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APPLICATION OF VASCULAR GRAFTS OF POLY(VINYL) ALCOHOL HYDROGEL ASSOCIATED WITH MESENCHYMAL STEM CELLS (MSCS) FROM WHARTON JELLY IN AN ANIMAL MODEL (SHEEP)

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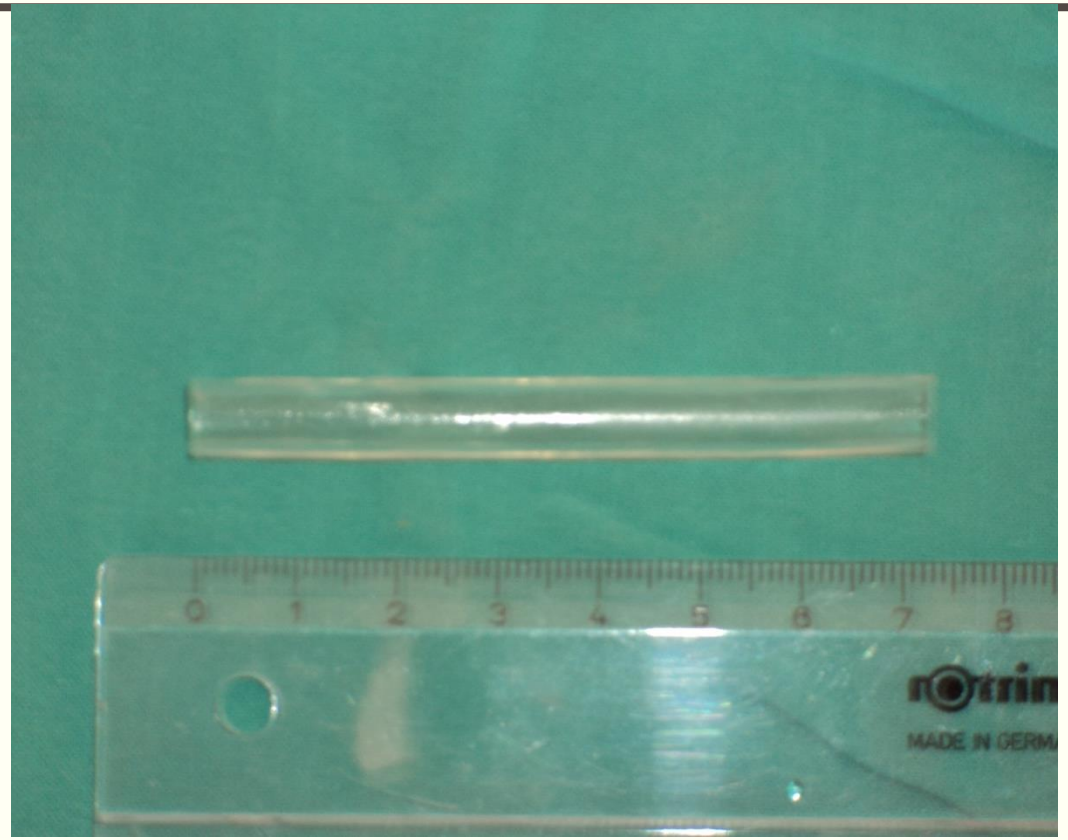


N. Alexandre,* M.A. Lopes, N.A.Nunes, I. Amorim, A.C. Mauricio, J.D. Santos, A.L.Luís

*Departamento de Zootecnia – Universidade de Évora
Pólo da Mitra – Valverde
7002-554 Évora

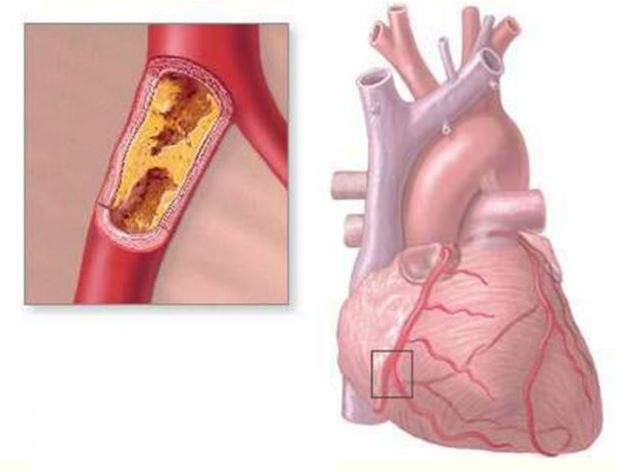
INTRODUCTION

- 3 million procedures related with heart of blood vessels are performed in the US each year *
- Most of these procedures are in small caliber vessels (< 6 mm)
- Cardiovascular disease accounts for a significant percentage of mortality and morbidity in the ageing population
  - Estimate increase in the coming years†
- Main reperfusion-based surgical intervention options included:
  - Angioplasty stenting, endarterectomy and bypass graft surgery

*American Heart Association, †British heart foundation

INTRODUCTION

- Arteries with degree of occlusion greater than 70% required a bypass graft surgery



- For small diameter bypass grafts (< 6 mm)
  - autologous bypass conduits are preferred
- 3-30% patients presented with no autologous vessels
  - Due to previous diseases or surgery
- Demand for artificial vascular grafts that performed like autologous gratfs

