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A phenotypical study of vascular smooth muscle cells in human arterial and venous stenosis

Etudiant
Swenn Maxence Krähenbühl

Tuteur
Professeur Jean-Marc Corpataux
Service de Chirurgie Thoracique et Vasculaire, CHUV

Co-tuteur
Docteur François Saucy
Service de Chirurgie Thoracique et Vasculaire, CHUV

Expert
Professeur Jacques-Antoine Haefliger
Service de Médecine Interne, CHUV

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Abstract

Introduction

The primary function of the contractile vascular smooth muscle cells (cVSMCs) is the regulation of the vascular contractility which means the adaptation of the vascular tonus in response to the modulation of the blood pressure and blood flow. The cVSMCs are essentially quiescent, and therefore their synthesis rate is very limited. They are characterized by the expression of contractile proteins specific to the muscular tissue including myosin, h-caldesmon and α-smooth muscle actin (α-SMA). These contractile cells are strongly represented in the media layer of the arterial wall and, in a smaller proportion, of the vein wall. Their typical stretched-out morphology allows recognizing them by a histological analysis. They do not produce any extracellular matrix (ECM), and do not migrate through the different layers of the vessel wall, and are not directly involved in the development of intimal hyperplasia (IH).

Neointimal formation occurs after endothelial disruption leading to complex molecular and biological mechanisms. The de-differentiation of cVSMCs into synthetic VSMCs (sVSMCs) is mentioned as a key element. These non mature cells are able to proliferate and produce ECM.

The characterization of the vascular smooth muscle cells (VSMCs) from healthy and stenosed vascular tissues will contribue to the understanding of the different biological processes leading to IH and will be useful for the development of new therapies to interfere with the cVSMCs growth and migration.

The aim of our research was to quantify the proportion of cVSMCs and sVSMCs into the healthy and pathologic human blood vessel wall and to characterize their phenotype.

Methods

We selected 23 specimens of arterial and venous segments from 18 patients. All these specimens were stored in the biobank from the thoracic and vascular surgery departement. 4 groups were designed (group 1 : arteries without lesions (n=3) ; group 2 : veins without lesions (n=1) ; group 3 : arteries with stenosis (n=9) ; group 4 : veins with stenosis (n=10)).

Histology : 5μm-sections were made from each sample embedded in paraffin wax and further stained with hematoxylin & eosin (HE), Van Gieson's stain (VGEL) and Masson's Trichrome (TMB). Pathologic tissues were defined using the label that was given to the macroscopic samples by the surgeon and also, based on the histological analysis with HE

and VGEL evaluating the presence of a thickened intima. The same was done to the control samples evaluating the absence of thickening.

Immunohistochemistry : The primary antibodies were used : α-SMA, vimentin, h-caldesmon, calponin, smooth muscle-myosin heavy chain (SM-MHC), tropomyosin-4, retinol binding protein-1 (RBP-1), non muscle-myosin heavy chain-B (NM-MHC-B), Von Willebrand factor (VWF). A semi-quantitative assessment of the intensity of each sample stained was performed.

Western Blot : Segments of arteries and veins were analyzed using the following primary antibodies : α-SMA, Calponin, SM-MHC, NM-MHC-B. The given results were then normalized with tubulin.

Results

Our data showed that, when using immunohistochemistry analysis we found that α-SMA was mostly expressed in control arteries, whereas NM-MHC-B in the pathologic ones. Using SM-MHC, calponin, vimentin and caldesmon we found no significative differences in the expression of these proteins in the control and in the pathologic samples. Western Blot analysis showed an inverse correlation between healthy and pathological samples as α-SMA was more expressed in the pathological samples, while NM-MHC-B in the control group ; SM-MHC and calponin were mostly expressed in the pathologic samples.

Conclusion

Our study showed no clear differences between stenotic and control arterial and venous segments using semi-quantitative assessement by immunohistochemistry. Western Blot showed a significant increased expression of α-SMA, calponin and SM-MHC in the arteries with stenosis, while NM-MHC-B was mostly expressed in the arteries without lesions.

Further studies are needed to track the lineage of VSMCs to understand the mechanisms leading to IH.

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Introduction

Peripheral arterial disease reprents a major clinical problem in the industrial countries. There are about 200'000 and 500'000 patients treated for this medical problem every year in Europe, and it won't stop increasing.[1] Lower limb revascularization by a surgical bypass is a therapeutic solution that is often used despite the increasing number of endovascular interventions. Doing such a bypass is generally sufficent to heal intermittent claudication or critical limb ischemia.[2] The results of primary and secondary permeability are relatively good in a short to mid-term.[3] Nevertheless, in 20-50% of all cases, we have to face restenosis linked to the development of myointimal proliferation at the anastomosis and/or inside the bypass.[4,5] Indeed, surgical or endovascular interventions create a vascular trauma triggering a remodelling process named intimal hyperplasia (IH). It can be sufficient to create a significant stenosis.[6] IH is the biological result of the increasing number of vascular smooth muscle cells (VSMCs) in the sub-intima region, followed by the proliferation of the extra-cellular matrix (ECM) which provokes a thickening of the blood vessel wall and thus a decrease of the lumen. VSMCs are the key-cells of this process.

The primary function of the contractile vascular smooth muscle cells (cVSMCs) is the regulation of the vascular contractility which means adaptation of the vascular tonus, blood pression and blood flow. They are essentially quiescent, which means that their synthesis rate is very limited.[2] They are characterized by the expression of contractile proteins specific to the muscular tissue including myosin, h-caldesmon and α-smooth muscle actin (α-SMA).[7] These contractile cells are strongly represented in the media layer from the arterial wall and, in a smaller proportion, from the vein wall.[8] Their typical stretched-out morphology make them easy to recognize by a histological analysis. They do not produce any ECM (connective tissue composed of proteoglycans (PG), non-PG polysaccharides, fibers, fibronectin and laminin), do not migrate through the different layers of the vessel wall, and are not directly implicated into the IH process.[2]

