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ANNUAL REPORT

Innovative molecule for vascular grafts enrichment: the role of Pleitrophin and SDF-1 in vascular tissue regeneration.

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Introduction

Vascular diseases are abnormal conditions of the blood vessels (arteries and veins). Any problems along the network of blood vessels could cause severe health problems. Among the different vascular diseases, vascular occlusion remains the leading cause of death in Western countries, despite advances made in the clinic treatment of this pathology (balloon angioplasty and conventional surgical intervention) [1]. Arterial bypass graft remains the primary therapy for patients with advanced vascular disease. Autologous vessels are preferred as graft materials, however, this approach requires multiple surgical procedures, and, a large part of the patients needing bypass surgery, often may not have healthy arteries useful as autograft [2]. Synthetic grafts have been developed as alternatives to autografts, but their low patency owing to short- and intermediate-term thrombosis still limits their clinical application [3].

Tissue-engineering approaches can be used to generate biologically-based conduits to address the need for vascular grafts. However, tissue-engineered vascular grafts either lack of adequate mechanical strength or require long culture periods to obtain the mechanical strength useful for their use as vascular graft [4, 5]. Hence, these approaches result cost- and labor-intensive. In recent years, an alternative tissue engineering approach, involving acellular native tissue, has gained a significant attention in the field of tissue engineering, resulting satisfactory in several applications. These substrates are amenable for tissue regeneration since they are expected to maintain the complex three-dimensional extracellular matrix structure and, thus, the original mechanical properties of the native tissue [6]. It is well established that the mechanical properties of a tissue are linked to its micro-anatomical structure, and this relationship is especially important for load-bearing tissues such as arteries [7]. Thus, the preservation of the native structure and composition of extracellular matrix during the decellularization process is highly desirable. In an attempt to use the inherent strength of a structurally organized ECM without an immunologic response, researchers have developed methods of decellularization able to remove all the antigenic components present in the native tissue, thus enable them for allogenic use [8]. Decellularized biomaterials have the potential to repair, growth, and remodeling *in vivo* and then can be seeded with various cardiovascular cells, including endothelial cells, progenitor cells and myocardial cells, in order to generate functional tissues [9, 10]. These findings suggest that decellularization of naturally available biomaterials is a promising approach to produce vascular grafts.

In the last years, the use of stem cells in regenerative medicine has been studied. The ability of embryonic and adult stem cells to differentiate into endothelial cells and vascular smooth muscle

cells has stimulated studies investigating their use as reparative therapy for vascular diseases. The ideal stem cell for vascular regeneration and repair should be easy to isolate, autologous, show proliferative restraint, and possess the ability of differentiating into the appropriate component of the vascular system in response to the pathophysiological circumstances. Two main categories of stem cells have so far been considered: embryonic and adult stem cells.

Human embryonic stem cells (hES) are isolated from the inner cell mass of the early developing embryonic blastocyst. At a single cell level, the hES are clonogenic, self-renewing and pluripotent capable of giving rise to differentiated, functionally-mature cell types derived from all the three germ layers [11]. These properties would render hES optimal for their use in regenerative medicine. However, the use of hES is associated with significant safety, legal and ethical challenges that restrict their application in the clinical setting.

Adult stem cells (ASCs) exist in mature tissues, in which they play replacement and regenerative roles throughout life. Compared with hES, which can make replacement cells for any tissue, ASCs are multipotent and not pluripotent. Furthermore ASCs, when isolated and placed in the culture dish, do not grow indefinitely as hES.

Despite these drawbacks, ASCs offer several potential advantages for tissue regeneration. First, they can be readily-isolated, expanded *ex vivo* from an initial tissue biopsy or from peripheral blood (PB), thus offering the possibility of autologous grafting, and obviating the need for immunosuppressive therapy that has its own inherent risks. Second, some of the ASCs may be amenable to allogenic transplantation [12]. Third, ASCs can be genetically-modified, rendering them suitable as vectors for delivery of therapeutic genes to enhance their therapeutic [13]. Finally, the therapeutic use of ASCs is not burdened by the legal and ethical restrictions placed on hES.

The hypothesis that tissue regeneration and/or repair may be achieved by cells circulating in the bloodstream was proposed as early as the middle of the nineteenth century by Cohnheim [14]. The most important and studied of the ASCs found in the bloodstream are the endothelial progenitor cells (EPCs). The isolation and characterization of EPCs from the peripheral blood was first reported by Asahara et al. in 1997 [15]. The $CD34^+$ fraction was isolated from human peripheral blood and cultured on fibronectin-coated plates. In presence of specifically enhanced culture medium the cells could grow and differentiate into endothelial-like cells, expressing a range of endothelial markers. Furthermore, these cells incorporated acetylated low-density lipoprotein, formed blood-island and tube-like structures *in vitro*, bound Ulex-lectin, and produced nitric oxide in response to vascular endothelial growth factor (VEGF). Since then, many groups have confirmed

the original findings and reported changes regarding the methods of isolation, characterization and culture of the EPCs [16, 17]. The EPCs play a key role in maintaining vascular homoeostasis, participating in vasculogenesis and repair of damaged vessels [18]. The relative abundance of circulating EPCs is low in basal conditions, but their number increases several fold after stimulation with cytokines, hormones, and statins or in pathological conditions [19].