INTRODUCTION

- Characteristics of the ideal vascular graft

Nonthrombogenic

- High biocompatibility

Resistant to infection

- Easy to suture

Resistant to intimal hyperplasia

- Compliant

Nontoxic

- Flexible, elastic without kinking

INTRODUCTION

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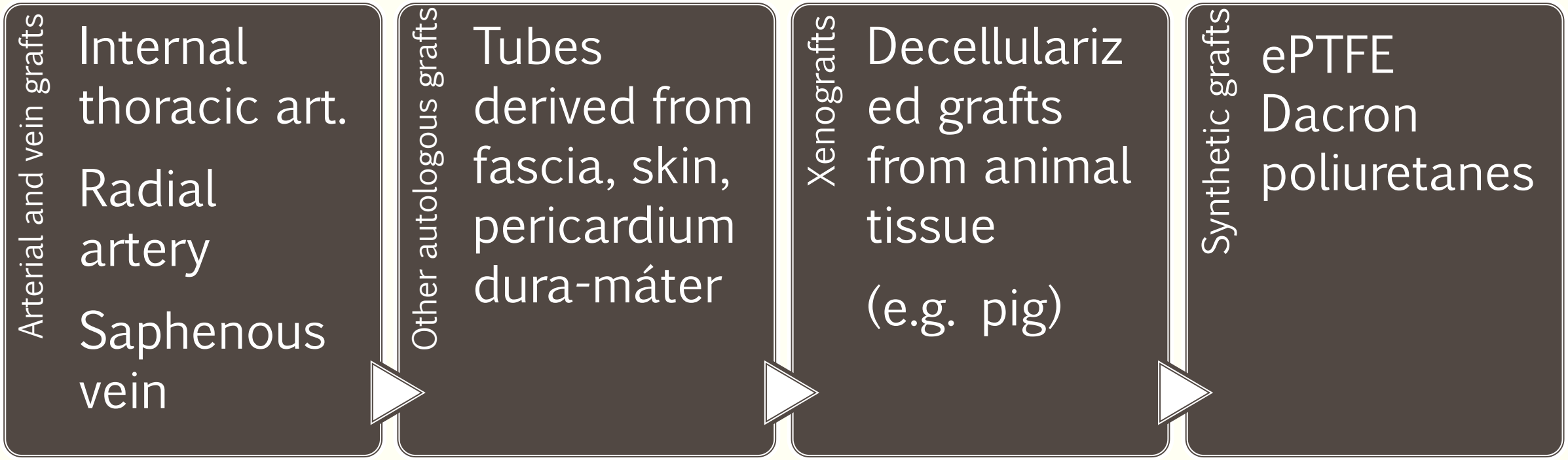
VASCULAR GRAFTS - options