In the contrary, synthetic vascular smooth muscle cells (sVSMCs) are thought to come from the de-differentiation of cVSMCs.[9,10] This mechanism, only partially understood, is triggered by the vascular trauma associated with an endothelial injury followed by platelets aggregation and their activation. A complicated enzymatic cascade permits the phenotypical modifications of VSMCs.[4,11] These cells can migrate from the media layer to the intima layer. They also have a very high proliferation rate, produce ECM and loose their vascular contractility capacity. We can differenciate them from the cVSMCs by the

expression of diverse proteins like non-muscle myosin heavy chain (NM-MHC) and retinol binding protein-1 (RBP-1).[7,12]

The conversion of cVSMCs into sVSMCs is thought to play a major role in the vascular diseases such as atherosclerosis and restenosis.[13-15] Most of the new therapies are centered on the aspect of de-differentiation of these cVSMCs into sVSMCs in the context of vascular lesions in order to significantly inhibit this biological process of IH.[16]

Nevertheless, the origin of sVSMCs is controversial, and the specific markers are lacking to trace the lineage of VSMCs.[17]

The evaluation of the proportion of VSMCs in healthy and stenosed vascular wall will be useful for the development of future new therapies focused on the de-differentiation of cVSMCs into sVSMCs.

The aim of this research was to quantify the proportion of cVSMCs and sVSMCs into the healthy and pathologic human blood vessel wall and characterize their phenotype.

Methods

Tissue sampling

The human veins and arteries were harversted during surgical interventions. The ethical committee of the University Hospital of Lausanne approved the experiments, which are in accordance with the principles outlined in the Declaration of Helsinki for use of human tissue. The tissue fragments were then divided into two equal lengths. One segment was directly fixed in formol and the other frozen in liquid nitrogen and stored at -80°C.

The specimens were characterized and described into an anonymised database.

The selected samples were divided in four groups :

- Group 1 : Arteries without lesions (named control arteries) (n=3)
- Group 2 : Veins without lesions (named control vein) (n=1)
- Group 3 : Arteries with stenosis (named pathologic arteries) (n=9)
- Group 4 : Veins with stenosis (named pathologic veins) (n=10)

A total of 23 specimens were harvested in 18 patients.

Histology

The different samples were first embedded in paraffin wax and consecutive 5μm-sections were made on each sample. The sections were further stained with hematoxylin & eosin (HE), Van Gieson's stain (VGEL) and Masson's Trichrome (TMB). HE will stain the nucleus in black/purple and the different cytoplasmic components in pink/red ; VGEL will stain the collagen fibers in red, in order to differentiate the different layers of the blood vessel wall as well as the presence of IH ; TMB will differentiate the connective tissue stained in blue from the nucleus (dark red/purple) and the cytoplasm stained in red/pink. Among the 30 samples initially chosen, we selected, based on the HE and VGEL stainings, the best samples for the immunohistochemistry analysis, 7 samples from the 30 were dicarded. This brings the count to 23 samples used as listed in the groups above.

Pathologic tissues were defined first, using the label that was given to the macroscopic sample by the surgeon and second, based on the histological analysis with HE and VGEL evaluating the presence of a thickened intima. The same was done to the control samples evaluating the absence of thickening.

Immunohistochemistry

The primary antibodies listed below were used. Binding sites of the different antibodies were visualized using an avidin-biotinylated horseradish peroxidase complex (Vectastain Elite ABC Kit, USA). After rinsing the sections in PBS, the precipitate obtained from the perdoxidase reaction was visualized using 0.1% 3,3-diaminobenzidine tetrahydrochloride dehydrate, dissolved in PBS containing 0.3% H_2O_2. Antibody binding was revealed using the avidin-biotin technique.

Antibodies used in the study :

1. α-SMA (α-Smooth Muscle Actin ; Sigma ; 1:50'000)
2. Vimentin (Dako ; 1:200)
3. H-Caldesmon (Dako ; 1:100)
4. Calponin (Dako ; 1:200)
5. SM-MHC (Smooth Muscle-Myosin Heavy Chain ; Milipore ; 1:200)
6. Tropomyosin-4 (Serotec ; 1:100)
7. RBP-1 (Retinol Binding Protein-1 ; Abnova ; 1:50)
8. NM-MHC-B (Non Muscle-Myosin Heavy Chain-B ; Abcam ; 1:100)
9. VWF (Von Willebrand Factor ; Dako ; 1:500)

Then a semi-quantitative assessment of the intensity of each sample stained was performed. An arbitrary scale was used : (**0**) : no expression ; (**+**) : light expression ; (**++**) : moderate expression ; (**+++**) : strong expression. The sections were analysed at 3 different time frame by the same author each and every time. Then a mean value was calculated on the basis of these 3 evaluations.

Western Blot

Segments of veins and arteries were reduced to powder and homogenized in lysis buffer as published. Samples of total vessel extracts (25μg) were resolved by SDS-PAGE (10%) and transferred to a polyviliden difluoride membrane (immobilon p, Millipore). The membrane was incubated with a primary antibody overnight at 4°C, washed in PBS-T 3 times 10 minutes and then incubated for 1h with horseradish peroxydase-linked antibody against mouse (Southern Biotech), rabbit (Thermo Scientific) or goat IgGs (Sigma), whichever adequate. Immunostaining was revealed by chemoluminescence, using ECL-plus kit (GE Healthcare).

The following primary antibodies were used :

1. α-SMA (Sigma ; 1:5'000)
2. SM-MHC (Milipore ; 1:1'000)
3. Calponin (Dako ; 1:2'000)
4. NM-MHC-B (Abcam ; 1:2'000)

Results

Patients Characteristics

Table 1 summarizes the demographic and clinical data characteristics of the patients in the four groups. All clinical data are based on the 23 samples used for the immunohistochemistry analysis; therefore, we had 14 men samples and 9 women samples (mean age : 67.5 ± 14.3 years). A particular attention was taken to list the different comorbidities and their medical treatements.

Variables (n=23)	n (%)
Age (mean ± SD)	67.5 ± 14.3
Men	14 (61)
Women	9 (39)
Comorbidities	
Hypertension	20 (87)
CVD	14 (61)
CDK	14 (61)
Diabetes	13 (57)
Dyslipidemia	12 (52)
Medical Treatment	
Statins	17 (74)
Aspirin	17 (74)
Acenocoumarol	12 (52)
Clopidogrel	4 (17)
Heparin	3 (13)

Table 1. Demographic & Clinical Data. (**CVD**) : cardiovascular disease ; (**CDK**) : chronic kidney disease ; (**SD**) : standard deviation.