- **Aim of the study**

The present PhD research project has the long term goal of developing and characterizing a decellularized matrix that display suitable mechanical and biological characteristics capable of providing structural and mechanical support as a vascular substitutes. Therefore, it is important to study the best source from which to obtain the matrices to be used as graft and the adequate method of decellularization to use in order to obtain a matrix with the desired biological and mechanical characteristics needful for a vascular substitute.

This matrix will also be additionally engineered by an enrichment with bioactive molecules (for example, growth factors, peptides, etc.), able to efficiently recruit both circulating EPCs from the bloodstream and resident endothelial cells (EC) and to promote their adhesion and growth in the matrix and to guide the optimal integration and functionality of the grafted vessel.

In literature, several molecules are indicated as chemoattractant for EPCs. This project focus on two molecules: stromal cell-derived factor 1 (SDF-1) and Pleitrophin. SDF-1, also known as C-X-C motif chemokine 12 (CXCL12), is a chemokine protein encoded by the CXCL12 gene in human. SDF-1 is strongly chemotactic for lymphocytes. During embryogenesis it directs the migration of hematopoietic cells from foetal liver to bone marrow and the formation of large blood vessels. In adulthood, CXCL12 plays an important role in angiogenesis by recruiting endothelial progenitor cells (EPCs) from the bone marrow through a CXCR4 dependent mechanism. It is this function of CXCL12 that makes it a very important factor in the neovascularisation process [20].

Pleiotrophin (PTN) is a growth/differentiation cytokine for various cell types with mitogenic, differentiating and angiogenic properties that is expressed during embryogenesis and in adults [21]. PTN has been described as a potent pro-angiogenic factor acting on EC by virtue of its expression by endothelial cells during healing from ischemic brain injury, and was found to stabilize the formation of tube structures by cultured capillary endothelial cells [22]. Interestingly, recent studies have also showed a PTN-induced transdifferentiation of monocytes into functional EC suggesting a role for PTN in inflammation-mediated neovascularization [23, 24].

Materials and methods

- **Cell Culture**

The human umbilical vein cell line EA.hy926 was used. Briefly, EA.hy926 endothelial cells were cultured in Dulbecco's modified Eagle's medium (D-MEM) with 10% foetal bovine serum (FBS), penicillin (100 U/ml), streptomycin (100 U/ml) and L-glutamine (2 mM). The cells were maintained at 37 ° C in a saturated atmosphere at 5% CO_2. Media was changed every two days until

an 85% - 90% of confluence was reached. At this point, cells were detached from the plate using trypsin and then replated at a ratio of 1:10.

- **Viability Assay**

Cell viability in the presence or not of SDF-1 and PTN was evaluated with the MTT Assay. The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple colour. The formazan product is than solubilized, and the absorbance at 570nm is measured. The absorbance at 570nm is directly proportional to viable cell number.

In our experiments, EA.hy926 cells were seeded at a concentration of 2500 cell/cm^2. After an overnight incubation at 37 ° C in a saturated atmosphere at 5% CO_2 to allow the cells to adhere to the wells surface, cells were treated with: 1) normal growth medium (CTRL); 2) three different concentration of SDF-1 (10 ng/ml, 50 ng/ml, 100 ng/ml); 3) three different concentration of PTN (10 ng/ml, 50 ng/ml, 100 ng/ml) and 4) both SDF-1 and PTN (50 ng/ml SDF-1 + 50 ng/ml PTN). Three time point were planned: 24 hour, 3 days and 7 days. At every time point, cells were incubated with the MTT reagent for 3 hours at 37°C in the dark. After the incubation, formazan was solubilized using dimethyl sulfoxide. Than the absorbance, at a wavelength of 570 nm, was measured with a Packard SpectraCount Absorbance microplate reader.

- **Wound Healing Assay**

Wound Healing Assay was performed to measure EA.hy926 cells migration and reparatory ability *in vitro* in the presence of SDF-1 and PTN. The basic steps involve creating a "scratch" in a cell monolayer, capturing the images at the beginning and at regular intervals (6 and 24 hour) during cell migration to close the scratch, and comparing the images to quantify the migration rate of the cells (**Figure 1**).

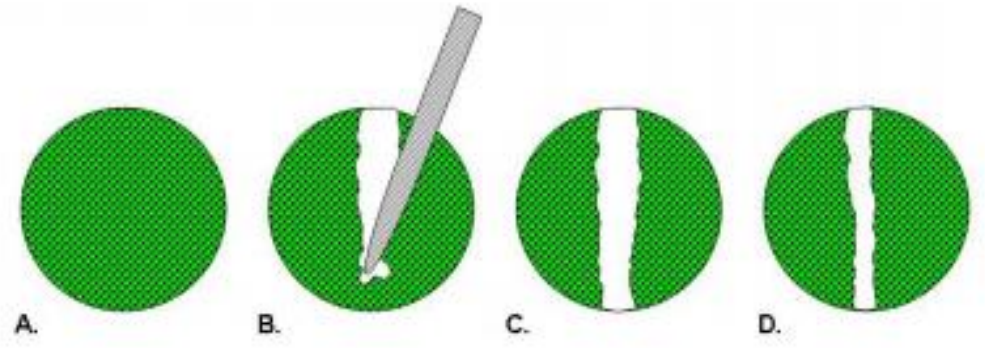

Figure 1: Wound Healing Assay. A wound is introduced in a monolayer of confluent cells (**A**) by drawing a tip across the cell layer (**B**). The denuded area is imaged to mesaure the boundary of the wound at pre-migration (**C**) and after cells have migrated inward to fill the wound (**D**).

