Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/d920wb20

A QUANTITATIVE ANALYSIS ON HUMAN MICROVASCULAR LYMPHATIC AND BLOOD ENDOTHELIAL CELLS PRODUCE FIBRILLIN

Dr Suresh Kumari*

*Associate professor, Department of Anatomy, Al Falah School of Medical Sciences and Research Center Dhauj Faridabad

***Corresponding Author:** Dr Suresh Kumari
*Associate professor, Department of Anatomy, Al Falah School of Medical Sciences and Research Center Dhauj Faridabad

Abstract
Fibrillin microfibrils serve as the anchoring filaments that join the lymphatic endothelium to the surrounding elastic fibers and provide a scaffold for the deposition of elastin in the arterial wall. Previous researches confirmed that thoracic duct endothelial cells produce an uneven web, while bovine arterial endothelial cells deposit fibrillin in a honeycomb pattern together with microfibril-associated glycoprotein (MAGP)-1. By sequentially using the pan-endothelial marker CD31 and the lymphatic-specific marker D2-40, the current immunohistochemistry investigation aimed to confirm whether lymphatic and blood human dermal microvascular endothelial cells (HDMECs) isolated from human foreskin deposit fibrillin and MAGP-1. Both fibrillin and MAGP-1 co-localized in both cell types and were deposited in the same culture in varying patterns of increasing complexity. Wide-mesh honeycomb was created by fibrillin microfibrils, leaving gaps that were subsequently filled by fibrillin. Blood and lymphatic HDMECs exhibited a fibrillin deposition mechanism that was essentially identical to that of bovine large artery endothelial cells. Fibrillin was first deposited in some lymphatic HDMECs as evenly distributed short fibrillin strands, most likely due to anchoring filaments that were carried over from the original capillaries. Our results demonstrate the involvement of lymphatic and blood endothelial cells in the deposition of fibrillin in human skin.

Keywords: Immunohistochemistry; lymphatic vessels; microfibril-associated glycoprotein-1; microvascular endothelial cells

Introduction

A functionally essential component of the circulatory tree, microvessels such as arterioles, capillaries, and venules control tissue perfusion and blood–tissue exchanges. Despite the fact that blood microcirculation has been the subject of several studies, lymphatic beginning vessels have been problematic until the last ten years due to their challenging identification in histological sections. Interest in this long-neglected topic has increased due to the discovery of certain lymphatic markers that enable their positive identification even when they are fully collapsed (Scavelli et al.).Jurisic & Detmar, 2009; 2004).The growing demand for tissue-engineered vessels that might be utilized to replace tiny arteries and surgically disrupted lymphatics is one of the reasons to broaden our understanding of the fundamentals of blood and lymphatic artery function. However, matrix proteins have a major role in the mechanical characteristics, specifically the elastic recoil and burst strength.

Stephan et al. (2006) stress the importance of precoating scaffolds with matrix proteins, such as fibrillin. The goal of the current study was to provide fresh information on how blood and lymphatic endothelial cells (ECs) deposit fibrillin, a crucial protein in the development of elastic fibers. The size of fibrillin's is enormous (around 350 kDa). Typically found in conjunction with elastin, cysteine-rich glycoproteins of the extracellular matrix are the main structural elements of 10–12 nm microfibrils that have a "bead on a string" appearance (Sakai et al. 1986). However, they have also been discovered to function as anchoring proteins in non-elastic tissues that are subjected to mechanical stress, such as the ciliary zonule of the eye (Wagenseil & Mecham, 2007). Fibrillin is believed to be essential for elastin deposition in elastic tissues. During development, fibrillin-1 and fibrillin-2 create a scaffold for the first assembly of elastic fibers, and in postnatal life, fibrillin-1 gives the adult tissue force-bearing structural support (Ramirez, 2000; Carta et al. 2006). Mice with fibrillin-1 knockout perish as newborns because their elastic tissues collapse (Carta et al. 2006). Apart from its "structural" role of support, fibrillin also plays a "instructive" role because it can sequester crucial signalling molecules like bone morphogenetic protein and transforming growth factor b in the extracellular matrix, which controls matrix formation and remodeling (Ramirez & Sakai, 2010). It has been demonstrated that fibrillin interacts with a number of molecules. These include MAGP-2 and microfibril-associated glycoprotein (MAGP)-1.

One of the main structural elements of microfibrils is MAGP-1. It is a 31 kDa glycoprotein that has been demonstrated to exist on microfibril beads (Henderson et al., 1996); also, its N-terminus may bind tropoelastin's C-terminus (Brown-Augsburger et al., 1994, 1996). It co-localizes with almost all microfibrils that contain fibrillin-1. Although MAGP-2 has a more limited tissue distribution in postnatal life (Gibson et al. 1998), its expression peaks at the commencement of elastic fibres during development and has recently been demonstrated to induce elastic fibre construction (Lemaire et al. 2007). The walls of arteries and lymphatic vessels contain fibrillin microfibrils.

