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ORIGINAL ARTICLE

Evaluation of the Structural and Functional Dynamics of Extracellular Matrix of the Skin and Histochemical Characterization of the Dermal Interstitium

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Abstract

Introduction: Skin is the largest organ in the body consists of three layers, epidermis, dermis and hypodermis. Skin along with its accessories like nails, hair follicles, sweat glands and sebaceous glands forms the integumentary system. Skin acts as a barrier between the external factors and internal environment and gives protection to deeper tissues in the body. Skin cancer is the fifth most common type of cancer. **Materials and Methods:** Samples for this study were taken from human cadavers. Histochemical study was done with Van Gieson's method for collagen fibers, Weigert's resorcin fuchsin for elastic fibers and Mallory's trichrome stain for connective tissue were used to study the connective tissue. Collagen, elastic fibers and cells in the interstitium of skin were analyzed. **Results:** Thick and thin collagen fibers were found to be arranged in vertical and horizontal direction in the dermis. Mast cells and macrophages were more in number in the interstitium near the vessels. Fibers surrounding the vessels transfer the mechanical pressure to the lymphatic vessels. Initial and collecting lymphatic vessels were identified. **Conclusion:** The arrangement of fibers and distribution of cells in the skin helps in understanding fields like regenerative medicine and tissue engineering.

Keywords: Skin, Interstitium, histochemistry, lymphatics, interstitial space

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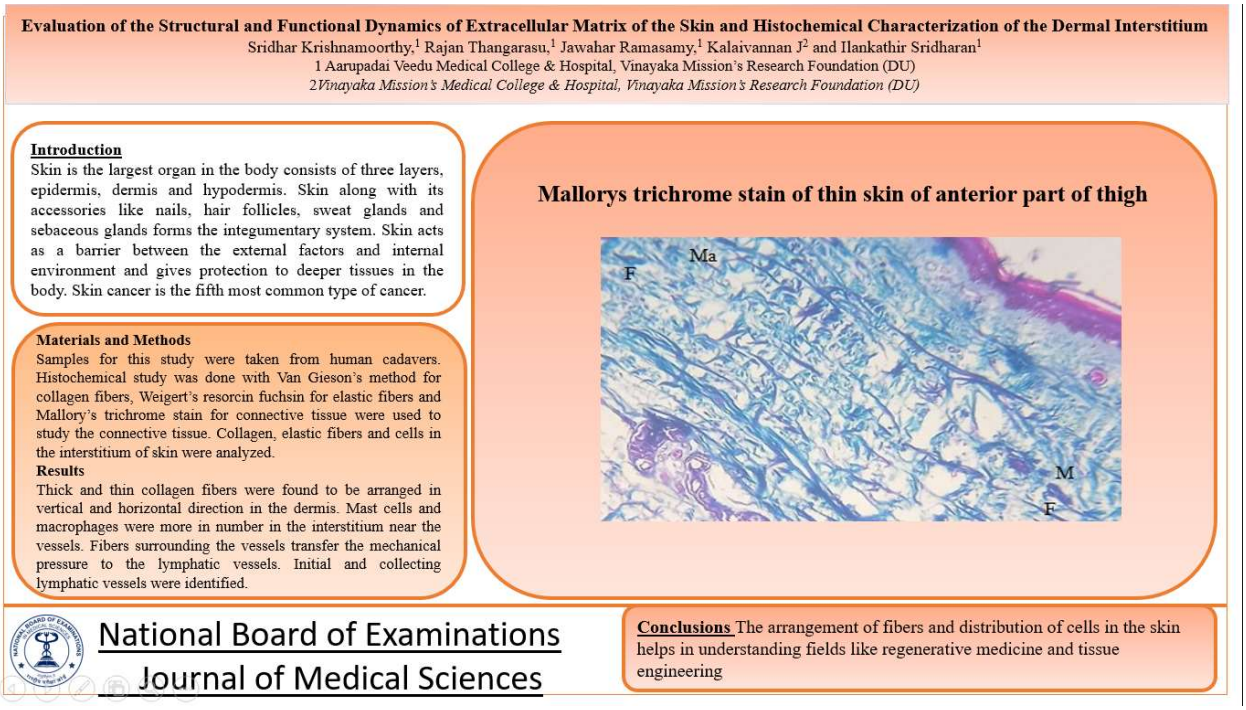
Graphical Abstract



