune mechanisms could reveal novel diagnostic and therapeutic opportunities for neurological disorders [42].

ABBREVIATIONS:

CNS: Central Nervous System,

CSF: Cerebrospinal Fluid,

ISF- Interstitial Fluid,

ECM: Extracellular matrix,

BM: Basement Membrane,

VZ: Ventricular Zone,

SLYM: Subarachnoid Lymphatic-Like Membrane,

BBB: Blood-Brain Barrier,

PDPN: Podoplanin

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