Marfan syndrome, in which a mutation of the fibrillin-1 encoding gene on chromosome 15 results in severe cardiovascular defects, such as aortic root dilatation and a propensity to aortic dissection, highlights the significance of fibrillin-1 in the structural organization of elastic arteries (Lee et al. 1991; Robinson et al. 2006). Except for sinusoids (Dubuisson et al. 2001), blood capillaries do not have fibrillin around them and are not linked to perivascular elastin (Gerli et al. 1990; Ryan, 2009).The microfibrils of anchoring filaments of human skin's lymphatic vessels (Solito et al., 1997) that join the lymphatic endothelium to the surrounding elastic fibers (Gerli et al., 1990) include fibrillin. Skin lymphatics' physiological activity in this particular area depends on their relationship with elastin fibres, and lymphatic dysfunction results from elastin degradation (Ryan, 2009). It has long been believed that the anchoring filaments of lymphatic vessels just tear apart interendothelial junctions in edema, promoting the production of lymph and the outflow of interstitial fluid (Casley-Smith, 1980). However, we have recently hypothesized that they may operate a mechanotransduction of mechanical signals from the extracellular matrix in cytoskeletal arrangements and biochemical signals inside the cell (Rossi et al. 2007) to adapt lymph formation to the physiological requirements. This is based on the immunohistochemical demonstration of the association of fibrillin with focal adhesion molecules in initial lymphatic vessels as well as experimental data on stretched thoracic duct and stretched lymphatic EC cultures. Numerous cell types, including fibroblasts, especially those isolated from the bovine nuchal ligament (Sakaiet al. 1986; Kielty & Shuttleworth, 1993), aortic smooth muscle cells (Kielty & Shuttleworth, 1993), osteoblasts (Kitahama et al. 2000), chondroblasts (Sakamoto et al. 1996), and keratinocytes (Haynes et al. 1997), have been demonstrated to deposit fibrillin in the extracellular matrix through in vitro studies. Similar to the nucleation of crystals, fibrillin deposition in vitro follows tracks carried over from the original tissue (Brenn et al. 1996)

Researchers has demonstrated that ECs isolated from the primary lymphatic channel, the thoracic duct, and the principal arteries of cows, the aorta and pulmonary arteries, generate fibrillin-1 in vitro (Weber et al. 2002, 2004). Deposition of fibrillin varies in lymphatic EC cultures and cultured blood, a dense, uneven web of fibrillin is formed in the underlying matrix, while aortic ECs deposit fibrillin in a honeycomb pattern with fibrillin-free gaps. we were able to isolate lymphatic and blood human dermal microvascular ECs (HDMECs) from human foreskin. We also confirmed whether lymphatic

and blood HDMECs deposit fibrillin-1 and MAGP-1 in vitro. Specifically, we wanted to know if lymphatic HDMECs deposit fibrillin before MAGP-1, as thoracic duct ECs, or if they deposit both simultaneously, as big vessel arterial cells.

Materials and methods

With the parents' informed consent, foreskins from children aged 1 to 5 undergoing circumcision for religious reasons were obtained at Department of Anatomy ,Al Falah School of Medical Sciences and Research Center Dhauj Faridabad. The foreskins were then incubated for one hour at 37 °C in phosphate-buffered saline (PBS) containing penicillin (100 U/mL), streptomycin (100 lg/mL),kanamycin (200 lg/mL), gentamycin (100 lg mL), and amphotericin B (0.5 lg mL). To make it easier to separate the epidermis from the dermis, foreskins were then sliced into tiny pieces (2 · 2 mm) and incubated for the whole night at 4°C in 3–5 mL of dispase I (2.5 U mL) PBS; Roche). After that, the epidermis was pulled off and thrown away and 0.25% type 1A collagenase [185 U mL], in Dulbecco's modified Eagle's medium nutrition mixture F-12 HAM(DME/F-12) was added to the residual dermis, which was then vigorously shaken every 30 minutes for 1 hour at 37°C. After diluting the cell solution with 10 milliliters of DME/F-12, it was filtered through a nylon mesh and spun. The pellet was seeded into flasks covered with gelatin (DIFCO, 0.1% in PBS) after being resuspended in endothelial growth medium 2-microvascularbullet kit. After 24 hours after seeding, the medium was changed and the flasks were cleaned with PBS to get rid of any non-adhering cells. Cells were trypsinized two to three days after seeding, and HDMECs were separated from contaminating cell types (fibroblasts and smooth muscle cells) using an immunomagnetic technique using CD31-conjugated microbeads in accordance with the manufacturer's instructions. In short, the cells were counted, treated with microbeads for 17 minutes at 4°C, and then passed through a magnetic column with a monoclonal antibody to CD31 coupled with the beads. CD31+ cells were seeded in gelatin-coated 75-cm² flasks after the process was repeated until all impurities were eliminated. The CD31+ cells represent a mixed population of lymphatic and blood HDMECs since CD31 is a pan-endothelial marker. Thus, lymphatic microvascular HDMECs were isolated using the lymphatic-specific monoclonal antibody D2-40. The cells were trypsinized, resuspended in DME/F-12, and run through a column to remove CD31+ cells that still had some beads attached before being incubated with D2-40. After spinning, the eluate was resuspended.in DME/F-12 containing a rat anti-mouse IgG1 antibody conjugated with the beads, incubated for 10 minutes at 4°C, spun, then incubated for 15 minutes at 4°C with 3% bovine serum albumin and D2-40 (final dilution 1:20). The cells were passed through a column that this time held CD31+ D2-40+ lymphatic HDMECs. From the eluate, blood HDMECs (CD31+ D2-40) were extracted.