Briefly, cells were seeded in 6-well multi-plate in order to obtain a confluent cell monolayer. The cells were then incubated over night at 37 ° C in a saturated atmosphere at 5% CO_2 to allow the cells to adhere to the wells surface. After the overnight incubation, the cell monolayer was scraped in a straight line to create a "scratch" with a pipet tip. The media containing cellular debris was then removed, the cells were washed 2 times with sterile PBS 1X and then treated as fellow: growth medium without the addiction of FBS (CTRL-), medium without FBS with added SDF-1 (50 ng/ml) and medium without FBS with added PTN (50 ng/ml). To obtain the same field during the image acquisition, markings were created to be used as reference points close to the scratch. After the reference points were made, the multi-well was placed under a phase-contrast microscope, and leave the reference mark outside the capture image field but within the eye-piece field of view. Acquire the first image of the scratch (T0). The multi-well was then placed in a tissue culture incubator at 37 °C for the desired time. Images at a magnification of 20X (n=5) were then taken after 6 hour and 24 hour of incubation. The images acquired for each sample have been further analyzed by using ImageJ software to quantify the scratch-area reduction over time.

- **Transwell Migration Assay**

The transwell migration assay is a commonly used test to study the migratory response of endothelial cells to angiogenic inducers or inhibitors. This assay is also known as the Boyden or modified Boyden chamber assay. During this assay, endothelial cells are placed on the upper layer of a cell permeable membrane and a solution containing the test agent is placed below the cell

permeable membrane. Following an incubation period, the cells that have migrated through the membrane are stained and counted (**Figure 2**).

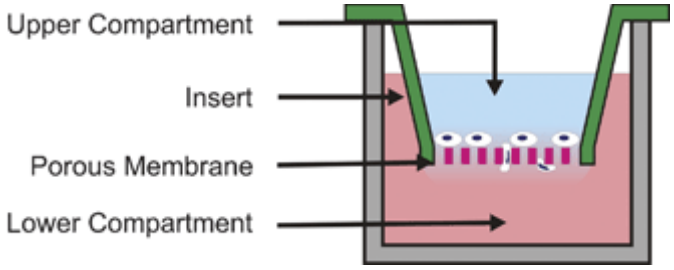

Figure 2: Transwell Migration Assay. Cells seeded on a porous membrane are placed in a well containing a chemoattractant solution. The chemoattractant in the lower compartment diffuses into the upper compartment forming a gradient across the membrane. Cells respond by migrating through the membrane to the bottom surface where they can be subsequently fixed, stained, and counted.

Briefly, 90000 cells were seeded in the upper compartment of 24 well-format Transwell with 8 µm pores in 250 µL of D-MEM without the addiction of FBS. In the lower compartment were added the different treatment compound: growth medium without the addiction of FBS (CTRL-); three different concentration of SDF-1 (10 ng/ml, 50 ng/ml, 100 ng/ml) and three different concentration of PTN (10 ng/ml, 50 ng/ml, 100 ng/ml). The cells were incubated at 37° C in a saturated atmosphere at 5% CO_2 for 6, 24, 48 and 72 hours. At every time point, cells on either faces of the porous membrane were fixed by incubation with formalin 4% for 20 minutes at room temperature. The cells were than stained with Crystal violet for 20 minutes at room temperature. Once stained, cells on the upper face of the porous membrane were gently removed using a cotton swab. The transwell insert were then placed under a phase-contrast microscope and images of different fields (n=3) were taken at a magnification of 20X. For every condition, the stained cells were counted to assess the migration rate. In addition, at every time point, the Crystal Violet stain incorporated by the migrated cells was solubilized with methanol 100% and the absorbance was recorded at a wave length of 540 nm with a PerkinElmer VICTOR™ X Multilabel Plate Reader.

- **Statistical Analysis**

Statistical significance was calculated using ANOVA nonparametric Kruskal-Wallis method through the software InStat ™. Values of $p < 0.05$ were considered significant.

Results and Discussion

- **Viability Assay**

The effects of the treatment with SDF-1 and PTN on the viability of EA.hy926 cells were assessed with the MTT Assay (**Figure 3**). At 24 hours, in presence of PTN a significant increase in cell viability was observed compared to all the other treatments ($p<0.001$ vs. all other treatments). At 3 days, in the presence of two out of three concentrations of PTN tested: 100 ng/ml ($p<0.05$ vs. CTRL and SDF-1 10 ng/ml) and 50 ng/ml ($p<0.01$ vs. CTRL and SDF-1 10 ng/ml and $p<0.05$ vs. SDF-1 50 ng/ml), a significant increase in cells viability was shown. At 7 days, all the concentration of SDF-1 and PTN significantly increase the cells viability with respect to the CTRL group ($p<0.01$). Moreover, the 100 ng/ml and 50 ng/ml of PTN significantly increase cells viability compared to SDF-1 and the treatment with both SDF-1 and PTN ($p<0.001$). The treatment with 10 ng/ml of PTN significantly increase the viability compared to the treatment with all the concentrations of SDF-1 and with both SDF-1 and PTN ($p<0.05$).