Introduction

Human skin is the largest organ, made up of three layers, epidermis, dermis and hypodermis. There are two types of skin, thin skin and thick skin. Outermost layer is epidermis, formed by stratified squamous non keratinized epithelium and is avascular in nature. Developmentally ectoderm layer gives rise to epidermis. Dense irregular connective tissue forms the dermis and is richly supplied with blood vessels and nerves. The connective tissue of both thin skin and thick skin consists of interstitial space in the interstitium. The structure and function of the skin is maintained by the extracellular matrix (ECM), cells and fibers. The ECM is a compound arrangement of macromolecules such as proteins, polysaccharides and adhesion molecules, which provides mechanical support and allows the passage for biochemical signals to the surrounding cells [1]. Fibroblast, Adipocytes (fat cells), mast cells and macrophages are the cells present in the connective tissue matrix. Along with these cells undifferentiated multipotent mesenchymal cells are present. Fibroblasts are the primary cells responsible for the production of ECM and the structural proteins are collagen, elastic and reticular fibers. Collagen provides firmness and tensile strength and elastic fibers allows the skin to widen and recoil by its elastic property. The gel like ECM is due to the hydration of hyaluronic acid present in proteoglycans and glycosaminoglycans [2]. Mast cells and macrophages are the immune cells respond directly to any infections, injuries or irritation of skin and both cells remove the pathogens, dead cells and regulate the tissue repair. Reticular fibers

forms mesh work like structure and support the surrounding cells and tissue.

Superficial part of the dermis is papillary layer made up of loose areolar tissue and it produces finger like projection into the epidermis called papillae [3]. Deeper layer of the dermis is called reticular layer formed by dense irregular connective tissue. The collagen fibers extend into the hypodermis for anchoring. Hypodermis is a loose areolar tissue consists of more adipose cells and also called as superficial fascia. Adipose cells store fat and provide insulation and cushioning effect for the skin. The space between the cells and fibers in the connective tissue is called as interstitial space. Fluid present in this space is called as interstitial fluid. The interstitial fluid provides a medium for the exchange of nutrients and waste materials between the blood vessels and cells.

Skin along with its accessories like nails, hair follicles, sweat glands and sebaceous glands forms the integumentary system. Skin plays a vital role and gives protection to deeper tissues in the body. Continuous exposure to ultra violet radiation causes melanoma and non melanoma skin cancer. Skin cancer is the fifth most common type of cancer worldwide with increasing incidence [4]. Skin produces vitamin D3, cholecalciferol from 7-dehyrocholesterol on exposure to ultra violet radiation [5]. Skin may be affected by melanomas, cancer and some genetic diseases like albinism and vitiligo. The fluid and structural atmosphere around cells is provided by the interstitial space that exists between blood arteries and cells. The fluid from the circulatory area continuously filters by means of microvessels through the interstitial space in most tissues under normal circumstances, without being reabsorbed [6]. Clinically some drugs are given through skin in topical routes [7]. Interstitium apart from providing a structural support, it also acts as a medium for transport of fluid and immune cells.

The purpose of this study is to identify the interstitium as dynamic structure, the extra cellular matrix and its significant role in fluid transport and immune cell trafficking. Understanding the function of the interstitium may help in identifying the process behind frequent skin disorders like inflammation, fibrosis and edema. The interstitium located beneath the skin is continuously exposed to infections and environmental assaults. Moreover, since the interstitium has a direct connection to the lymphatic system, studying its function may reveal the connections between the disorders such as cancer metastasis, autoimmune disorders and the interstitial space of the skin.

In the present study, we have studied the fibers and cells in the interstitium of skin involved in immune surveillance. Knowledge of interstitium helps in disease pathology, immune response, wound healing process, tissue regeneration and possible clinical applications in the management and prevention of skin associated disease conditions.

Materials and Methods

Histochemical study was conducted on the human cadaver skin. Skin samples were collected from both male and female. Samples were obtained from scalp, arm,

front of thigh, pectoral region and anterior abdominal wall. Study design was experimental study, conducted in department of Anatomy, Aarupadai Veedu Medical College & Hospital, Pondicherry, India. Ethical clearance was obtained from the Institution ethical committee (AV/IEC/2022/023).

Tissue Processing

Human skin tissues were obtained from both male and female cadavers. The tissues were processed for light microscopy. To prevent putrefaction and autolysis the collected tissues were fixed in formal saline at room temperature. Formal saline contains 50ml of formalin, 450ml of water and 1.8g of sodium chloride. After primary fixation, tissues were washed under running tap water overnight. Dehydration was done by passing the tissues from ascending grade of 50% to absolute isopropyl alcohol. Clearing was done with two changes in xylene. Infiltration was done with paraffin wax at 60^0C in wax bath. Each tissue was embedded in separate paraffin block. The sections were taken in rotary microtome of 5µm thickness. The sections were taken in clean glass slide.