In order to prevent unspecific binding sites, cells to be examined for the expression of particular markers were seeded onto round coverslips coated with gelatin, let to achieve confluence, fixed with cold acetone at 20°C for 7 minutes, and then rinsed and incubated for 40 minutes in a solution containing 3% BSA in PBS. Von Willebrand factor (polyclonal, diluted 1:50 after a 2-min permeabilization with 0.2% Triton), CD31 (monoclonal, diluted 1:20), and CD34 (monoclonal, diluted 1:20 after a 2-min permeabilization with 0.2% Triton) were the blood endothelial markers that were examined. Vascular Endothelial Growth Factor Receptor-3 (monoclonal, diluted 1:100), Prox-1 (polyclonal, diluted 1:250 after a 5-min permeabilization with 0.5% Triton), D2-40 (monoclonal, IgG-1, diluted 1:20), and LYVE-1 (monoclonal, diluted 1:50 after a 2-min permeabilization with 0.2% Triton) were the lymphatic markers that were tested. PBS containing 0.5% BSA (henceforth referred to as buffer) was used to dilute each antibody. Secondary antibodies labeled with fluorescein isothiocyanate-labeled goat anti-rabbit or anti-mouse IgG (diluted 1:100) or tetramethylrhodamine isothiocyanate-labeled rabbit anti-mouse IgG (diluted 1:200) were employed as needed. Monoclonal mouse anti-calbindin-D-28K (vitamin D-dependent calcium binding protein, IgG1) anti-bovine was used to replace the primary antibody in order to obtain isotypic controls for fibrillin-1 antibody, D2-40, Vascular Endothelial Growth Factor Receptor-3, and LYVE-1. A rabbit polyclonal antibody anti-calsequestrin-2 was used to create an isotypic control for Prox-1. Additionally, negative controls were carried out by leaving out main antibodies. Cells cultured on gelatine-coated coverslips were tested

for fibrillin deposition at confluence and on days two and four after confluence. To inhibit unspecific binding sites, cells were fixed for seven minutes in cold acetone, rinsed, and then incubated for forty minutes in a solution containing three percent BSA in PBS. The first labeling was completed followed by a 90-minute incubation with a fluorescein isothiocyanate-conjugated goat anti-rabbit IgG antibody (Sigma) diluted 1:100 after an overnight incubation at 4°C with a polyclonal antibody to MAGP-1 (MFAP-2; Sigma-Aldrich) diluted 1:25 in buffer. A 40-minute incubation with PBS containing 3% BSA once more prevented nonspecific binding sites. Following cleaning, cells were treated for two hours with a 1:100 diluted monoclonal antibody to fibrillin-1 in buffer. The secondary antibody, tetramethyl rhodamine isothiocyanate-conjugated goat antimouse IgG , diluted 1:200 in buffer, was incubated for 90 minutes to show the response. Coverslips were cleaned and then placed upside down on glass slides using Sigma's DABCO mounting media. At confluence (day 0), as well as on days two and four following confluence, fibrillin deposition was noted. Using a 40 objective and careful attention to set exposure conditions, coverslips containing adherent cells were taken in randomly chosen fields. After choosing the appropriate threshold, fibrillin deposition was assessed using a morphometric program called NIS Elements (Nikon) on a minimum of 50 photos per condition. The program measured the mean length of fibrillin fibers per photographic field, the mean number of branching points per photographic field, and the amount of fibrillin deposited expressed as sum density.