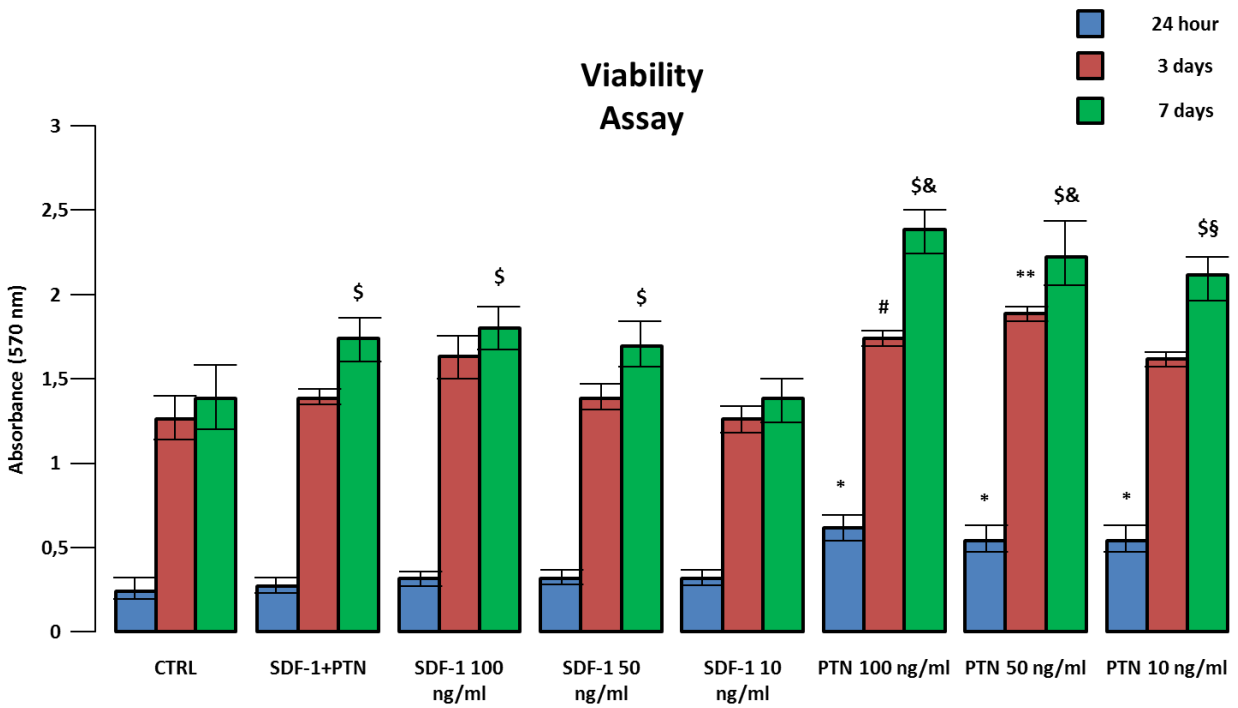

Figure 3: Viability Assay. Ea.hy926 cells were treated over a seven days period with: CTRL medium; three concentration of SDF-1 (100, 50 and 10 ng/ml); three concentrations of PTN (100, 50 and 10 ng/ml) and with both SDF-1 and PTN (50 ng/ml SDF-1 + 50 ng/ml PTN). Cell viability was measured at every time point (24 hour, 3 and 7 days) with the MTT Assay. * $p<0.001$ vs. all condition (except all PTN concentrations); ** $p<0.001$ vs. CTRL and SDF-1 10 ng/ml and $p<0,05$ vs SDF-1 50 ng/ml; # $p<0.05$ vs. CTRL and SDF-1 10 ng/ml; $ $p<0.01$ vs CTRL; & $p<0.001$ vs. all three concentrations of SDF-1 and SDF-1+PTN; § $p<0.05$ vs. all three concentrations of SDF-1 and SDF-1+PTN.

- **Wound Healing Assay**

In order to evaluate the effects of SDF-1 and PTN on the migratory and reparatory capacity of EA.hy926 cells, wound healing assay was performed. EA.hy926 were cultured up to confluence. After washing, a linear wound was created using a pipette tip, and the cells underwent the treatments earlier described. The bright field images were taken after 6 and 24 hours at a 20X magnification (**Figure 4**).