Histochemical staining

Van Gieson's method for collagen fibers

Van Gieson Solution was prepared by mixing 100ml of saturated aqueous picric acid solution with 10ml of 1% aqueous acid fuchsin solution. To this 100ml of distilled water was added. Procedure: The sections were deparaffinized in xylene for 5 minutes with two changes. The slides were dipped in descending grades of alcohol and washed in water. Nuclei were stained by the Celestine blue-hematoxylin sequence. Differentiated with 1% acid alcohol and washed in tap water. Counterstained in Van Gieson solution for 3 minutes and dehydrated through ascending grades of alcohol. Cleared in xylene and mounted with DPX [8].

Weigert's resorcin fuchsin for elastic fibers:

Preparation of reagents: 1 g of basic fuchsin and 2 g of resorcin was added to 100 ml of distilled water. Boiled and 12.5 ml of freshly prepared 30 % ferric chloride solution was added. Boiling continued for 5 minutes. Cooled and filtered, and the filtrate was discarded. The whole precipitate was dissolved in 100 ml of 95% ethanol using water bath and 2 ml of concentrated hydrochloric acid was added. The precipitate in a solvent prepared by mixing 50ml of 2-methoxyethanol with 50ml of distilled water. To this 2ml of concentrated hydrochloric acid was added. Procedure: Sections were deparaffinized and taken to alcohol. Slides were placed in resorcin fuchsin solution for 3 hours at room temperature and rinsed in tap water. Background staining was removed by treating with 1% acid alcohol. Rinsed in tap water. Counterstained with Van Gieson solution and dehydrated through ascending grades of alcohol. Cleared in xylene and mounted [8].

Mallory's trichrome stain for connective tissue

Reagents prepared were Acid fuchsin: 1g of acid fuchsin and 0.9g of Biebrich scarlet was added with 100ml of

distilled water. Phosphomolybdic acid: 1g of phosphomolybdic acid was mixed with 100ml of distilled water. Orange G: Orange G 2g, Oxalic acid 2g and Aniline blue 0.5g was added to 100ml of distilled water. Procedure: The slides were deparaffinized with Xylene and the sections brought to water. Slides were kept in acid fuchsin solution for 2 minutes and rinsed with the distilled water. Again the slides were dipped in phosphomolybdic acid for 2minutes and washed in distilled water. Sections were kept in orange G solution for 15 minutes. Slides were washed in running tap water and dehydrated with ethanol. Cleared with xylene and mounted [8].

Results

Histochemical analysis of skin samples were analyzed for the pattern of arrangement of fibers and formation of interstitial space and its importance. In the reticular part of dermis, thick collagen fibers are found to be horizontally arranged and between the horizontal fibers thin collagen fibers are arranged vertically. Interstitial spaces are found between the collagen fibers. Fibroblasts (F) were found nearer to the mast cells (M) and macrophages (Ma). Arterioles and venules are more in number in the papillary part of dermis (Figure 1).

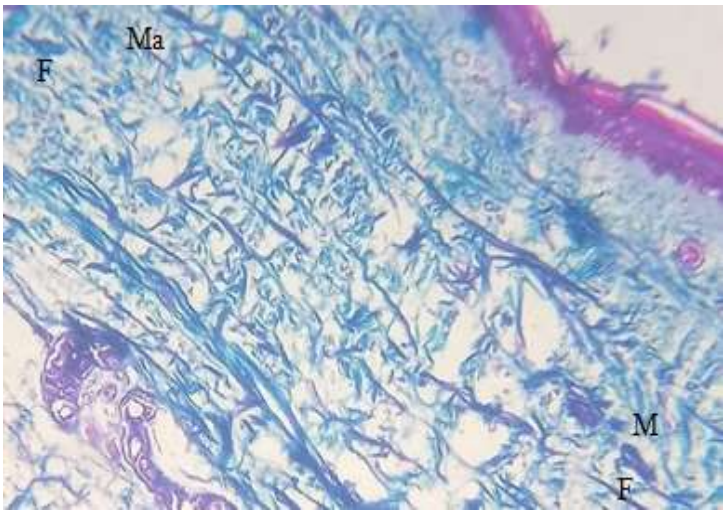


Figure 1. Mallorys trichrome stain of thin skin of anterior part of thigh.