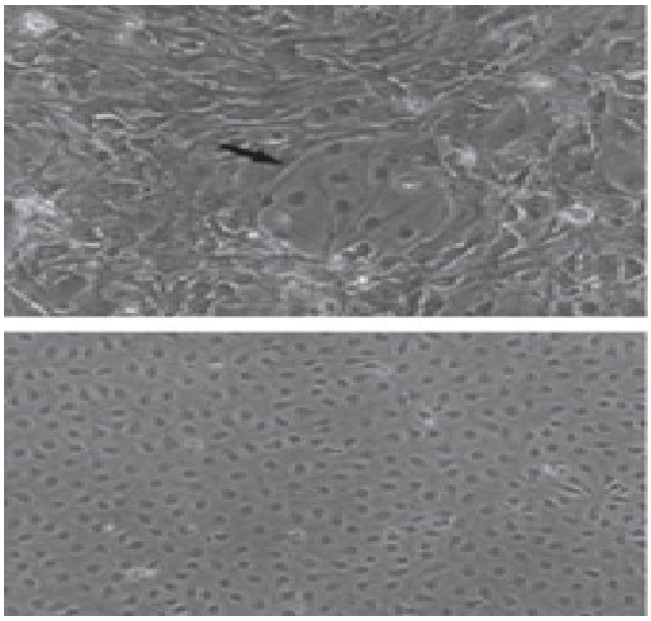

Fig 1 -Phase contrast microscopy of human dermal microvascular endothelial cells (HDMECs).
(A) Prior to purification, contaminants constitute the majority of the obtained cell population.
(B) An isolated group of HDMECs after purification with CD31. The cells have a polygonal shape.

Results

Collagenase's first digestion of the human foreskin dermis produced a mixed population of HDMECs and contaminants, primarily fibroblasts and smooth muscle cells (Fig. 1A). Following CD31 purification, isolated HDMEC clusters were visible because to their polygonal form (Fig. 1B). In three to six days, these cells multiplied and came to confluence. At this point, D2-40 was used to separate the lymphatic and blood HDMECs. The latter made up over 30% of all those who tested positive for CD31.Approximately 4.5 * 104 cells cm^2 were present in the lymphatic and blood HDMECs at confluence, which had a characteristic cobblestone shape (Fig. 1). This number did not rise noticeably on days two and four following confluence because ECs are contact inhibited.All evaluated blood endothelial markers including the pan-endothelial marker CD31 showed strong labeling of over 95% of blood HDMECs (CD31+ D2-40) (Fig. 2).

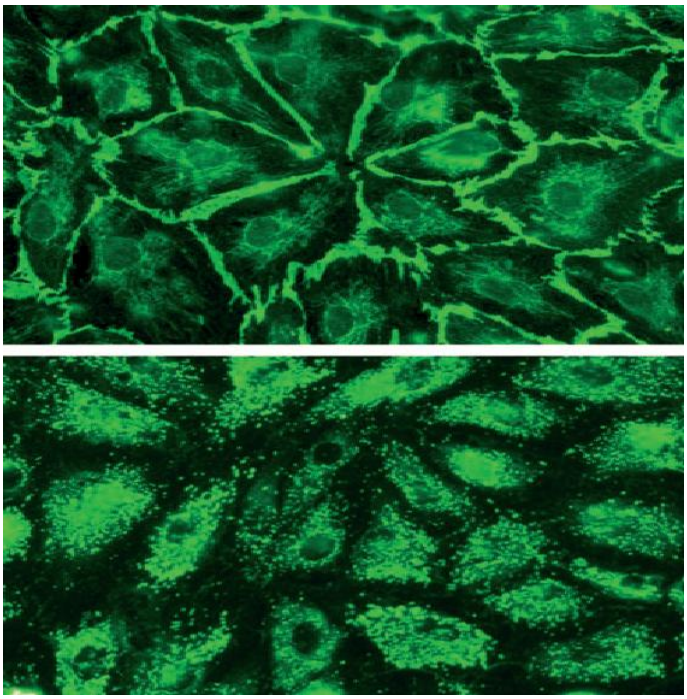

Fig 2 - Blood microvascular human dermal microvascular endothelial cells are characterized. The panendothelial marker CD31 (A) stains cell boundaries, whereas Vonvon Willebrand factor fluorescence (B)

While CD31 stained cell boundaries, Von Willebrand factor fluorescence was in the cytoplasm and appeared punctate, and the transmembrane glycoprotein CD34's punctate fluorescence was especially noticeable in the perinuclear area.The pan-endothelial marker CD31 and all examined lymphatic markers strongly labeled over 90% of lymphatic HDMECs (CD31+ D2-40+) (Fig. 3). While LYVE-1 and Vascular Endothelial Growth Factor Receptor-3 fluorescence was dispersed throughout the cell membrane and appeared punctate, CD31 and D2-40 mostly stained the cell boundaries. Cell nuclei were usually stained by Prox-1. Short, thin strands of fibrillin were first deposited, and they eventually came together to form linear arrays.