We can observe (Figure 1) that 100% of the pathologic arteries are from the lower limb, whereas 100% of the pathologic veins originate from the upper limb. And all pathologic arteries come from the external iliac artery.

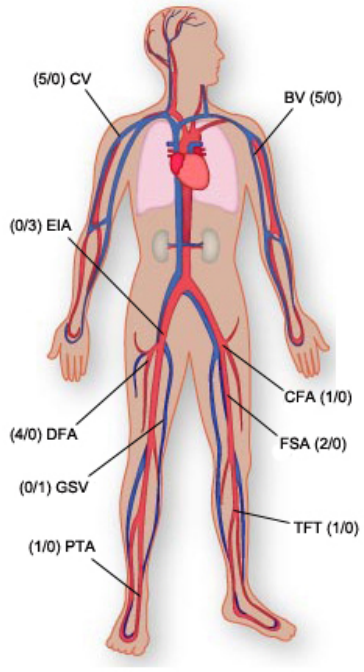

Figure 1. Anatomic Origins of Vascular Segments. Simplified anatomic plan showing the origins of the different vascular segments listed, with for each origin, the number of pathologic and control samples that were harvested from (**number of pathologic samples/number of control samples**). For example : (5/0) CV means 5 pathologic samples and 0 control sample harvested from the cephalic vein. (**SFA**) : superficial femoral artery ; (**DFA**) : deep femoral artery ; (**CFA**) : common femoral artery ; (**PTA**) : posterior tibial artery ; (**TFT**) : tibio-fibular trunk ; (**EIA**) : external iliac artery ; (**CV**) cephalic vein ; (**BV**) : basilic vein ; (**GSV**) : great saphenous vein.

Histology

The sections of arteries and veins from control and pathologic vessels, were first stained with the standard immunostainings (Figures 2 & 3), to visualize the different layers of the vessel wall, which also permitted to categorize them into the control or pathologic groups.

The Figures 2 & 3, comparing respectively a control artery with a pathologic artery (Figure 2) and a control vein with a pathologic vein (Figure 3) stained for the standard histological exam, show, in the control group, a better organized architecture of the vessel wall than in the pathologic one. It also shows the presence of a thickened intima in the pathologic group, which is typical of IH.

While selecting the samples, the « best » ones corresponding to each group were selected. By « best », we mean most appropriate to the group description ; for example a control sample would be a vein or an artery without a thickened intima. Note that the control vein does not fit the criterias as perfectly as the control artery does due to the light presence of IH ; we had to face some availability troubles concerning the healthy veins. So we had to choose the « healthiest » one.

TMB will differentiate the connective tissue stained in blue from the nucleus (dark red/purple) and the cytoplasm stained in red/pink. Compared to the control group, the pathologic one shows a slight predominance of blue into the intima, for both veins and arteries.

VWF staining was included in the standard histological exam figures, although it is a immunohistochemical staining, because it allows to see the continuity of the vascular endothelium of the lumen. It was important in this study, to assess if the endothelium was preserved despite the IH ; and as shown in Figures 2 & 3, it is somehow well preserved in each sample stained, a bit thinner or less expressed in the pathologic group.