Below the dermis, lymph collecting space (Ls) was present. From this space the lymphatic collecting channel (Lc) was draining the fluid in oblique direction between the adipose cells of hypodermis. It drains into another area present in the lymphatic space above deep fascia (Df). Between the adipose tissue mast cells (M) and macrophages (Ma) were present nearer to each other (Figure 2).

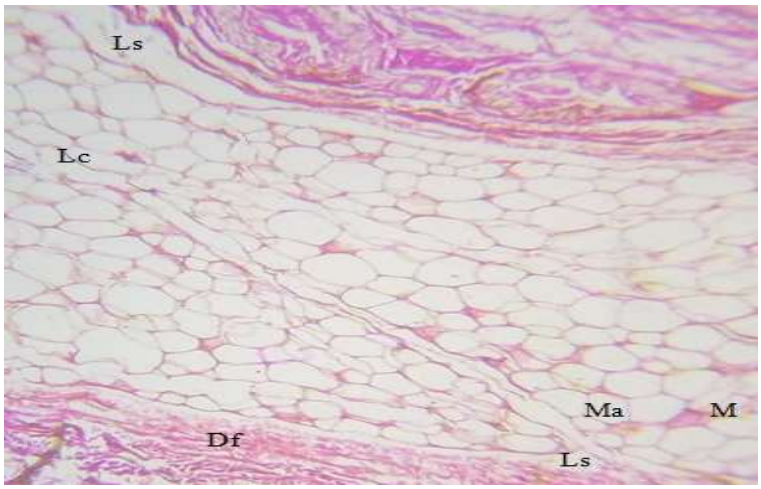


Figure 2. Thin skin of pectoral region stained with resorcin.

Hypodermis consisting of polyhedral adipose cells Lymphatic collecting vessels (Lc) was observed in the hypodermis. They are found to be formed by endothelium. Under the dermis lymphatic space (Ls) were observed which collects fluid from the interstitial space in the dermis. Macrophage (Ma) and fibroblast (F) was found in the dermis and hypodermis. Dendritic cell (D) also found in the dermis (Figure 3).

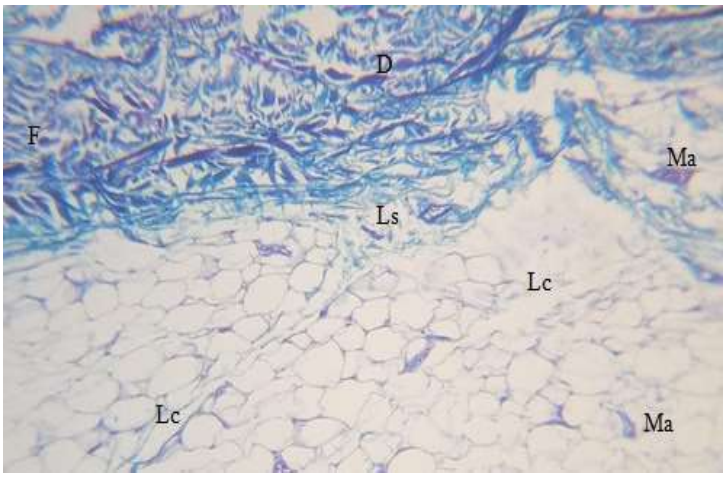


Figure 3. Mallorys trichrome staining of skin of pectoral region.

In hypodermis (Figure 4) the adipose tissue was surrounded by lymphatic space (Ls) formed by collagen fibers. Around the artery, thin collagen fibers (Cf) are arranged in a circular manner forming the interstitial space. Near the artery (A) smaller lymph vessels (Lv) are found. In the dermis the thick collagen fibers are arranged both vertically and horizontally forming the interstitial space. Dendritic cell (Dc) was found near the mast cell. Fibroblast (F) was observed in the interstitial space.

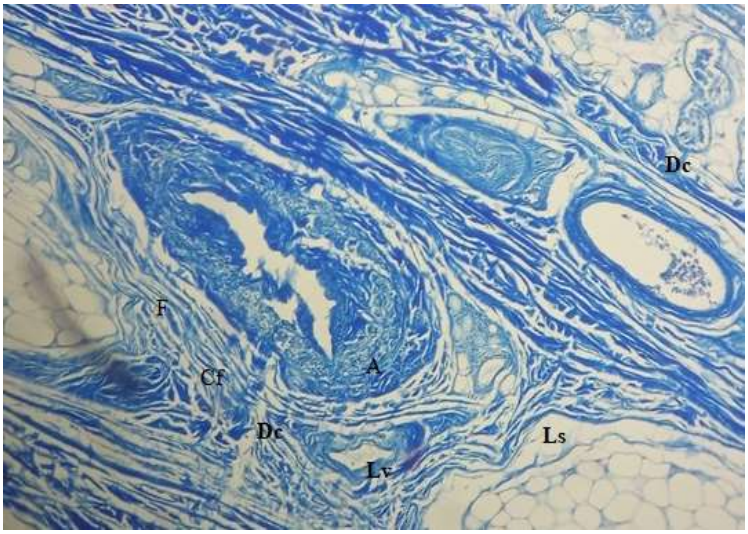


Figure 4. Mallory trichrome stain of abdomen skin.