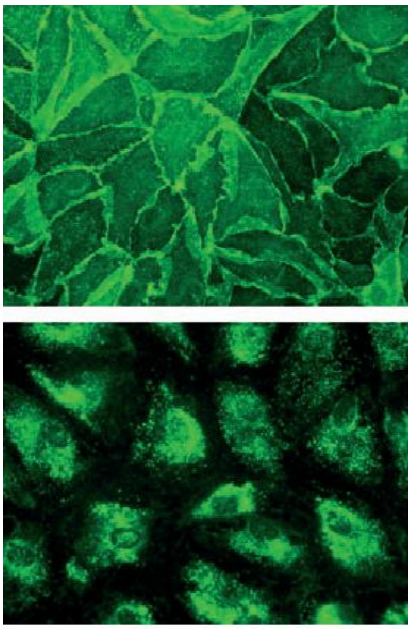

Fig 3 Characterization of human dermal endothelial cells in the lymphatic microvascular system. a) D2-40 stains the cell boundaries, whereas b) LYVE-1 and Vascular Endothelial Growth Factor Receptor 3 fluoresce.

The vast fibrillin-free areas of the honeycomb, which were encircled by fibrillin strands, showed HDMECs. Thin fibrillin strands that emerged from the honeycomb borders and divided the fibrillin-free areas into smaller ones later altered this original wide-mesh honeycomb. In addition, a thin network of fibrillin fibers filled the spaces between the various honeycombs, allowing some tiny honeycombs to develop (Pattern B, Fig. 4B).

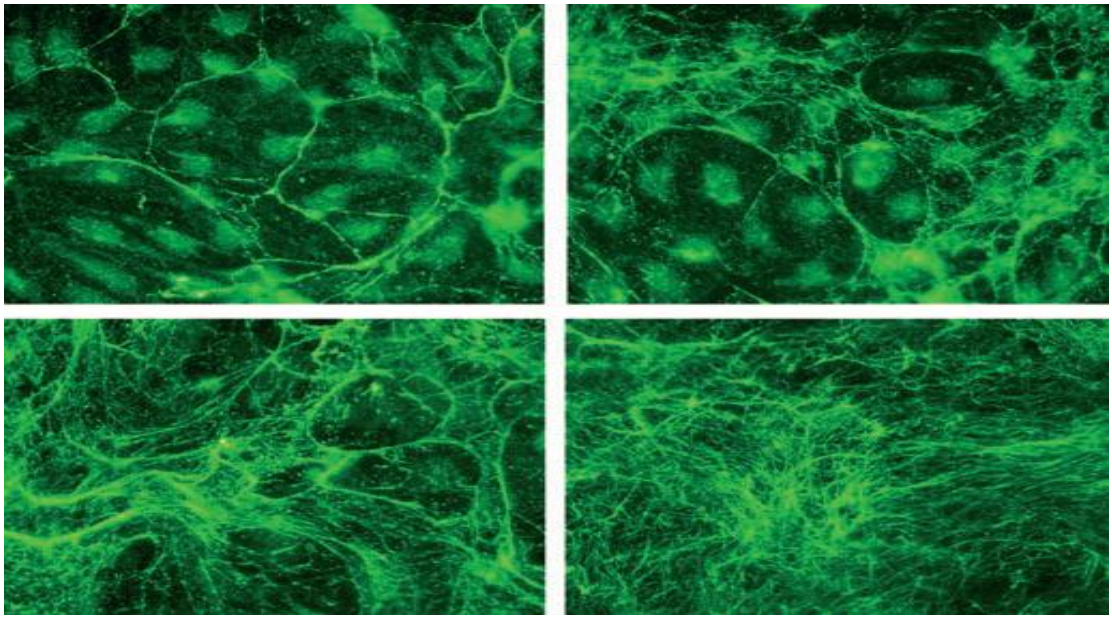

Figure 4: Patterns of fibrillin deposition. (A) design A: honeycomb design with broad mesh. The huge fibrillin-free areas show humandermal microvascular endothelial cells (HDMECs). (B) Pattern B: fibrillin-free areas are divided into smaller ones by thin fibrillin strands that emerge from the honeycomb boundaries. (C) Pattern C: an almost continuous net of fibrillin fills the vast honeycomb mesh gaps. The original honeycomb design is still discernible in part. (D) Pattern D: fibrillin creates an unevenly thick, almost continuous network.