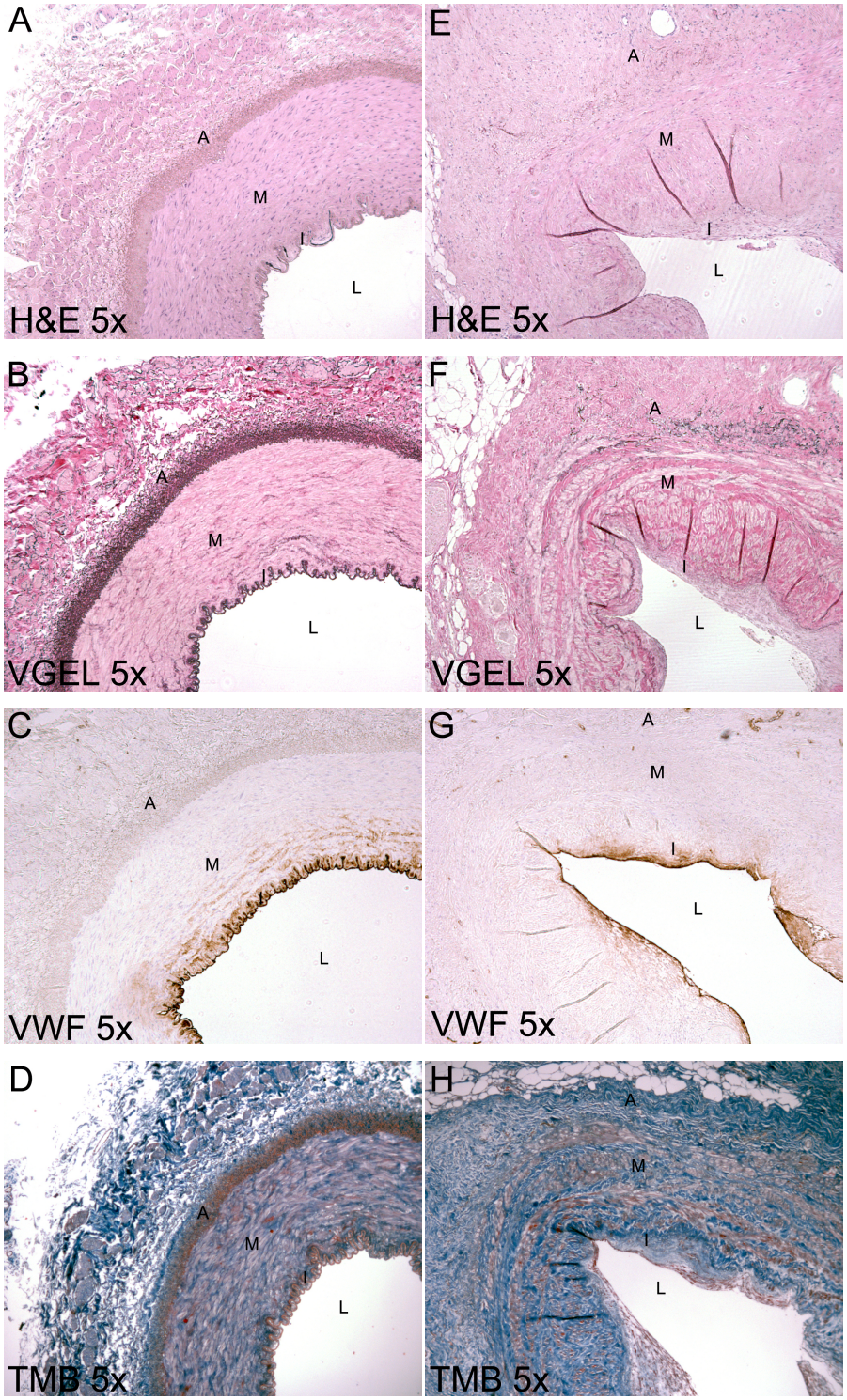

Figure 2. Healthy vs Pathologic Artery, original magnification x5. (**A-D**) : control, healthy artery ; (**E-H**) : pathologic, stenosed artery ; (**VGEL**) : Van Gieson's stain ; (**HE**) Hematoxylin & Eosin stain ; (**VWF**) : Von Willebrand Factor stain ; (**TMB**) Masson's Trichrome ; (**L**) : lumen ; (**I**) : intima ; (**M**) : media ; (**A**) : adventitia.

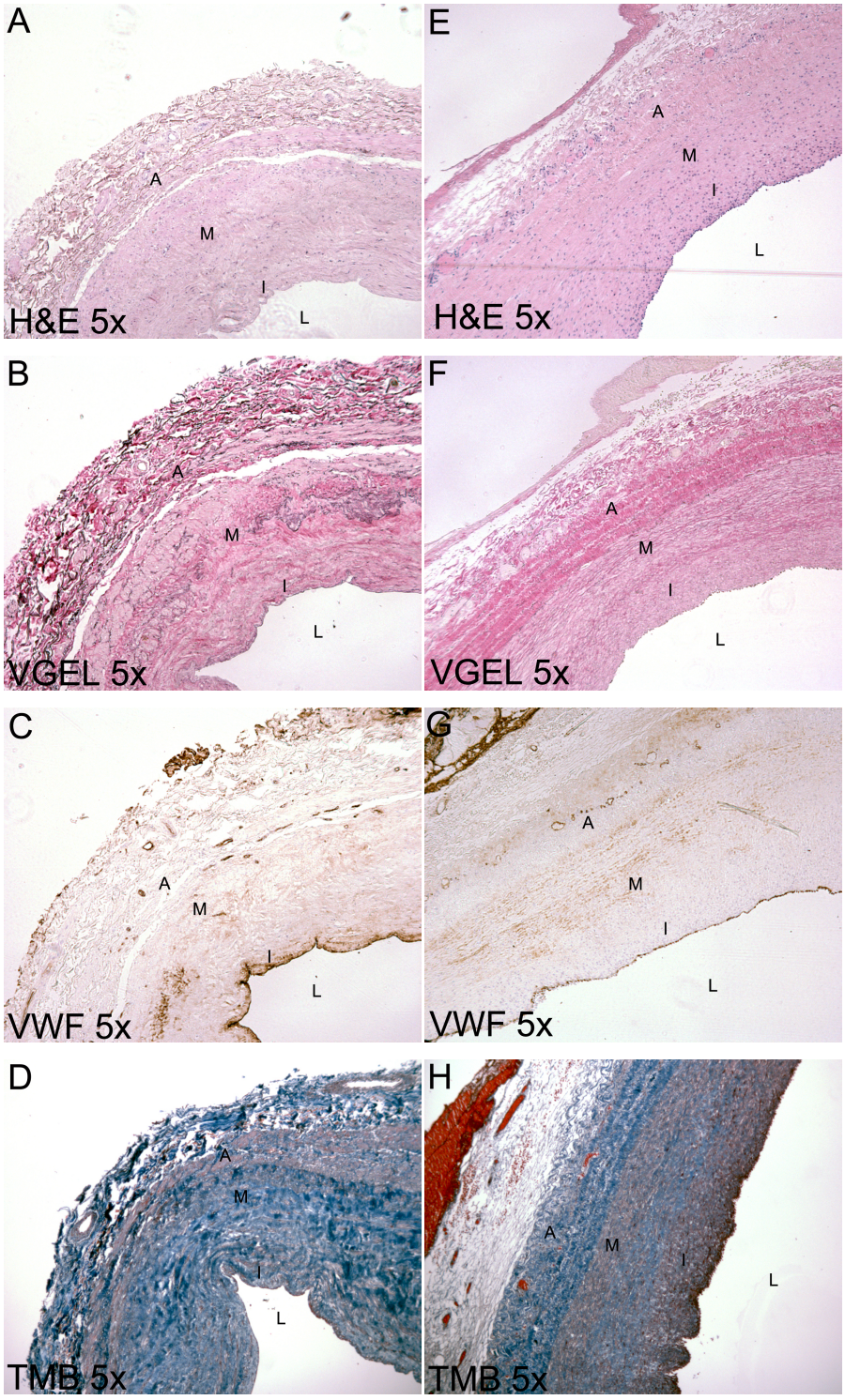

Figure 3. Healthy vs Pathologic Vein, original magnification x5. (**A-D**) : control, healthy vein ; (**E-H**) : pathologic, stenosed vein. (**VGEL**) : Van Gieson's stain ; (**HE**) Hematoxylin & Eosin stain ; (**VWF**) : Von Willebrand Factor stain ; (**TMB**) : Masson's Trichrome ; (**L**) : lumen ; (**I**) : intima ; (**M**) : media ; (**A**) : adventitia.

Immunohistochemistry

Figures 4 & 5 compare respectively a control artery with a pathologic artery (Figure 4) and a control vein with a pathologic vein (Figure 5) stained with the primary antibodies listed above for the immunohistochemical exam. The VSMCs were positive for vimentin, SMA, h-caldesmon, calponin, SM-MHC, tropomyosin-4, RBP-1, NM-MHC-B.