Van Gieson stain of scalp shows thick collagen fibers arranged horizontally below the epidermis (Ep). Papillary layer (Pl) was not prominently projecting into the under surface of epidermis. More number of fibroblast (F) was present in the papillary layer. We observed a small space below the epidermis and found to be interstitial space. Hair follicle was present in the dermis. Macrophage (Ma) was present near the hair follicle. The collagen fibers are vertically arranged throughout the entire dermis. Few elastic fibers are present in horizontal direction. Between the fibers, wide spread interstitial space was present. A long lymphatic vessel (Ll) with endothelium was observed passing through the entire layer of dermis which is draining into another large vessel (Figure 5).

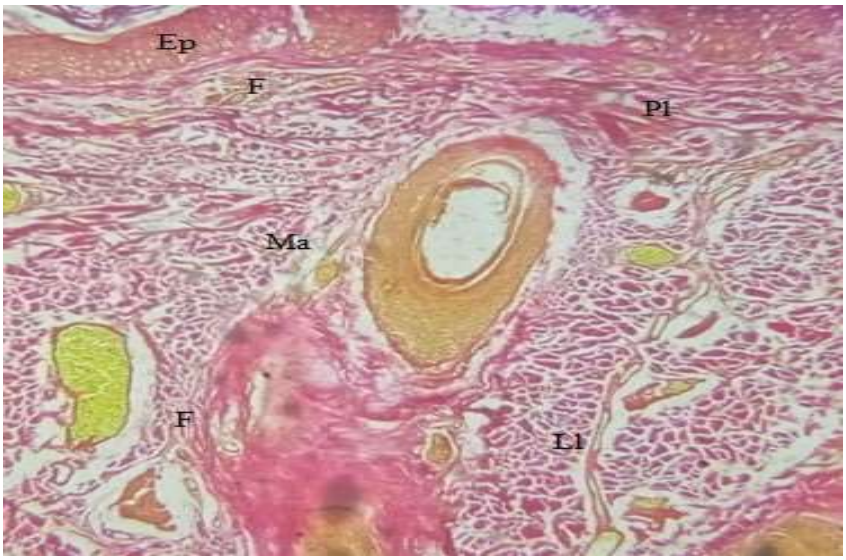


Figure 5. Scalp stained with Van Gieson stain

Collagen fibers were arranged in both horizontal and vertical direction. Sweat glands (Sg) were also surrounded by interstitial space. Macrophages (Ma) were found near the sweat glands. Surrounding the veins (V) and arteries (A) interstitial space was present. In the interstitial space smaller arterioles, venules and lymphatic vessels were found. Fibroblast and mast cells were present in the interstitial space. Interstitial space in the dermis was directly connected to the interstitial space around the vessels. Larger lymphatic space (Ls) was found which drains into lymphatic vessels (Lv) (Figure 6).

Figure 6. Mallorys trichrome staining of thin skin of shoulder.

The sebaceous gland (Se) was surrounded by thick collagen fibers arranged as bundles with thin interstitial space. Sebaceous duct (Sd) was observed near the gland. Arrector pili muscle (Ap) was present nearer to the sebaceous gland. Arrector pili muscle compresses the gland to release the sebum from the gland. In between the collagen fibers numerous smaller arterioles and venules are more in number. Mast cells (M) and macrophages (Ma) were more in number around the sebaceous gland confirms the existence of interstitial space around the gland. Our observation suggests the collagen fibers surrounding the sebaceous gland may compress the gland to release the secretion. Collagen fibers are arranged irregularly and connected by thin elastic fibers in the dermis (Figure 7).