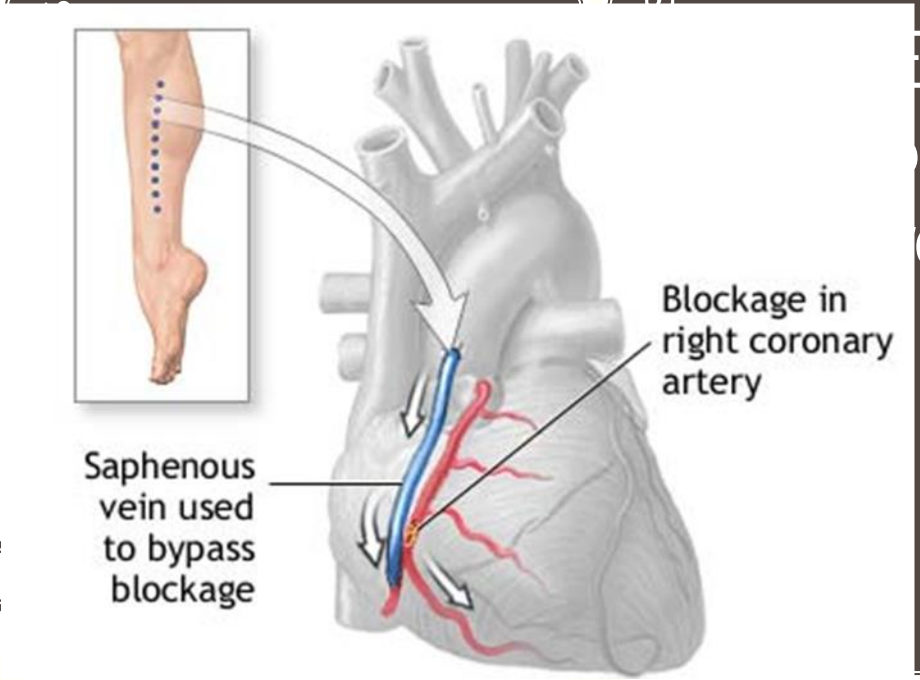
VASCULAR GRAFTS - options

Arterial and vein grafts

- Internal thoracic art.
- Radial artery
- Saphenous vein

Other autologous grafts

- Tubes derived from fascia, skin, pericardium dura-máter



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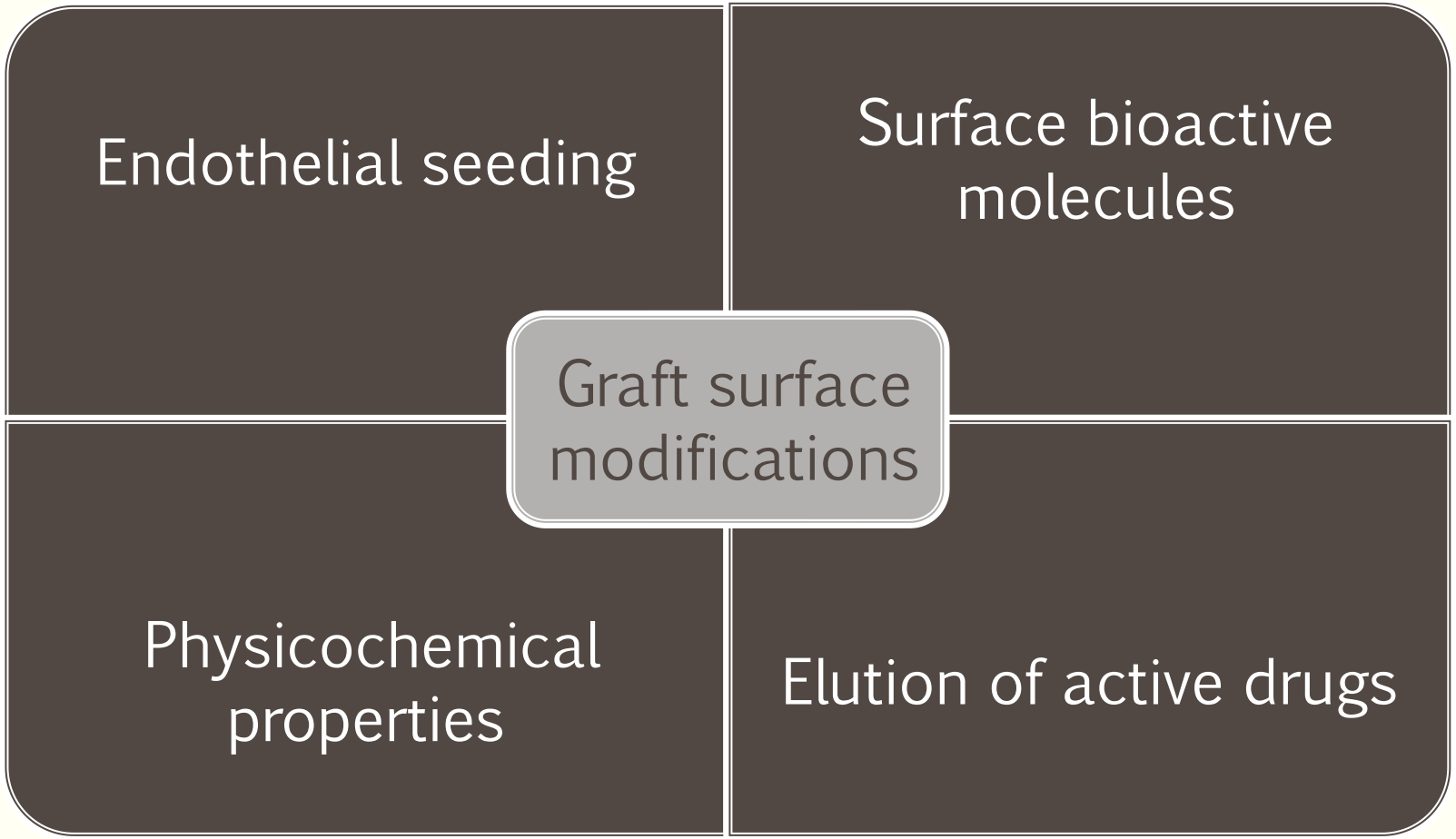
VASCULAR GRAFTS

- Main problems of small diameter synthetic grafts (< 6 mm)
  - Early thrombosis – thrombotic occlusion
  - Development of a fibrinous pseudointima with gradual thickening - thrombotic occlusion
  - Dilation of vascular graft wall - aneurisma
- Large diameter synthetic grafts (> 12 mm)
  - Development of a fibrinous pseudointima with gradual thickening - occlusion