Spaces devoid of fibrillin were progressively filled until, underneath the cell layer, a nearly continuous net of fibrillin developed, retaining part of the original honeycomb design (design C, Fig. 4C). Large strands of fibrillin coexisted with a mesh of tiny fibers to produce an almost continuous network of varying thickness (Pattern D, Fig. 4D).In the same culture, there were distinct patterns, or "hot spots" (Patterns C and D), which most likely matched the original tracks left by the original vessel. Pattern A predominated in confluent cultures and gradually declined on days two and four, Pattern B rose from confluence to day two and then declined concurrently with the rise of Pattern C on day four. On day two, Pattern D was insignificant at confluence; on day four, it grew to 11.4% of instances. In general, civilization gradually changed throughout time to increasingly intricate patterns. The patterns of fibrillin deposition by lymphatic HDMECs were identical to those reported for blood HDMECs. In addition, two further preliminary patterns were noted in place of Patterns A and B: Fibrillin created a dense network of short segments in Pattern A (Fig. 4E), which progressively came together to produce longer filaments grouped in semicircular arrays in Pattern B (Fig. 4F).

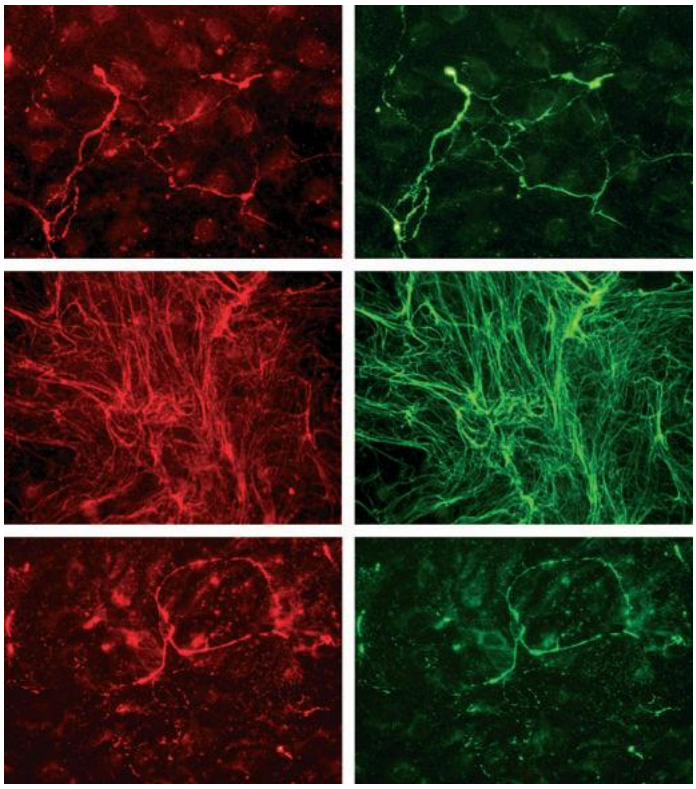

Fig 5 - Co-localization of fibrillin and microfibril-associated glycoprotein (MAGP)-1. Human dermal microvascular endothelial cells at day 2 (A, B, C) and day 4 (D, E,F) following confluence showed fibrillin (left) and MAGP-1 (right) in the blood (A–C) and lymphatic (D–F).·40 was the initial magnification.

At later phases of deposition, Patterns C and D were easily identifiable as the fibrillin mesh progressively got denser.The identical glass coverslips showed varying deposition patterns (Fig. 5B). At confluence, Pattern A was dominant, declining on days two and four in tandem with Patterns B and C's linear rise. At day 4, Pattern D, which was not present at confluence, rose to 6.1%. From the time of confluence until day four, patterns a and b steadily declined. Figure 5 shows a schematic illustration that summarizes the patterns of fibrillin deposition in lymphatic and blood ECs. At confluence, MAGP-1 had already been deposited in the underlying matrix by both blood and lymphatic HDMECs. During every test, MAGP-1 and fibrillin-1 co-localized flawlessly in all blood (Fig. 5A–C) and lymphatic (Fig. 5D–E) HDMEC cells. Two independent observers, E.G. and M.V., visually assessed the area covered by fibrillin. They found that in blood HDMECs, it was roughly 25% at confluence, 60% at day 2, and 80% at day 4 after confluence, and in lymphatic HDMECs, it was roughly 60% at confluence, 65% at day 2, and 70% at day 4 after confluence. At confluence, both blood and lymphatic HDMECs displayed a constitutive amount of fibrillin, which grew steadily over time in both cell types (Fig. 5).