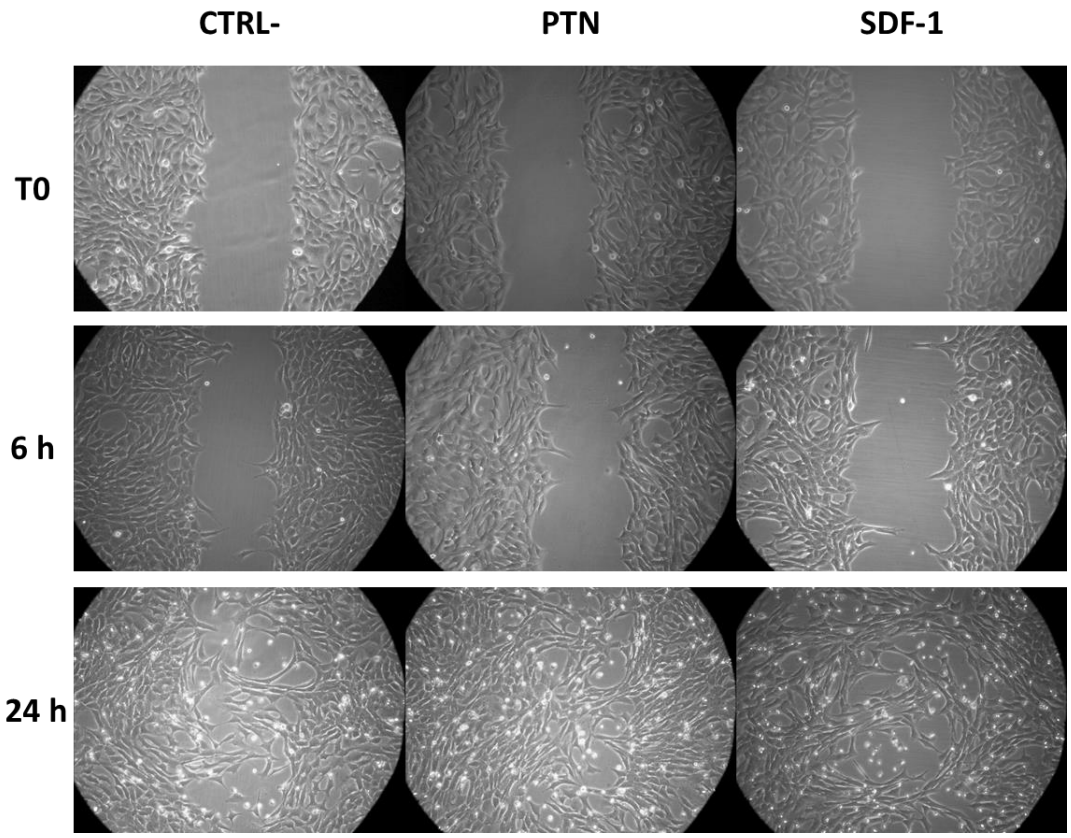

Figure 4: Wound Healing Assay. The images show the migration of EA.hy926 induced by treatment with: growth medium without the addiction of FBS (CTRL-); medium containing 50 ng/ml of PTN (PTN) and medium containing 50 ng/ml of SDF-1(SDF-1). The pictures were taken right after the scratch (T0) and 6 and 24 hours later. Magnification 20X.

The percentage of wound closure was calculated measuring the area compared to T0. As shown, the CTRL- treatment show a 11,2%±7,4 reduction after 6 hours and a 39,6%±8,5 reduction after 24 hours. The presence in the medium of SDF-1 and PTN increased the rate of wound closure compared to the CTRL-: with SDF-1, the reduction is up to 13,1%±6,9 after 6 hours and 67,5%±15,4 after 24 hour ($p<0.01$ vs. CTRL-); with PTN, we achieved a 19,1%±8,9 reduction after 6 hours and 71%±14,5 after 24 hours ($p<0.01$ vs. CTRL-) (**Figure 5**).

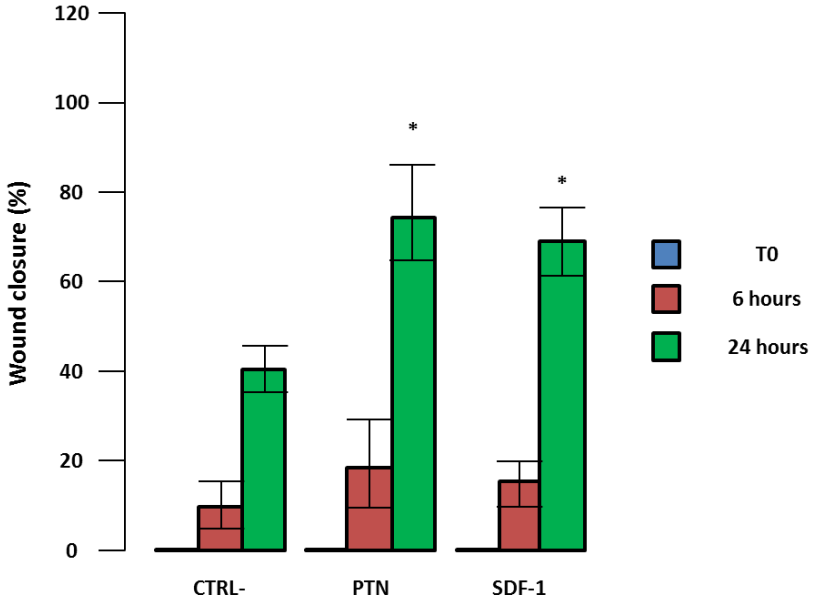

Figure 5: Wound Healing Assay. Quantification of wound closure. Graphic represents the area as the mean ± SD of the % of the closure of original wound at three time points with different treatments: growth medium without the addiction of FBS (CTRL-); medium containing 50 ng/ml of PTN (PTN) and medium containing 50 ng/ml of SDF-1(SDF-1). * p<0.01 vs CTRL-.

- **Transwell Migration Assay**

In order to determine the migratory response of EA.hy926 to the treatment with SDF-1 and PTN, we employed the Transwell assay for cell migration. The average number of migrated cells per field counted for the CTRL- group was of 103,3 cells. In response to the concentration of 10 ng/ml of both PTN and SDF-1, the average number of migrated cells were significantly different compared to the CTRL- group (294±56,5 cells/field with PTN 10 ng/ml, p<0,001 vs. CTRL-; 295,3±25,6 cells/field with SDF-1 10 ng/ml, p<0,001 vs. CTRL-). However, for both PTN and SDF-1, the response of EA.hy926 peaked at the 10 ng/mL concentration and decreased at higher doses, with only the 50 ng/ml concentration of SDF-1 able to significantly increase the migration rate compared to the CTRL- group (264±18,4 cells/field, p<0,01 vs. CTRL-) (**Figure 6**).