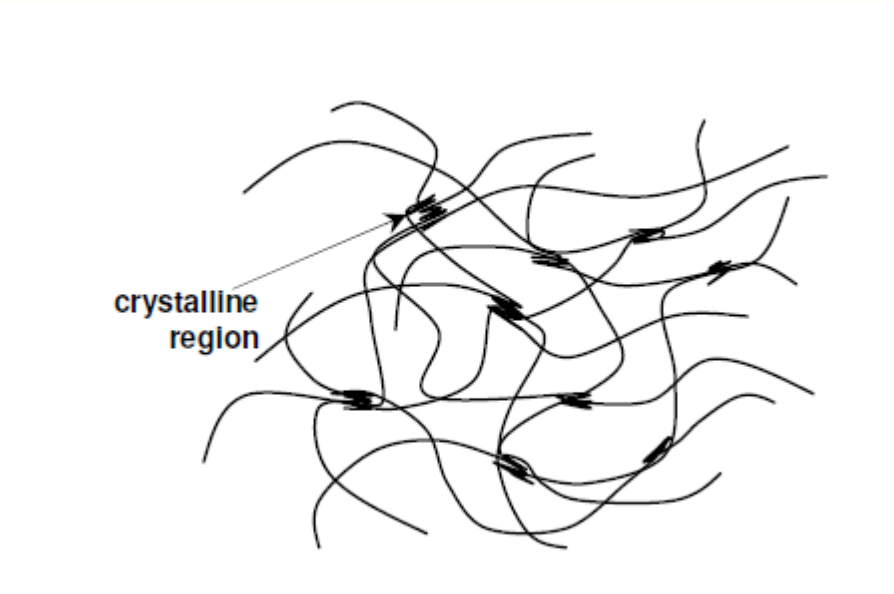
VASCULAR GRAFTS



Stategies to improve patency rate in synthetic vascular grafts

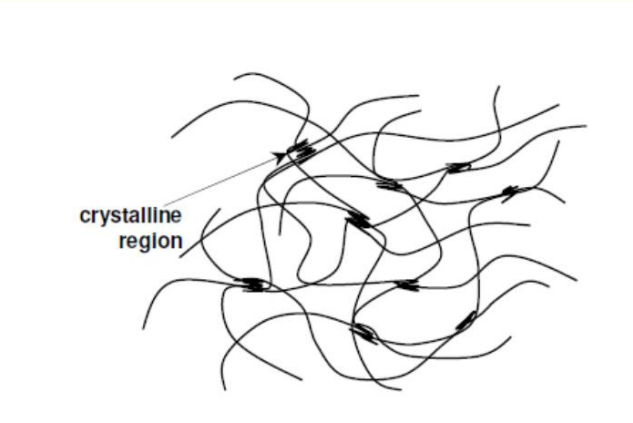
Biomaterial -poly(vinyl) alcohol hydrogel (PVA)

- PVA hydrogels are formed:
- by the crosslinking of linear PVA polymer chains to create an insoluble, swellable network
- monomer vinyl alcohol does not exist in a stable form, therefore PVA is synthesized by the hydrolysis of poly(vinyl acetate).



Biomaterial -poly(vinyl) alcohol hydrogel (PVA)

- PVA used in its linear as well as crosslinked forms:
  - crosslinks is a way to increase the strength of PVA polymer
- Method of polymer crosslinking is a major factor that influences the final properties
- Physically crosslinked hydrogels are formed by introducing crystalline regions that behave as crosslinks
  - freeze/thawing method
- Advantage of this method is that crosslinks are n of chemical reagent
  - Ideal for biomedical applications



Biomaterial - poly(vinyl) alcohol hydrogel (PVA)

- PVA hydrogels have been used in:
- Contact lenses, controlled release matrices, bioadhesives and wound dressings*
- Other biomedical applications included:
  - Scaffold for biosynthetic cartilage, artificial meniscus, artificial intervertebral discus*
- Other synthetic hydrogels:
  - poly(ethylene oxide) (PEO), and poly(ethylene glycol) (PEG)

***Bichara et al, 2010.** Porous Poly(Vinyl Alcohol)-Hydrogel Matrix-Engineered Biosynthetic Cartilage.Tissue engineering part **A**
Bourke et al, **2007**. A Photo-Crosslinked Poly(vinyl Alcohol) Hydrogel Growth Factor Release Vehicle for Wound Healing Applications. AAPS Phar
Kobayashi et al, 2005. A two year in vivo study of polyvinyl alcohol-hydrogel (PVA-H) artificial meniscus. *Biomaterials*
Kokabi et al, 2007. PVA–clay nanocomposite hydrogels for wound dressing. European Polymer Journal
Chun Ying, **2008**. Poly(vinyl alcohol) PVA hydrogel characterization as a potential nucleus pulposus replacement candidate MSc thesis

Biomaterial -poly(vinyl) alcohol hydrogel (PVA)

- Resistant to protein adsorption
- Low toxicity
- Resistant to cell attachment
- Non carcinogenic
- Hydrophylic
- Biocompatible

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- Hydrophylic
- Biocompatible

Biomaterial -poly(vinyl) alcohol hydrogel (PVA)

- Lack recognition sites that would enable cells to adhere
- Chronic arterio-venous canine shunt model - platelets were reactive upon contact with PVA
  - not adherent to the hydrogels after activation
- Adsorption of thrombin was also shown to be unaffected by the presence of heparin

Biomaterial -poly(vinyl) alcohol hydrogel (PVA)

- Use of PVA hydrogels as vascular prosthesis covalently coated with heparin as an antithrombotic agent
  - had no effect on platelets, rather, the PVA dominated the interaction with the platelets
- Chronic arterio-venous canine shunt model using PVA
  - platelets were found to be reactive upon contact with PVA but they did not remain adherent to the hydrogels after activation

Biomaterial - poly(vinyl) alcohol hydrogel (PVA)

- Hydrophobic scaffolds encourage better protein deposition.
  - hydrophobic poly(lactic acid)has been grafted as side chains onto PVA backbone and shown to improve the adhesion of valve interstitial cells to the hydrogels

- Fibronectin and cell binding domain RGD (Arg-Gly-Asp) - covalently incorporated in polymers
  - Create cell binding surfaces
  - Cell-adhesion molecules reduced the production of ECM by the attached cells
  - subsequent processes such as cell growth and tissue formation relie on an array of growth factors.