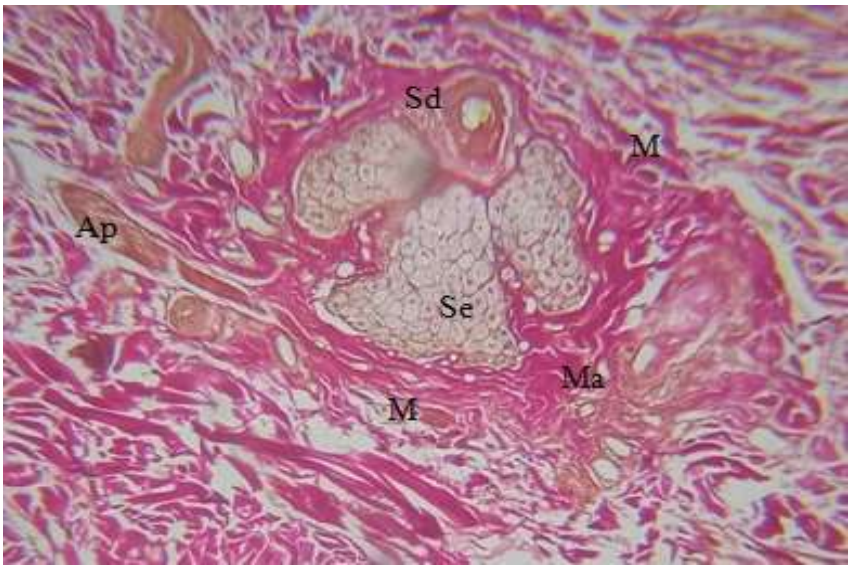


Figure 7. Skin of arm stained by Van Gieson.

Discussion

Rudolf Virchow in 1858 identified that fibroblast was present in the connective tissues [9]. Fibroblast is a spindle shaped cell present in the interstitial space of dermis part of the skin. Fibroblast has abundant rough endoplasmic reticulum by which it actively secretes extracellular matrix and proteins [10]. We observed more number of fibroblast in thick skin and less number of fibroblast in thin skin. In a study conducted on keratinocyte proliferation the researchers identified that fibroblast secretes keratinocyte growth factor and granulocyte macrophage colony stimulating factor and acts as paracrine regulator [11]. Fibroblast also directly communicates with the immune cells in the interstitium. The extracellular matrix (ECM), is the collective term for the structural components of the interstitial space, mostly composed of glycosaminoglycans, elastic fibers, collagen fibers, and microfibrils [12]. ECM withstands mechanical pressure and protects from minor trauma. Elastic and collagen fibers in dermis are produced by fibroblast. Another study suggests that Smooth muscles, endothelial and adventitial cells found in the vessel wall also produce collagen [13]. The elastic and collagen fibers by mechanical contraction exert pressure to the smaller arterioles and venules to manage the interstitial fluid pressure and interstitial fluid volume. This may be due to the basket weave like network of collagen fibers. Depending on the elastin quantity there are three types of elastic fibers in skin namely, elaunin oxytalan and mature elastic fibers. Oxytalan fibers are present near the dermoepidermal junction, elaunin fibers are found in the papillary layer and mature elastic fibers are present in the reticular layer [14]. Presence of elastic fibers in the reticular layer makes the skin to maintain its elasticity and recoil to its original position [15]. There are two types of elastic fibers, vertical elastic fibers reaches upto the basement membrane of epidermis and horizontal fibers are present in the reticular layer of the dermis [16]. Elastic fibers are in

close contact with the interstitial cells and involved in cell proliferation, migration and differentiation. Further elastic fibers are also involved in regulation of cellular phenotypes, production of matrix and cytokines [17].

The collagen fiber being thick in nature has the capacity to pass on the energy during mechanical stress and provides resistance. We found thin collagen fibers at the junction of epidermis and papillary layer and thick collagen fibers are predominantly present in the deeper layers of dermis which is similar to another study [18]. We have also observed thick collagen fibers are horizontally present in the thigh region whereas in the shoulder area the collagen fibers are in vertical direction. Collagen fibers help in migration of cells, morphogenesis of the tissue, involves in tissue repair and regulates the function of inflammatory and resident cells [19]. Collagen and elastic fibers are densely packed in thick skin to withstand mechanical stress whereas in thin skin collagen and elastic fibers are loosely arranged to permit broad range of motion.

The interstitial fluid formed in the interstitial space is removed by the lymphatic vessels and returned to the venous blood. Lymphatic vessels play major role in the interstitium in maintaining the fluid transport, proteins and immune cells. The lymphatic vessels are anchored to the extracellular matrix. The interstitial fluid formed also referred as lymph, is collected by initial lymphatics which starts as a blind end formed by endothelial cells and without basement membrane. We have observed the lymphatic vessels in hypodermis were passing in oblique direction. The muscle contraction and external force on the skin may cause pressure on the oblique lymphatics to drain the interstitial fluid towards the lymph node. The fluid then transferred to collecting lymphatic vessel and drained into lymph trunk. Another study on adaptive immunity suggests that the endothelium of lymphatic vessels control its own flow and communicates with the immune cells [20]. The interstitial space allows the exchange of gas, nutrients and waste products for maintaining the homeostasis.