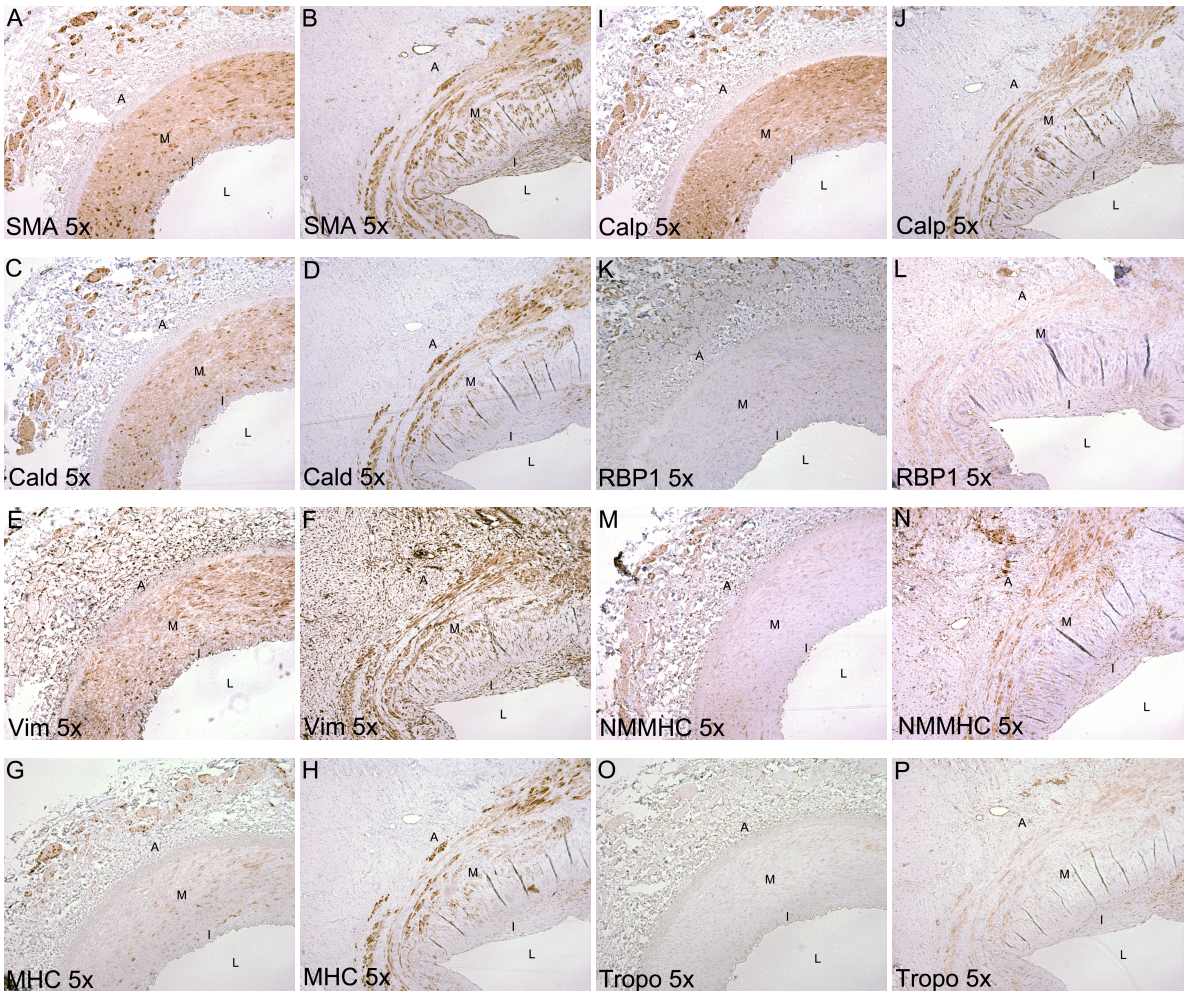

Figure 4. Healthy vs Pathologic Arteries with the Primary Antibodies Tested, original magnification x5. (**α-SMA**) : α-Smooth Muscle Actin ; (**Calp**) : Calponin ; (**Cald**) : H-Caldesmon ; (**RBP1**) : Retinol Binding Protein-1 ; (**Vim**) : Vimentin ; (**NMMHC**) : Non-Muscle Myosin Heavy Chain ; (**MHC**) : Smooth-Muscle Myosin Heavy Chain ; (**Tropo**) : Tropomyosin-4 ; (**L**) : lumen ; (**I**) : intima ; (**M**) : media ; (**A**) : adventitia. The first and the third column (**A, C, E, G, I, K, M, O**) represent the control artery. The second and last column (**B, D, F, H, J, L, N, P**) represent the pathologic artery.

	Vimentin	SMA	H-Caldesmon	Calponin	SM-MHC	Tropomyosin 4	RBP-1	NM-MHC-B
Pathologic Arteries	+++	++	++	++	+	+	+	+
Control Arteries	+++	+++	++	++	+	0	+	0
Pathologic Veins	+++	++	++	++	++	++	++	++
Control Veins	++	+++	++	++	++	0	++	+

Table 2. Semi-quantitative Assessment of the Expression of the Antibodies with Immunohistochemical analysis. (**0**) : no expression ; (**+**) : light expression ; (**++**) : moderate expression ; (**+++**) : strong expression.