Biomaterial -poly(vinyl) alcohol hydrogel (PVA)

- Combination of PVA with polysaccharides with anticoagulation properties
  - Decrease the antithrombogenicity of the polymer surfaces
  - Improve blood compatibility
- Dextran – bacterial pollysaccharide
  - glycosaminoglycans
  - Influencing different plasmatic protein systems such as coagulation and complement system

Biomaterial - dextran

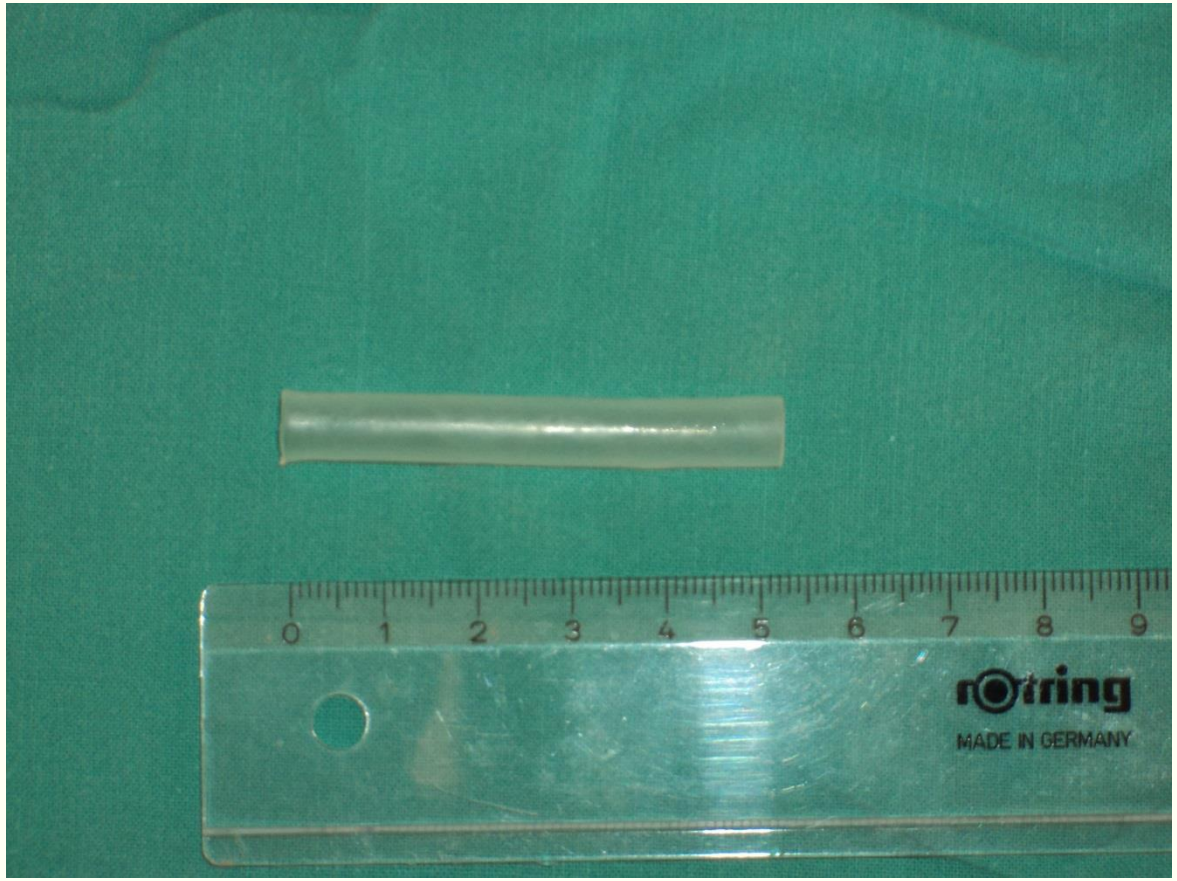
- Low-molecular weight dextran sulfate 5000 (5 kDa) is a sulfated polysaccharide and to the family of the glycosaminoglycans (GAGs) (e.g. heparin)
  - GAGs were reported to induce platelet dysfunction*
  - reported to accelerate the inhibition of thrombin by both antithrombin and heparin cofactor II *
  - enhance the inhibition of FXIa by C1 inhibitor*
  - other contact activation factors, such as factor XIIa and kallikrein, by C1 inhibitor remained unaffected*

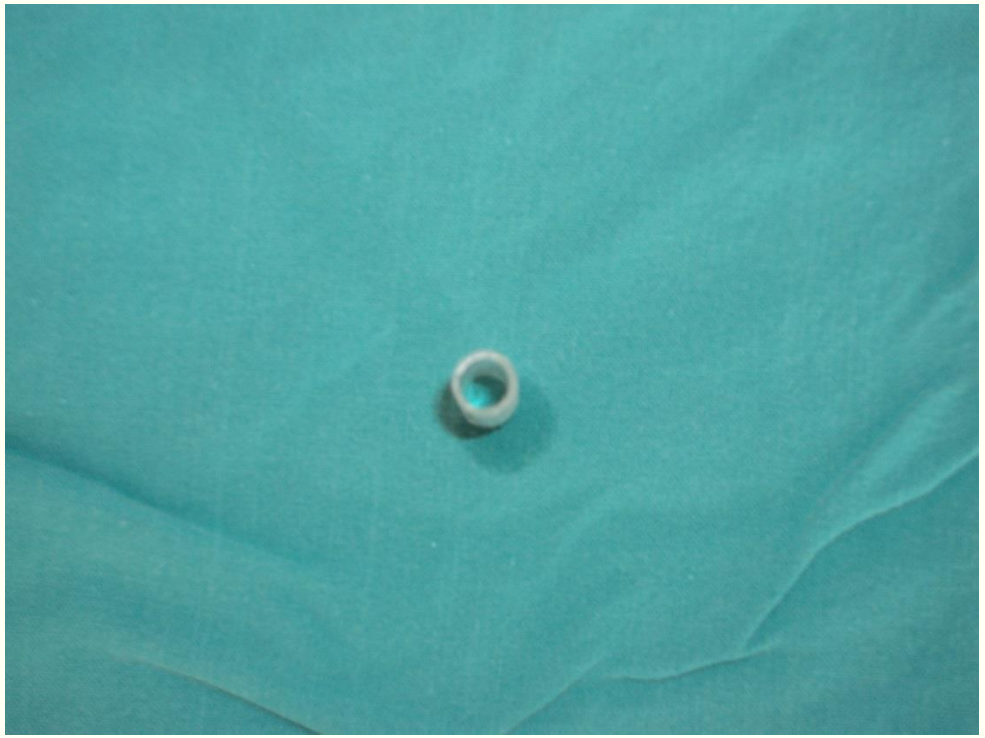
*Zeerleder, Sacha, et al. "Effect of low-molecular weight dextran sulfate on coagulation and platelet function tests." Thrombosis Research 105.5 (2002): 441-46.