Macrophages are the first line of defense mechanism. During any type of tissue injury or inflammation it migrates to that particular area and involve in phagocytosis [21]. Paul Ehrlich first coined the term mast cells in 1878 [22]. Mast cells are present mostly in the connective tissue closer to the blood vessels. Heparin and histamine are the secretory granules present in the mast cells [23]. Histamine is formed after the removal of the carboxyl group from the amino acid histidine by the enzyme histidine decarboxylase. Histamine H_1 receptors act on the skin to produce allergic reactions. H_1 receptor is present in chromosome 3 at 3p25 location [24]. Histamine acts on the endothelial lining of the blood vessels causing weakness in between the endothelial cells. Due to this action, cells and proteins leak into the surrounding connective tissues.

Steinman and Cohn in 1973 discovered the dendritic cells from the mouse spleen [25]. Dendritic cells are produced in bone marrow and have the capacity to migrate to other parts of the

body. It has multiple cytoplasmic processes. Dendritic Cells are heterogeneous groups of leukocytes. Dendritic cell identifies the pathogens and are called as antigen presenting cells. The lymphatic vessels transfer the dendritic cells to the circulation through lymph node. Capturing, analyzing, and distributing antigens to stimulate T-cell activation and differentiation, dendritic cells constitute a diverse group of cells that mediate the relationship between the innate immune response and the adaptive response [26]. The macrophages and lymphocytes escape from the capillaries and circulate in the interstitial space. During any inflammation these cells move towards the epidermis for immunological action and in normal stage the cells will move towards lymphatic channels then to regional lymph nodes. Along with the immune cells extracellular sodium ions are stored in the interstitium for the regulation of blood pressure [27].

Conclusion

The interstitium of the skin plays an important role in the protection and maintain equilibrium of the body by regulating the volume and composition of the interstitial space. During inflammation the interstitium gives free space to circulate large proteins and immune cells. Collagen and elastic fibers involves in proliferation of cells, angiogenesis and wound healing. Further the pattern of arrangement of fibers, distribution of cells and lymphatic vessels helps in understanding fields like regenerative medicine, tissue engineering and suggest that the interstitium may function as organ.

Ethical Approval

Ethical clearance was obtained from the Institution ethical committee (AV/IEC/2022/023).

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Conflicts of interest

The authors declare that they do not have conflict of interest.

References

1. Nikos K Karamanos, Achilleas D Theocharis, Zoi Piperigkou, Dimitra Manou, Alberto Passi, Spyros S Skandalis, Demitrios H Vynios, Véronique Orian-Rousseau, Sylvie Ricard-Blum, Christian E H Schmelzer, Laurent Duca, Madeleine Durbeej, Nikolaos A Afratis, Linda Troeberg, Marco Franchi, Valentina Masola and Maurizio Onisto. A guide to the composition and functions of the extracellular matrix. The FEBS journal. 2021;288(24):6850-6912.
2. Vitor H. Pomin and Barbara Mulloy. Glycosaminoglycans and Proteoglycans. Pharmaceuticals (Basel). 2018;11(1):27.
3. Michael Sorrell. J and Arnold I. Caplan. Fibroblast heterogeneity: more than skin deep. Journal of Cell Science. 2004;177(5):667-675.
4. Seokyung An, Kyungsik Kim, Sungji Moon, Kwang-Pil Ko, Inah Kim, Jung Eun Lee and Sue K. Park. Indoor