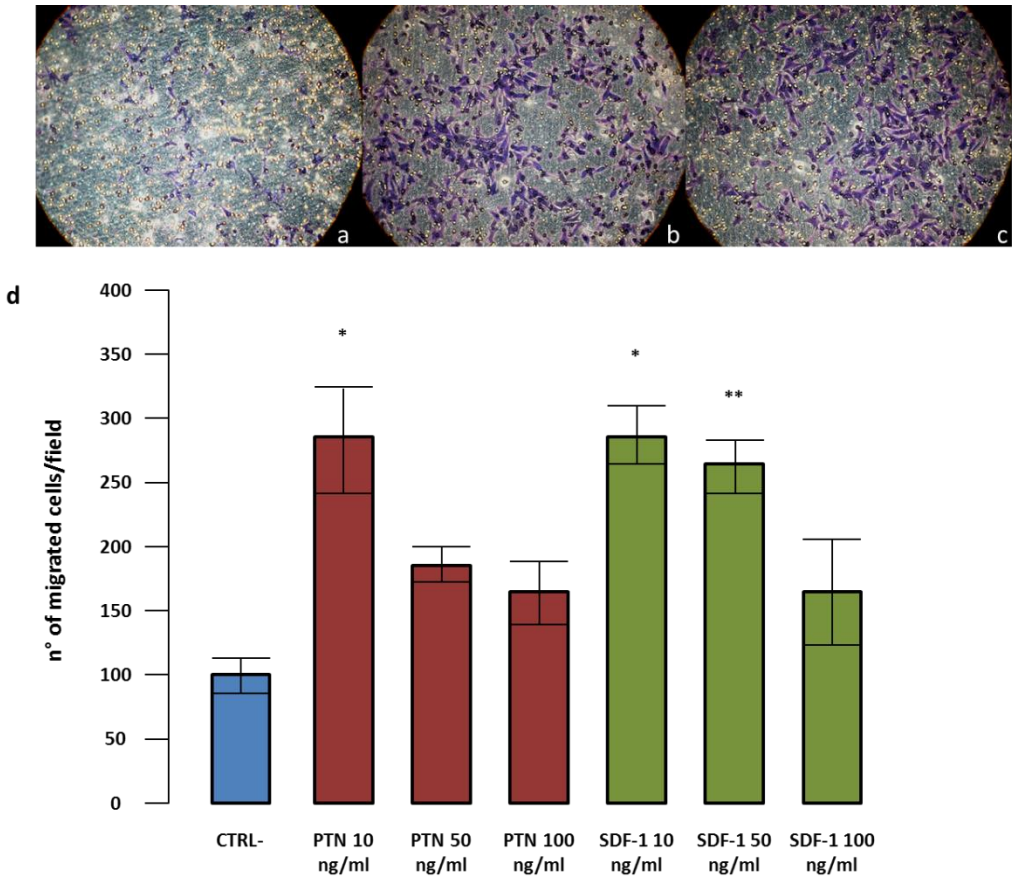

Figure 6: Transwell Migration Assay. The migratory activity of EA.hy926 cells after different treatments was estimated based on the number of cells migrated through the filter inserts. Representative images of cell migration are given: Untreated (a); PTN 10 ng/ml (b); SDF-1 10 ng/ml (c). Quantitative analysis of the number of migrated cells expressed as the mean ± SD (d). *p<0.001 vs. CTRL-, **p<0.01 vs. CTRL-.

At longer time points, due to the large amount of cells that migrated trough the porous membrane, it was impossible to count the migrated cells. However, the Crystal Violet stain incorporated by the migrated cells was solubilized with methanol and the absorbance was recorded at a wave length of 540 nm. At every time point, an increase in the absorbance value recorded was shown in the presence of all the tested concentration for both PTN and SDF-1 compared to CTRL-. In particular, the treatment with PTN seems to cause a time-dependent response on cell migration compared to SDF-1, which shows no particular changes between the tested concentrations at the single Time Point-level (**Figure 7**).