Methods – PVA + dextran 1% grafts

- PVA + dextran 1% production:
- PVA/Dextran - solutions proportion 10/90
  - low molecular weight Dextran (4-7Kda) (Sigma Aldrich®)
  - PVA (Sigma Aldrich® Mowiol® 10-98)
  - PVA solution 20%
- PVA physical crosslinking - 3 cycles of freezing/thawing method (-30ºC/25ºC) plus annealing to increase pressure resistance
- Graft desinfection – imersion in etanol 70% for 5 minutes
- Grafts dimensions
  - 5 cm lenght
  - 5 mm ID
  - 1 mm wall thickness

Methods – PVA + dextran 1% grafts





Methods - Experimental design

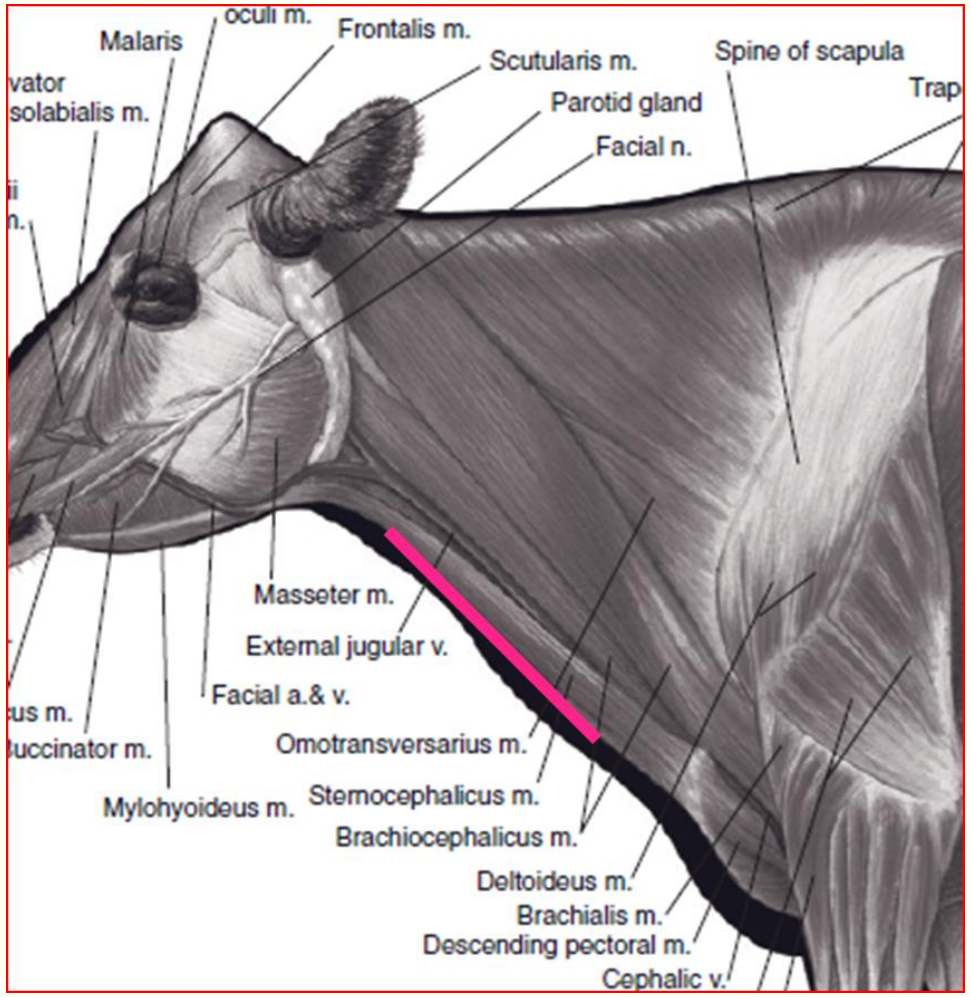
- Experimental group of six sheep
- Implanted with PVA + Dextran 1% grafts
  - 5 cm lenght
  - 5 mm ID
  - 1 mm wall thickness
- Functional performance of the prosthesis evaluated by vascular ultrasound in Doppler and B mode by measuring parameters such as: peak systolic/diastolic blood flow velocity, vascular diameters at implantation and at the periphery.
- measurements are performed at various time points
  - 24 hours, 4 weeks, 8 weeks, 12 weeks
- followed by euthanasia immediately sample collection for further techniques: histopathology, immunohistochemistry, morphometry and scanning electronic microscopy