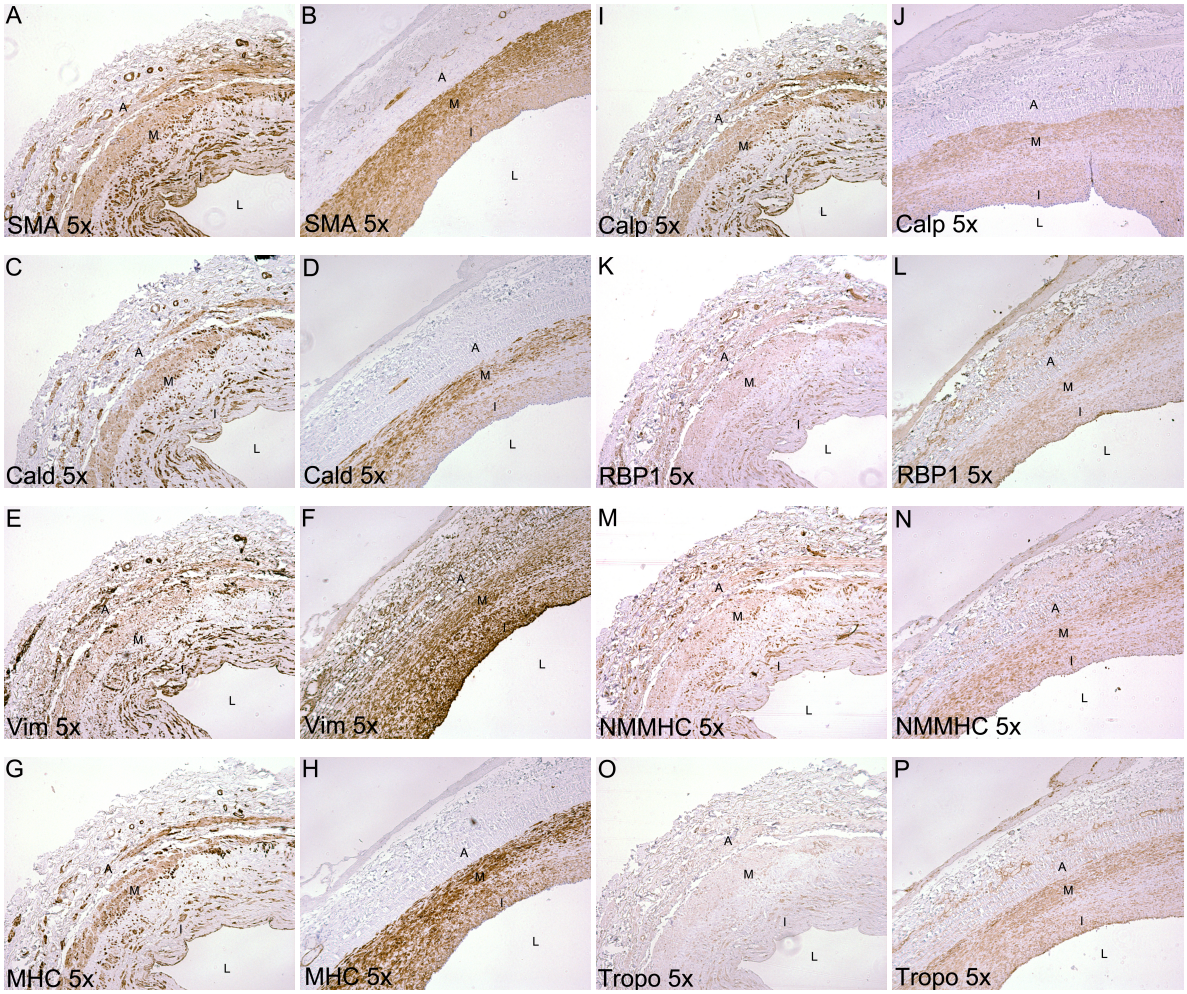

Figure 5. Healthy vs Pathologic Veins with the Primary Antibodies Tested, original magnification x5. (**α-SMA**) : α-Smooth Muscle Actin ; (**Calp**) : Calponin ; (**Cald**) : H-Caldesmon ; (**RBP1**) : Retinol Binding Protein-1 ; (**Vim**) : Vimentin ; (**NMMHC**) : Non-Muscle Myosin Heavy Chain ; (**MHC**) : Smooth-Muscle Myosin Heavy Chain ; (**Tropo**) : Tropomyosin-4 ; (**L**) : lumen ; (**I**) : intima ; (**M**) : media ; (**A**) : adventitia. The first and the third column (**A, C, E, G, I, K, M, O**) represent the control vein. The second and last column (**B, D, F, H, J, L, N, P**) represent the pathologic vein.

Based on the immunohistochemical analysis of the samples above, a semi-quantitative assessment of the intensity was performed (Table 2):

The major differences that can be observed, are the antibodies' expressions between each group stained with tropomyosin-4 and NM-MHC-B in both group veins and arteries. Indeed, comparing stainings with tropomyosin-4 and NM-MHC-B, the differences in expression between the pathologic and control artery are the same : the pathologic artery has a light expression, while the control one has no expression. Concerning the veins' groups : the pathologic vein has for both tropomyosin-4 and NM-MHC-B stainings a moderate expression, whereas control vein has no expression when stained with tropomyosin-4 and a light expression when stained with NM-MHC-B. Note that SMA was mostly expressed in the control artery, whereas NM-MHC-B in the pathologic one.

Western Blot

Figure 6 shows the WB of the antibodies listed above tested on the arteries. There was only one control vein, therefore we did not do a WB analysis on the veins.

It is a semi-quantitative assessment showing the differences in expression of the antibodies tested, normalized with a commonly used antibody (tubulin). It showed that the expression of SMA, SM-MHC and calponin were much stronger into the pathological group compared to control. Concerning NM-MHC-B, it was more expressed into the control group than in pathologic.