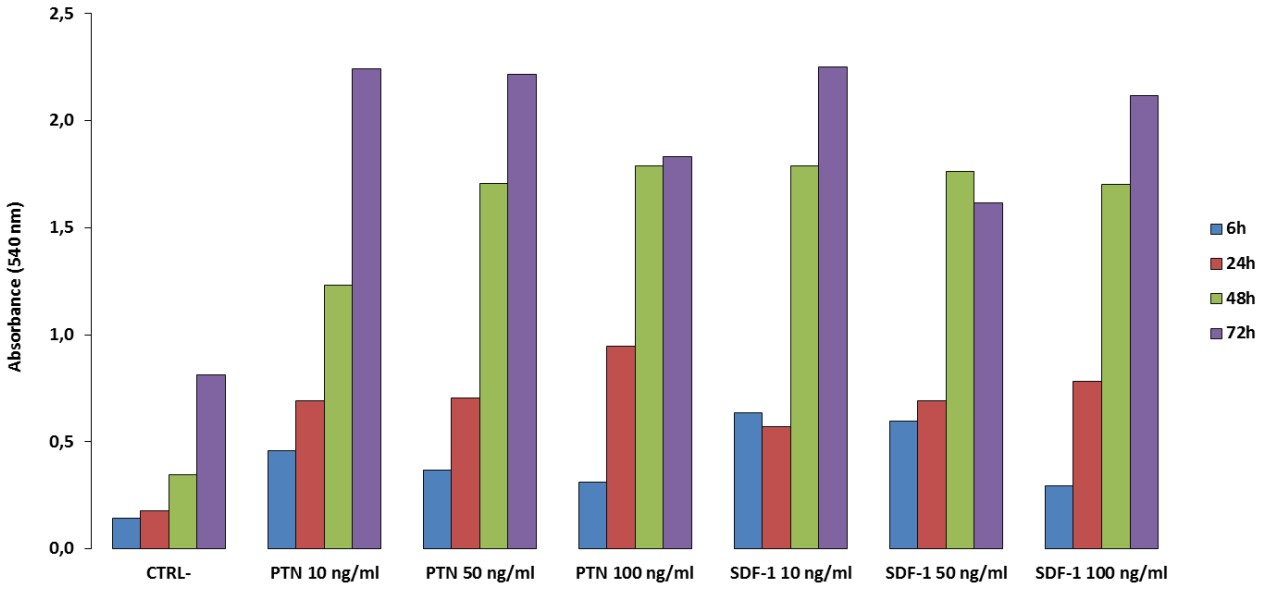

Figure 6: Transwell Migration Assay. The migratory activity of EA.hy926 cells was measured solubilizing the Crystal violet stain incorporated by the migrated cells after different treatments and then the absorbance was recorded at a wave length of 540 nm.

Conclusions and future perspectives

The aim of the preliminary experiments we conducted was to determine the effects on endothelial cells exerted by the two selected molecules, PTN and SDF-1. For this purpose, EA.hy926 cells have been used to assess cell viability, migration and repair capability in response to treatment with PTN and SDF-1.

The viability assay have shown that, already at the 24 hours time point, all the tested concentration of PTN are able to significantly increase the cell viability compared to the other conditions tested. At 3 and 7 days, this increase in cell viability it's maintained by all the tested concentrations of PTN. SDF-1, although only at 7, is able to significantly increase the cell viability compared to controls in the presence of all the tested concentrations.

The wound healing assay, in which we have investigated the effects of the two molecules on migration and repair ability of the EA.hy926, have highlighted how the treatment with either PTN or SDF-1 is able to significantly increase the migration rate of the endothelial cells after 24 hours of treatment. Similarly, the Transwell assay has shown an increase in the migration rate of EA.hy926 following treatment with PTN or SDF-1. After 6 hour of incubation, both PTN and SDF-1, especially at the lower concentration, were able to significantly increase the migration rate of the endothelial cells. With longer incubation times, this effect was maintained, even with the higher concentration tested for both the molecules.

In conclusion, preliminary data have shown that both the molecules tested, PTN and SDF-1, are able to improve the viability and functionality of the treated endothelial cells. Further studies are necessary in order to clarify the signalling pathways and molecular mechanisms in which PTN and SDF-1 are involved. Moreover, after the development of an effective protocol for the isolation of EPCs from peripheral blood, the effects of PTN and SDF-1 will also be tested on EPCs.

Finally, since my PhD is held in joint supervision with the group of Professor Diego Mantovani at Laval University in Quebec City, Canada, I will focus on the engineering aspect of my thesis project. Taking advantage of the expertise the group of Professor Mantovani has developed in the bio-engineering field, we aim to develop a vascular substitute based on biologic decellularized matrix enriched with one, or both, the molecules that we have characterized, in order to: increase the ability to recruit both circulating EPCs and resident endothelial cells (EC); to promote their adhesion and growth in the matrix and to guide the optimal integration and functionality of the grafted vessel.

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List of attended lessons and seminars

Lessons

1. “Tissue engineering: the state of the art” – 14 November 2014 – Dott.ssa Francesca Boccafoschi - Department of Health Sciences, University of Eastern Piedmont.
2. “Regenerative Medicine” – 21 November 2014 – Prof. Maria Prat - Department of Health Sciences, University of Eastern Piedmont.
3. “Ribosomopathies” – 25th May 2015 – Prof. Steve Ellis – Medical School, University of Louisville (Kentucky)
4. “Basis of scientific research” – 10th June 2015 – Prof. Nicoletta Filigheddu – Università del Piemonte Orientale (Italy)

Seminars

5. “Dysregulated antigen receptor signaling: molecular lessons from two congenital lymphoproliferative disorders” – 06 November 2014 - Prof. Andrew L. Snow - Department of Pharmacology Uniformed Services University of the Health Sciences Bethesda (Maryland, USA).
6. “Optical coherence tomography from bench to bedside shening the light during percutaneous vascular intervention” – 17 November 2014 - Dott. Secco Gioel Gabrio – Department of Health Sciences, University of Eastern Piedmont.
7. “Nuove sfide ed opportunità dell'epidemiologia molecolare per lo studio dei tumori” – 27 November 2014 - Prof. Laura Baglietto - Inserm - Centre for Research in Epidemiology and Population Health, Unit: Nutrition, Hormones and Women’s Health, Paris.
8. “Humoral responses to HCV infection and clinical outcomes” – 28 November 2014 - Dott. Arvind Patel - Programme Leader, MRC Centre for Virus Research, University of Glasgow (UK).
9. "Uncovering the role of β-HPV in field cancerization: a collaboration in progress" – 4 December 2014 – Dott. Girish Patel - European Cancer Stem Cell Research Institute, Cardiff (UK).
10. “Focus on the liver: from basics of NAFLD to hot topics in HBV & HCV infections” – 5 December 2014 – Prof. Rifaat Safadi M.D, Chairman of the Israeli Association for the Study of Liver. Director of Liver Unit. Institute of Gastroenterology and Liver Diseases.