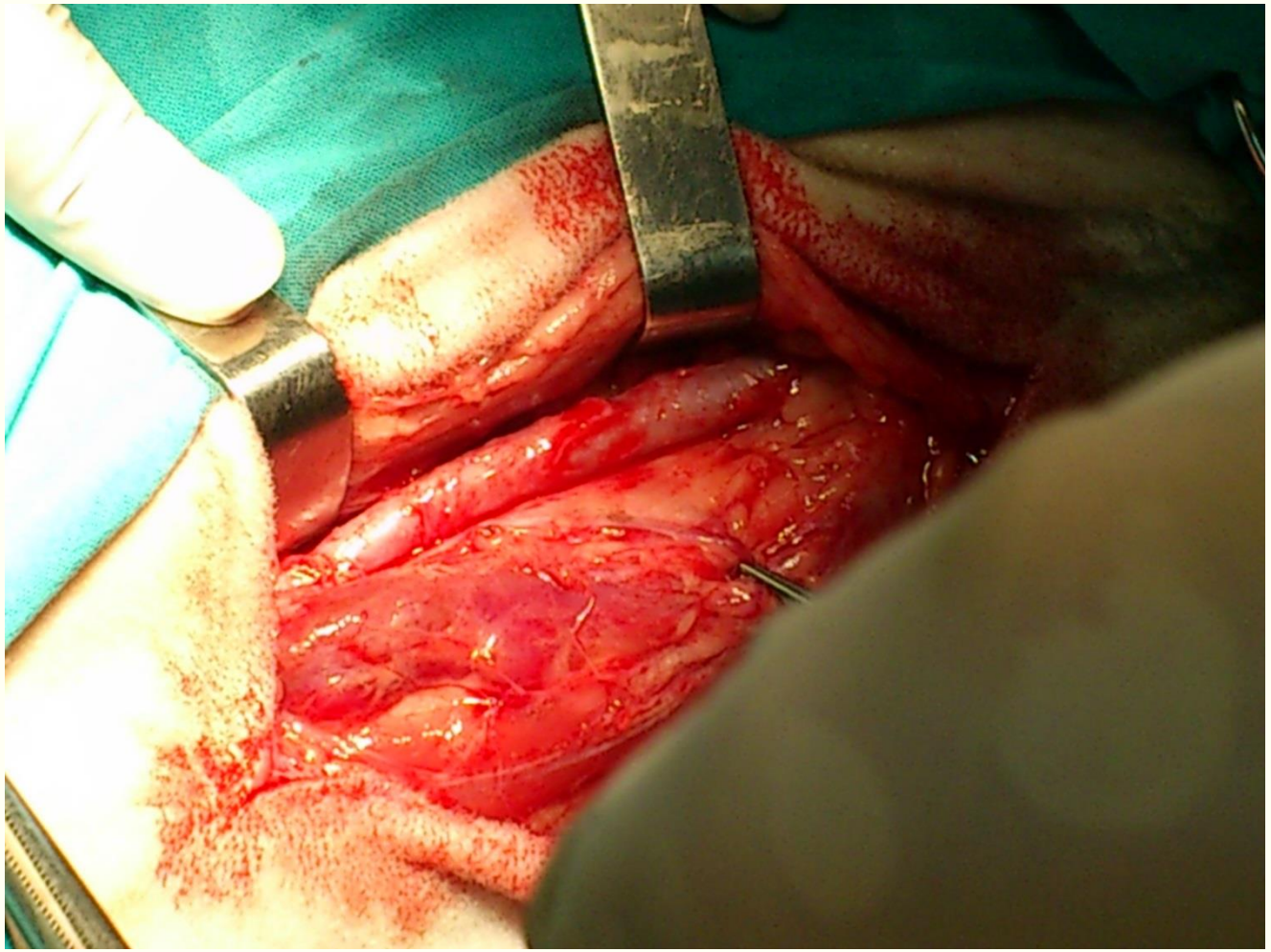
Methods - Experimental model

- Sheep – Adult female, merino branco breed
- Surgical acess to the left common carotid artery
- Segment of the carotid artery was removed and end-to-end anastomosis was made with the PVA graft using 6/0 USP polypropylene suture
- MSCs were isolated from Wharton's jelly of umbilical cord and multiplied *in vitro* and placed into syringes
  - 1 ml with an average concentration of 1 x 10^6 cells/ml and were injected perivascularly
- Anticoagulation protocol with the objective of reducing prosthesis thrombosis*:
  - Clopidogrel 150 mg PO SID
  - Warfarin 0,3 mg/Kg PO SID
  - Heparin 200 UI/Kg SC SID