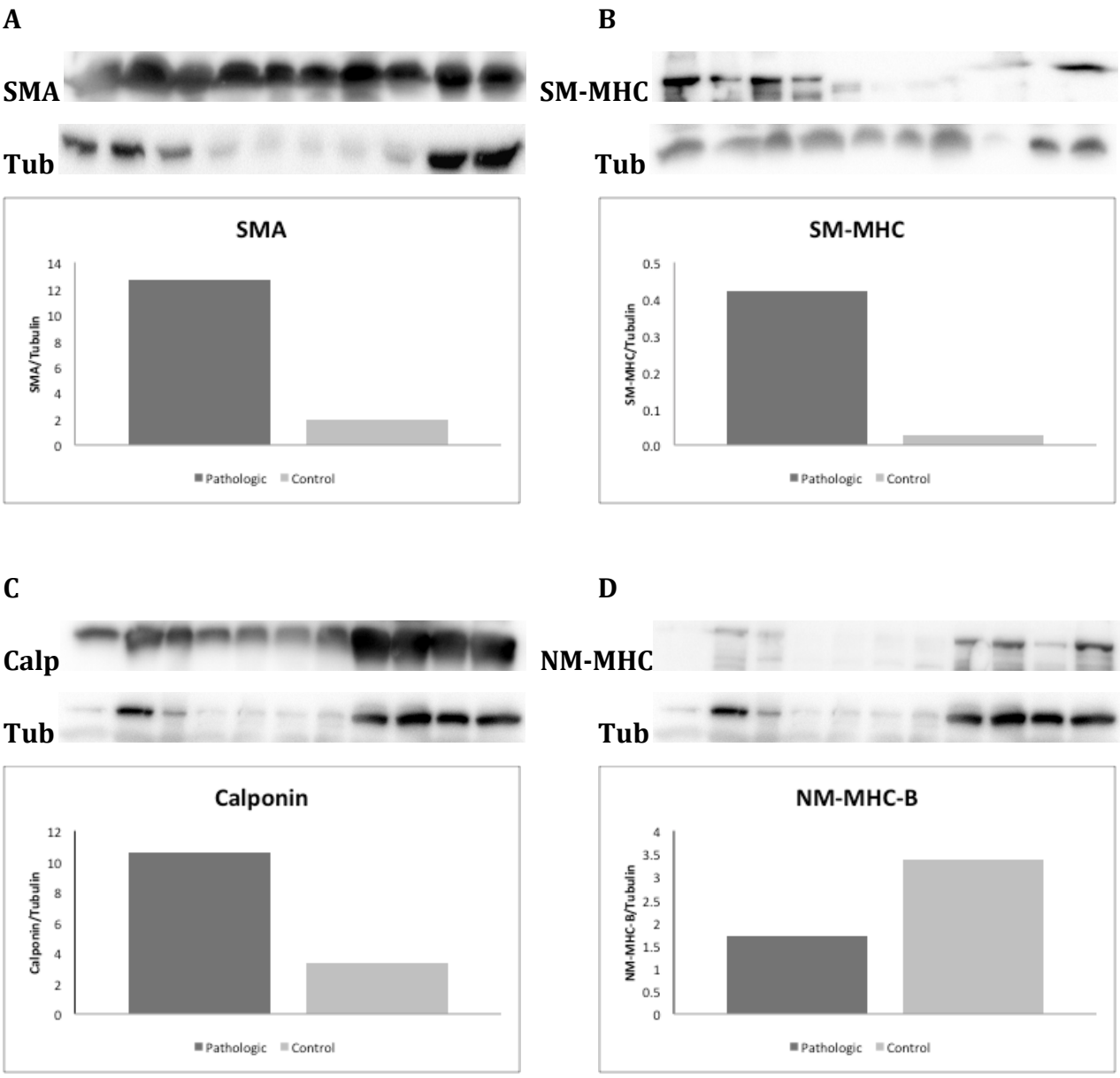

Figure 6. Western blots analysis. This analysis and the results shown were done on the control and pathologic arteries. Example of WB using antibodies against (**A**) : α-smooth muscle actin (**SMA**) ; (**B**) : smooth muscle-myosin heavy chain (**SM-MHC**) ; (**C**) : **calponin** ; (**D**) : non muscle-myosin heavy chain-B (**NM-MHC-B**). For each WB, tubulin was used as second antibody to normalize the results.

Discussion

The present work was undertaken to examine the proportion of cVSMC and sVSMC into the healthy and pathologic human blood vessel wall and characterize their phenotype. We took advantage of the availability of tissue samples of vascular lesions removed during the operations.

VSMCs biology has been studied *in vitro* and *in vivo* extensively in the past 50 years since the method for SMCs culture was established. The concept of « phenotypical modulation » of SMCs was originated from the ultrastructural characterization of SMCs culture in 1960s-1970s, which has been summarized in the comprehensive review by Chamley-Campbell and Ross.[18] The contractile SMCs are characterized by the cytoplasm largely filled with thick and thin myofilaments, while synthetic SMCs have few myofilaments but a larger amount of rough endoplasmic reticulum, free ribosomes and Golgi plasma. Depending on the method used during this work, the results were difficult to explain. Using immunohistochemistry analysis we found that α-SMA was mostly expressed in control arteries, whereas NM-MHC-B in the pathologic ones. Using SM-MHC and calponin, we found no significative differences in the expression of these proteins in the control and in the pathologic samples. Western blot analysis showed an inverse correlation between healthy and pathological samples as α-SMA was more expressed in the pathological samples, whereas NM-MHC-B in the control group ; SM-MHC and calponin were mostly expressed in the pathologic samples. If we think that stenotic lesions are mainly due to the proliferation of sVSMCs, we found a controversial result showing that SM-MHC is mainly expressed in pathologic specimens. Indeed SM-MHC is the most specific marker of differenciated cVSMCs identified to date.[19] Although extensive studies have identified mechanisms that control the process of SMCs phenotypic switching in cultured cells,[8] there are still major ambiguities regarding the definitive identification of altered VSMCs phenotypes during vascular remodelling, including vascular injury-induced proliferation and atherosclerotic plaque progression since a key feature of this process is the loss of expression SMCs selective gene products such as SM-MHC and α-SMA.[20] It is now widely accepted that VSMCs have a phenotypic plasticity and that mature and cVSMCs can de-differenciate into proliferative and synthetic VSMCs. However, this de-differentiation process has not been directly demonstrated by tracking the fate of mature or contractile VSMCs. Indeed, there is a critical need for definitive *in vivo* VSMCs lineage tracking studies.[20,21]

Tang et al recently published that the differentiation of multipotent vascular stem cells rather than the de-differentiation of VSMCs, contributes to vascular remodelling and diseases.[22] The presence of large cells body in the neointima that secrete ECM and express lower levels of the smooth muscle-specific contractile proteins is consistent with the fact that the de-diffentiated cells is present in the neointima.[23]

Our results of semi-quantitative analysis of SM-MHC and α-SMA showed a high expression of these two markers of differentiated cells in the media of the vessels in the control group and also located in the subintimal space in the pathologic specimens. These cells are differentiated even if there were previously de-differentiated. Nevertheless, they differentiated few days after the principal injury lesion. All the pathological specimens were harvested in patients with long lasting lesions. They have consistent stenotic lesions with already differentiated VSMCs. Shy et al showed that during the remodelling of an autologous vein graft, a proportion of neointimal cells derived from adventitial myofibroblasts. We did not find a high expression of VSMCs markers in the adventitial area, probably by the fact that this phenomenom occured in the beginning of the neointima formation. It has also been showed that an important degree of apoptosis takes place in the vein graft VSMCs, needing probably the recruitment of myofibroblasts. Unfortunately the smoothelin marker, which is specific for VSMCs was not expressed in the pathological and control vessels probably due to technical problem (data not shown).[24]