Tanning and the Risk of Overall and Early-Onset Melanoma and Non-Melanoma Skin Cancer: Systematic Review and Meta-Analysis. Cancers. 2021;13:5940.
5. Ligia J. Dominguez, Mario Farruggia, Nicola Veronese and Mario Barbagallo. Vitamin D Sources, Metabolism, and Deficiency: Available Compounds and Guidelines for Its Treatment. Metabolites. 2021;11(4):255.
6. Randolph H. Stewart. A Modern View of the Interstitial Space in Health and Disease. Frontiers in Veterinary Science. 2020;7.
7. Caramori G, Mumby S, Girbino G, Chung KF, Adcock IM. Corticosteroids. 2019 Feb 23.
8. Kim Suvarna. S, Christopher Layton and John D. Bancroft. Bancroft's Theory and Practice of Histological Techniques. 8th ed. Elsevier; 2019. p. 56-175.
9. Virchow Rudolf. Cellular pathology based on physiological and pathological tissue theory. 1859: Berlin: August Hirschwald Publishing House.
10. Maksim V Plikus, Xiaojie Wang, Sarthak Sinha, Elvira Forte, Sean M Thompson, Erica L Herzog, Ryan R Driskell, Nadia Rosenthal, Jeff Biernaskie, Valerie Horsley. Fibroblasts: origins, definitions, and functions in health and disease. Cell. 2021;184(15):3852–3872.
11. Sabine Werner and Hans Smola. Paracrine regulation of keratinocyte proliferation and differentiation. Trends in Cell Biology. 2001;11(4):143-146.
12. Rolf K. Reed, Åsa Lidén and Kristofer Rubin. Edema and fluid dynamics in connective tissue remodelling. Journal of molecular and cellular cardiology. 2010;48(3):518–523.
13. Twardowski. T, A. Fertala, J. P.R.O. Orgel and J. D. San Antonio. Type I Collagen and Collagen Mimetics as Angiogenesis Promoting Superpolymers. Current Pharmaceutical Design. 2007;13(35):3608-3621.
14. Thaís P. Amadeu, André S. Braune, Luís C. Porto, Alexis Desmoulière and Andréa M. A. Costa. Fibrillin-1 and elastin are differentially expressed in hypertrophic scars and keloids. Wound Repair and Regeneration. 2004;12(2):169-174.
15. Lowell A. Goldsmith, Stephen I. Katz, Barbara A. Gilchrest, Amy S. Paller, David J. Leffell, Klaus Wolff. Fitzpatrick's Dermatology in General Medicine. 8 ed. 2012, New York: McGraw-Hill.
16. Frances. C, M.C. Branchet, S. Boisnic, C.L. Lesty and L. Robert. Elastic fibers in normal human skin. Variations with age:a morphometric analysis. Archives of Gerontology and Geriatrics. 1990;10:57-67.
17. Balakrishnan Sivaraman, Chris A. Bashur and Anand Ramamurth. Advances in biomimetic regeneration of elastic matrix structures. Drug Delivery Translational Research. 2012;2(5):323-350.

18. Junqueira L.C.U, G.S. Montes, J.E.C. Martins and P.P. Joazeiro. Dermal Collagen Distribution. A Histochemical and Ultrastructural Study. Histochemistry. 1983;79:397-403.
19. Sara Sprangers and Vincent Everts. Molecular pathways of cell-mediated degradation of fibrillar collagen. Matrix Biology. 2019;75:190-200.
20. Catherine M. Card, Shann S. Yu and Melody A. Swartz. Emerging roles of lymphatic endothelium in regulating adaptive immunity. The Journal of Clinical Investigation. 2014;124(3):943-952.
21. Uwe Lendeckel, Simone Venz and Carmen Wolke. Macrophages: shapes and functions. ChemTexts. 2022; 8(2):12.
22. Enrico Crivellato, Carlo Alberto Beltrami, Franco Mallardi, Domenico Ribatti. Paul Ehrlich's doctoral thesis: A milestone in the study of mast cells. British Journal of Haematology. 2003;123:19-21.
23. Zmorzynski, Szymon, Aleksandra Kimicka-Szajwaj, Angelika Szajwaj, Joanna Czerwik-Marcinkowska, and Jacek Wojcierowski. Genetic Changes in Mastocytes and Their Significance in Mast Cell Tumor Prognosis and Treatment. Genes 2024;15(1):137.
24. Stephany Micallef, Holger Stark and Astrid Sasse. Polymorphisms and genetic linkage of histamine receptors. Life Sciences. 2013;93(15):487-494.
25. Ralph M. Steinman and Zanvil A.Cohn. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. The Journal of experimental medicine. 1973;137:1142-1162.
26. Natalie C. Wilcox, Golnar Taheri, Katherine Halievski, Sebastien Talbot, Jaqueline R. Silva, Nader Ghasemlou. Interactions between skin-resident dendritic and Langerhans cells and pain-sensing neurons. Journal of Allergy and Clinical Immunology. 2024;154(1):11-19.
27. Wiig. H, F C Luft and J M Titze. The interstitium conducts extrarenal storage of sodium and represents a third compartment essential for extracellular volume and blood pressure homeostasis. Acta Physiologica. 2018;222(3).