Division of Medicine. Hadassah Medical Organization, Hadassah Hebrew University Medical Center, Jerusalem.

11. "From the legend of Prometheus to regenerative medicine" – 16 December 2014 – Prof. Antonio Musarò, DAHFMO-Unit of Histology and Medical Embryology Sapienza University of Rome.
12. "Different molecular mechanisms regulate hepatocyte differentiation during the transitions between epithelial and mesenchymal states" – 20 January 2015 - Dott. Tonino Alonzi, PhD, Lab. Of Gene Expression and Experimental Hepatology, Istituto Nazionale per le Malattie Infettive "L. Spallanzani" IRCCS, Rome.
13. "Targeting the liver to cure myocarditis: a lesson from a model of STAT3-dependent auto-immune myocarditis" – 21 January 2015 - Prof. Valeria Poli - Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Centre, University of Turin.
14. "Myeloid cells as therapeutic target in cancer" – 27 January 2015 - Prof. Antonio Sica - DiSCAFF, UPO, Novara.
15. "Proof of principle for cell therapy: from autologous transplantation of tissue specific progenitors to gene corrected patient specific injured pluripotent stem cells" – 11 March 2015 – Prof. Darko Bosnakovski - Associate Professor, University "Goce Delcev" Stip, Faculty of Medical Sciences, Krste Misirkov bb, 2000 Stip R. Macedonia.
16. "Signal control in iNKT cell development and function" – 09 April 2015 - Prof. Xiaoping Zhong, MD, PhD - Associate Professor, Department of Pediatrics-Allergy and Immunology Duke University, Medical Center, Durham (North Carolina, USA).
17. "Actin-based mechanisms in the control of gene expression and cell fate" – 21st April 2015 – Prof. Piergiorgio Percipalle – Associate Professor, Department of Cell and Molecular Biology, Karolinska Institutet (Solns, Sweden).
18. "An integrated approach to the diagnosis and treatment of ovarian cancer" – 7th May 2015 – Prof. John McDonald, MD, PhD – Integrated Cancer Research Center, School of Biology and Parker H. Petit Institute of Bioengineering and Biosciience, Georgia Institute of Technology, Georgia Tech University, Georgia (Atlanta, USA).
19. "Recent developments in (cutaneous) Human Polyomavirus research" – 5th June 2015 – Mariet C.W. Feltkamp – Associate Professor of Medical Virology, Department of Medical Microbiology, Leiden University Medical Center (Leiden, The Netherlands).
20. Miniworkshop on "Biotechnology for Dermatology" – 9th July 2015 - Dr Gwenaël ROLIN, PhD - Clinical Research Engineer - Thomas LIHOREAU - Ingénieur

hospitalier, Research and Studies Center on the Integument (CERT), Department of Dermatology, Clinical Investigation Center (CIC INSERM 1431), Besançon University Hospital; INSERM UMR1098, FED4234 IBCT, University of FrancheComté, Besançon, France.

21. "High-tech product preservation and operator protection: two apparently opposite requirements in different fields of medicine and biotechnology: the emerging glove box approach" – 15th July 2015 - Dr. Ing. Marco Fatta, Phd – COMECER Group (Italy).
22. "Le cellule staminali nel danno renale acuto e nel trapianto di rene" – 28th July 2015 - Dr. Vincenzo Cantaluppi, MD – Facoltà di Medicina e Chirurgia, Università di Torino (Italy).
23. "Cell based models for studying molecular mechanisms of Facioscapulohumeral Muscolar Distrophy (FSHD)" , "Toward animal model for Facioscapulohumeral Muscolar Distrophy (FSHD)" – 3rd September 2015 - Prof. Darko Boshnakovski, PhD – University Goce Delcev Stip, Faculty of Medical Sciences (Stip, R. Macedonia).

List of attended congress

- Boccafoschi F, Botta M, Fusaro L, Ramella M, Torri F, **Copes F**, Azzimonti B, Cannas M, *Decellularized matrices for tissue engineering enriched with antibiotics: a promising approach for tissue regeneration*. SIB, Ancona, 3-5 June 2015.

List of publication

- Boccafoschi F, Botta M, Fusaro L, **Copes F**, Ramella M, Cannas M, *Decellularized biological matrices: an interesting approach for cardiovascular tissues repair and regeneration*. J Tissue Eng Regen Med 2015; in press (Review)
- Pisano F, Altomare C, Cervio E, Barile L, Rocchetti M, Ciuffreda MC, Malpasso G, **Copes F,** Mura M, Danieli P, Viarengo G, Zaza A, Gnecchi M, *Combination of miRNA499 and miRNA133 exerts a synergic effect on cardiac differentiation.* Stem Cells 2015; 33(4): 1187-1199
- Danieli P, Malpasso G, Ciuffreda MC, Cervio E, Calvillo L, **Copes F**, Pisano F, Mura M, Kleijn L, de Boer RA, Viarengo G, Rosti V, Spinillo A, Roccio M, Gnecchi M, *Conditioned medium from human amniotic mesenchymal stromal cells limits infarct size and enhances angiogenesis.* Stem Cells Transl Med 2015; 4(5): 448-458