A number of recent studies have provided evidence showing that circulating cells, presumably derived from bone marrow can contribute to neointima formation and repair the vascular injury.[25] Owens et al have found that the primary antibody employed in these studies cross reacts with a nonmuscle isoform of SM-MHC, NM-MHC-B.[8] Frid et al used a panel of antibodies specific for different markers of VSMCs differentiation including α-SMA, SM-MHC, calponin to perform immunofluorescence labelling studies on cryosections of adult and fetal bovine main pulmonary arteries. In addition, they performed Western Blot analyses of these markers in three different layers of the adult bovine artery. They reported the presence of what they categorized as four distinct populations or clusters of VSMCs based on morphology, cell orientation, pattern of elastic lamellae and immunostaining patterns and speculated that these distinct populations may represent unique lineages that may serve different functions within the artierial media, and respond differently to pathophysiological stimuli.[26]

The origin of VSMCs observed in the neointima formation is probably multiple. The perivascular myofibroblasts are able to translocate into the media and exhibit sustained α-SMA expression. In the tunica media, mesenchymal stem cells (MSC)-like cells can be isolated from VSMCs population, which express CD29 and CD44, and have multilineage potential for osteogenic and chodrogenic differentiation.[27,28] In adventitia, Sca-1+ progenitors can diffenrentiate into VSMCs and contribute to atherosclerosis of vein grafts in ApoE-deficient mice.[29] Furthermore, evidence exists that vascular endothelial cells (ECs) can be converted into MSC-like cells by an activin-like kinase-2 receptor-dependant mechanism.[30] However, the characterization of these vascular progenitors have an important role in the generation of proliferative or synthetic VSMCs and the development of vascular diseases remain unclear.

In summary, whereas we cannot rule out that SM-MHC may be expressed at least transiently under certain circumstances in cells other than VSMCs *in vivo*, to our knowledge, conclusive evidence of SM-MHC expression in a non-VSMC *in vivo* does not exist, and, at present it is the most specific marker to discriminate the VSMC.[8]

Contractile VSMC express many proteins such as α-SMA, SM-MHC, calponine h1, h-caldesmon, smoothelin, etc.[8]

While α-SMA serves as a « general » marker for the identification of VSMCs, α-SMA is also expressed by immature or modulated VSMCs. Furthermore other non-SM cell types, called VSMC-like, that share similar morphology and behaviors, such as myofibroblasts, can also express α-SMA.[31] Indeed, activated myofibroblast and VSMCs common markers : α-SMA, h1-calponin, and possibly, SM-MHC. These observations are not surprising. Indeed, it has been hypothesized that the fibroblats/myofibroblasts represent an alternative phenotype of the VSMCs and/or a progenitor/precursor of fully differenciated mature VSMCs.

α-SMA and some other, is not unique to this cell type. Watch out false positive and false negative.[32] It is important to make a difference between differentiation markers and a lineage markers. One serve as indice of the relative state of diffenrenciation of VSMCs and, respectively, the others serve to identify VSMCs to the exclusion of all other cell types. Using this kind of categorization, α-SMA found to be expressed by VSMCs an VSMC-like cells and represents around 40% of the total cell proteins, is not a good lineage marker because it is expressed by to many different cells.[8]

Neointimal hyperplasia is a major obstacle to the long term success of percutaneous interventions as well as surgical therapies (e.g bypass grafting). The drug eluting

technology showed promising result in peripheral arteries. Nevertheless it remains still many unknown biological mechanisms that trigger the proliferation of cells in the neointima followed by deposition of ECM. Defining the origin of neointimal VSMCs, the functional implications of interactions between resident VSMCs and infiltrating or circulating progenitor cells remain an important issue for the design of more effective therapeutics to control restenosis.

Conclusion

The aim of our research was to quantify the proportion of cVSMCs into the healthy and pathologic human blood vessel wall and to characterize their phenotype. It showed, on one hand, no clear difference between stenotic and control arterial venous segments using semiquantitative assessement by immunohistochemistry. On the other hand, Western Blot analysis showed a significant expression of α-SMA, calponin and SM-MHC in the arteries with stenosis, while NM-MHC-B was mostly expressed in the arteries without lesions. Further studies are needed to track the lineage of VSMCs to understand the mechanisms leading to IH.

Guide of the Abbreviations

BV : Basilic Vein

CDK : Chronic Kidney Disease

CV : Cephalic Vein

CVD : Cardio-Vascular Disease

DFA : Deep Femoral Artery

EC(s) : Endothelial Cell(s)

ECM : Extra-Cellular Matrice

EIA : External Iliac Artery

GSV : Great Saphenous Vein

HE : Hematoxylin & Eosin stain

IH : Intimal Hyperplasia

MSC(s) : Mesenchymal Stem Cell(s)

NM-MHC(-B) : Non Muscle-Myosin Heavy Chain(-B)

PBS : Sodium Perborate

PBS-T : Phosphate Buffered Saline-Tween

PG : Proteoglycans

PTA : Posterior Tibial Artery

RBP-1 : Retinol Binding Protein-1

SD : Standard Deviation

SDS-PAGE : Sodium Dodecyl Sulfate-Polyacrilamide Gel Electrophoresis

SFA : Superficial Femoral Artery

α-SMA : α-Smooth Muscle Actin

SMC(s) : Smooth Muscle Cell(s)

SM-MHC : Smooth Muscle-myosin Heavy Chain

TFT : Tibio-Fibular Trunk

TMB : Masson's Trichrome stain

Tub : Tubulin

VGEL : Van Gieson's stain

VSMC(s) : Vascular Smooth Muscle Cell(s)

cVSMC(s) : contractile Vascular Smooth Muscle Cell(s)

sVSMC(s) : synthetic Vascular Smooth Muscle Cell(s)

VWF : Von Willebrand Factor

WB : Western Blot

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