Discussion

To the best of our knowledge, this is the first report of fibrilli being deposited in the underlying matrix by human-cultured blood and lymphatic microvascular ECs. Due to varying levels of fibrillin that were carried over from the original vessel, fibrillin was deposited in both cell types in various patterns that coexisted in the same culture (Brenn et al. 1996). We suggest that Pattern A might be an early stage of deposition and that a progressive shift from the simplest Pattern A to the most complex Pattern D occurs over time in culture, as the percentage of Patterns A and B gradually decreased from confluence to days 2 and 4, while Patterns C and D simultaneously increased. Similar to ECs isolated from auricular chondrocytes (Brown-Augsburger et al. 1996) and bovine major arteries (Weber et al. 2004), blood HDMECs deposited fibrillin in a honeycomb pattern. In contrast to bovine thoracic duct ECs (Weber et al. 2004), lymphatic HDMECs exhibited characteristics akin to those of their blood

counterparts. Overall, fibrillin was more evenly distributed and more plentiful at confluence in lymphatic than in blood HDMECs, as patterns a and b specifically show. This could be the consequence of fibrillin microfibrils, which are likely to correlate to anchoring filaments seen in all lymphatic ECs, more widely passing over from the vessel of origin. Nonetheless, fibrillin deposition in the underlying extracellular matrix would gradually rise, indicating a particular need of the lymphatic starting channel rather than of the surrounding tissue. Blood HDMECs showed the opposite pattern, producing much more fibrillin over time in culture while having less fibrillin at confluence. Their fast rise in fibrillin deposition would suggest that blood microvascular ECs generally possess the innate capacity to generate significant quantities of fibrillin when needed. Regarding the deposition of fibrillin, we think that the reason lymphatic HDMECs act more like their blood counterparts than like lymphatic ECs separated from the thoracic duct might be because the two cell types express MAGP-1 differently. We previously hypothesized that only when fibrillin-1 and MAGP-1 are both already expressed at confluence can a honeycomb pattern arise in vitro. When MAGP-1 is not present, as in the thoracic duct. Fibrillin is deposited in ECs in an asymmetrical web. According to Raghunath et al. (1996), fibrillin microfibrils have been demonstrated to precede and direct elastin deposition in the healing skin of burnt infants. It makes sense that multiple cell types contribute to the production of fibrillin given its strategic importance in the skin both during skin repair and under normal conditions. Fibroblasts (Sakai et al. 1986; Kielty & Shuttleworth, 1993) and keratinocytes (Haynes et al. 1997) likely account for fibrillin deposition at the dermo-epidermal junction, while blood and lymphatic ECs may also be responsible for fibrillin and, consequently, elastin deposition in the remaining dermis.

References

1. Brenn T, Aoyama T, Francke U, et al. (1996) Dermal fibroblast culture as a model system for studies of fibrillin assembly and pathogenetic mechanisms: defects in distinct groups of individuals with Marfan's syndrome. Lab Invest 75, 389–402.
2. Brown-Augsburger P, Broekelmann T, Mecham L, et al. (1994) Microfibril-associated glycoprotein binds to the carboxylterminal domain of tropoelastin and is a substrate for transglutaminase. J Biol Chem 269, 28443–28449.
3. Brown-Augsburger P, Broekelmann T, Rosenbloom J, et al. (1996) Functional domains on elastin and microfibrilassociated glycoprotein involved in elastic fibre assembly. Biochem J 318, 149–155.
4. Carta L, Pereira L, Arteaga-Solis E, et al. (2006) Fibrillins 1 and 2 perform partially overlapping functions during aortic development. J Biol Chem 281, 8016–8023.
5. Casley-Smith JR (1980) Are the initial lymphatics normally pulled open by the anchoring filaments? Lymphology 13, 120–129.
6. Dubuisson L, Lepreux S, Bioulac-Sage P, et al. (2001) Expression and cellular localization of fibrillin-1 in normal and pathological human liver. J Hepatol 34, 514–522.
7. Gerli R, Ibba L, Fruschelli C (1990) A fibrillar elastic apparatus around human lymph capillaries. Anat Embryol 181, 281–286.
8. Gibson MA, Finnis ML, Kumaratilake JS, et al. (1998) Microfibrilassociated glycoprotein-2 (MAGP-2) is specifically associated with fibrillin-containing microfibrils but exhibits more restricted patterns of tissue localization and developmental expression than its structural relative MAGP-1. J Histochem Cytochem 46, 871–886.
9. Haynes SL, Shuttleworth CA, Kielty CM (1997) Keratinocytes express fibrillin and assemble microfibrils: implications for dermal matrix organization. Br J Dermatol 137, 17–23.
10. Henderson M, Polewski R, Fanning JC, et al. (1996) Microfibrilassociated glycoprotein-1 (MAGP-1) is specifically located on the beads of the beaded-filament structure for fibrillincontaining microfibrils as visualized by the rotary shadowing technique. J Histochem Cytochem 44, 1389–1397.
11. Jurisic G, Detmar M (2009) Lymphatic endothelium in health and disease. Cell Tissue Res 335, 97–108.

12. Kahn HJ, Marks A (2002) A new monoclonal antibody, D2-40, for detection of lymphatic invasion in primary tumors. Lab Invest 82, 1255–1257.
13. Kielty CM, Shuttleworth CA (1993) Synthesis and assembly of fibrillin by fibroblasts and smooth muscle cells. J Cell Sci 106,167–173.
14. Kitahama S, Gibson MA, Hatzinikolas G, et al. (2000) Expression of fibrillins and other microfibril-associated proteins in human bone and osteoblast-like cells. Bone 27, 61–67.
15. Kriehuber E, Breiteneder-Geleff S, Groeger M, et al. (2001) Isolation and characterization of dermal lymphatic and blood endothelial cells reveal stable and functionally specialized cell lineages. J Exp Med 194, 797–808.
16. Lee B, Godfrey M, Vitale E, et al. (1991) Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. Nature 352, 330–334.
17. Lemaire R, Bayle J, Mecham RP, et al. (2007) Microfibril associated MAGP-2 stimulates elastic fiber assembly. J Biol Chem 282, 800–808.
18. Raghunath M, Ba¨ chi T, Meuli M, et al. (1996) Fibrillin and elastin expression in skin regenerating from cultured keratinocyte autografts: morphogenesis of microfibrils begins at the dermo-epidermal junction and precedes elastic fiber formation. J Invest Dermatol 106, 1090–1095.
19. Ramirez F (2000) Pathophysiology of the microfibril／elastic fiber system: introduction. Matrix Biol 19, 455–456.
20. Ramirez F, Sakai LY (2010) Biogenesis and function of fibrillin assemblies. Cell Tissue Res 339, 71–82.
21. Robinson PN, Arteaga-Solis E, Baldock C, et al. (2006) The molecular genetics of Marfan syndrome and related disorders. J Med Genet 43, 769–787.
22. Rossi A, Weber E, Sacchi G, et al. (2007) Mechanotransduction in lymphatic endothelial cells. Lymphology 40, 102–113.
23. Ryan TJ (1995) Mechanical resilience of skin: a function of blood supply and lymphatic drainage. Clin Dermatol 13, 429–432.
24. Sakai LY, Keene DR, Engvall E (1986) Fibrillin, a new 350-kD glycoprotein, is a component of extracellular microfibrils. J Cell Biol 103, 2499–2509.
25. Sakamoto H, Broekelmann T, Cheresh DA, et al. (1996) Cell-type specific recognition of RGD- and non-RGD-containing cell binding domains in fibrillin-1. Biol Chem 271, 4916–4922.
26. Scavelli C, Weber E, Agliano` M, et al. (2004) Lymphatics at the crossroads of angiogenesis and lymphangiogenesis. J Anat 204, 433–449.
27. Schacht V, Dadras SS, Johnson LA, et al. (2005) Up-regulation of the lymphatic marker podoplanin, a mucin-type transmembrane glycoprotein, in human squamous cell carcinomas and germ cell tumors. Am J Pathol 166, 913–921.
28. Solito R, Alessandrini C, Fruschelli M, et al. (1997) An immunological correlation between the anchoring filaments of initial lymph vessels and the neighboring elastic fibers: a unified morphofunctional concept. Lymphology 30, 194–202.
29. Stephan S, Ball SG, Williamson M, et al. (2006) Cell-matrix biology in vascular tissue engineering. J Anat 209, 495–502.
30. Wagenseil JE, Mecham RP (2007) New insights into elastic fiber assembly. Birth Defects Res C Embryo Today 81, 229–240.
31. Weber E, Rossi A, Solito R, et al. (2002) Focal adhesion molecules expression and fibrillin deposition by lymphatic and blood vessel endothelial cells in culture. Microvasc Res 64, 47– 55.
32. Weber E, Rossi A, Solito R, et al. (2004) The pattern of fibrillin deposition correlates with microfibril-associated glycoprotein 1 (MAGP-1) expression in cultured blood and lymphatic endothelial cells. Lymphology 37, 116–126.