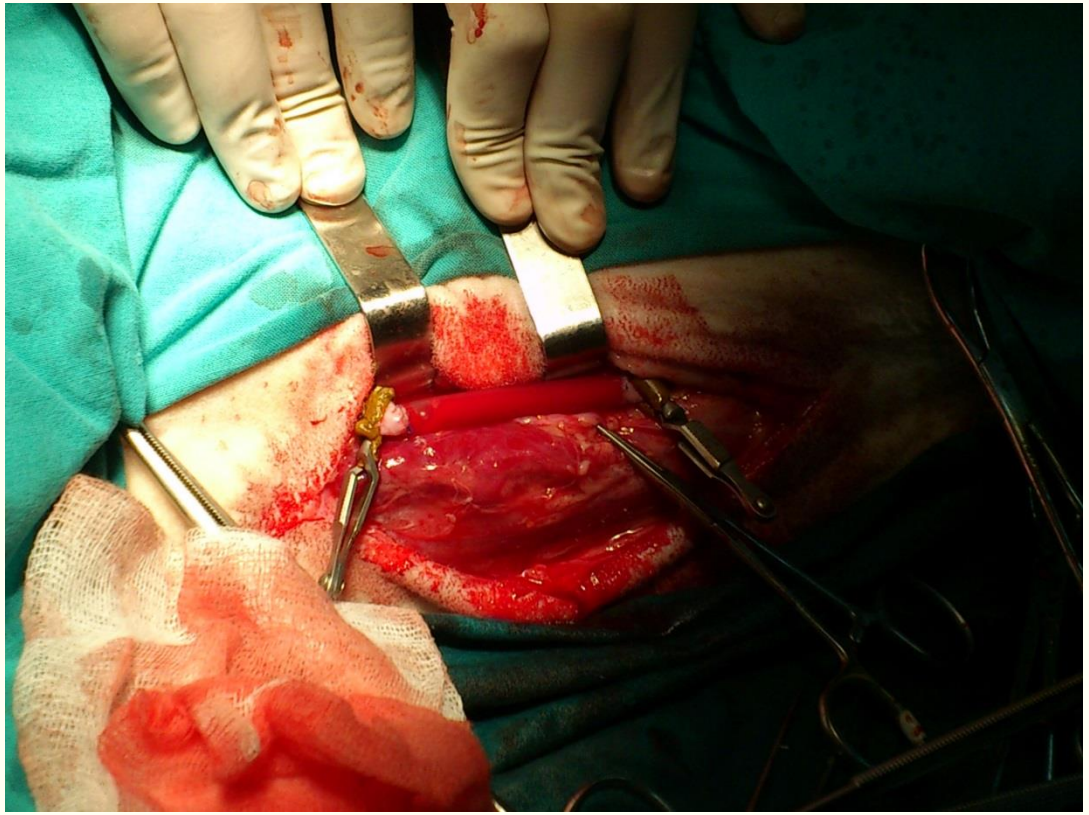
*Connell, John M., et al. "Anticoagulation of Juvenile Sheep and Goats With Heparin, Warfarin, and Clopidogrel." ASAIO Journal 53.2 (2007).

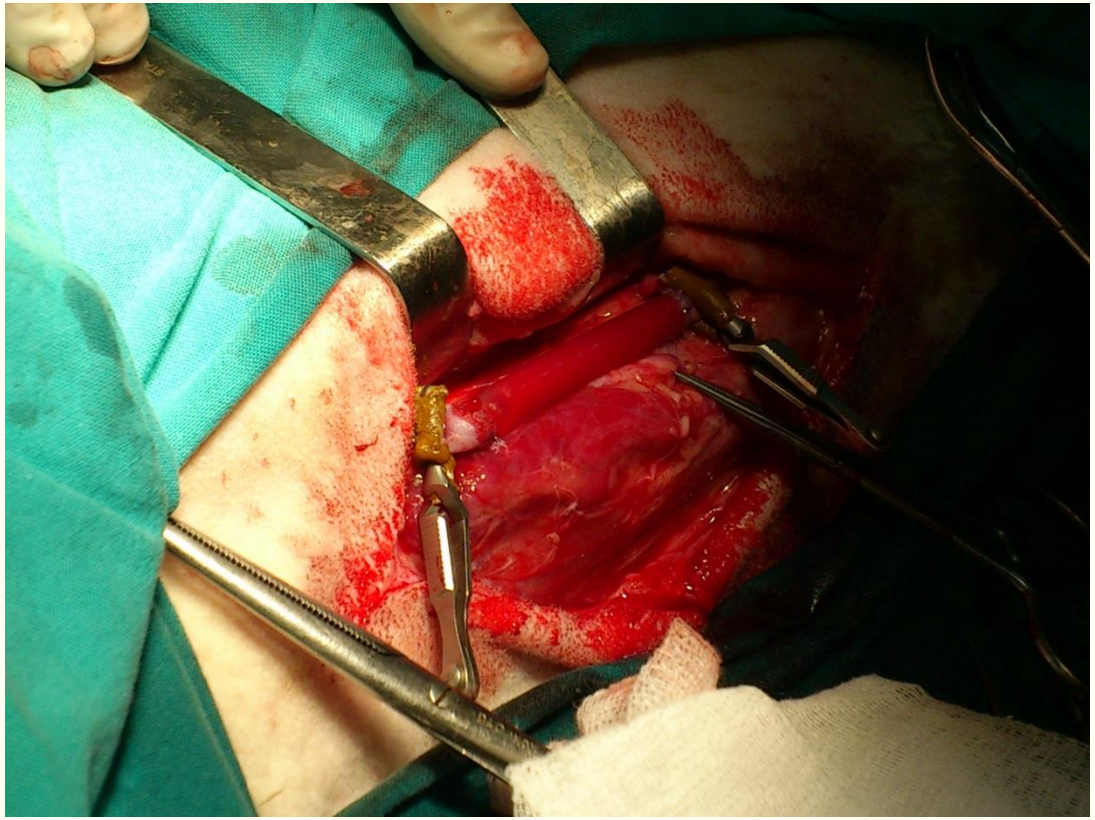
Methods - Experimental model



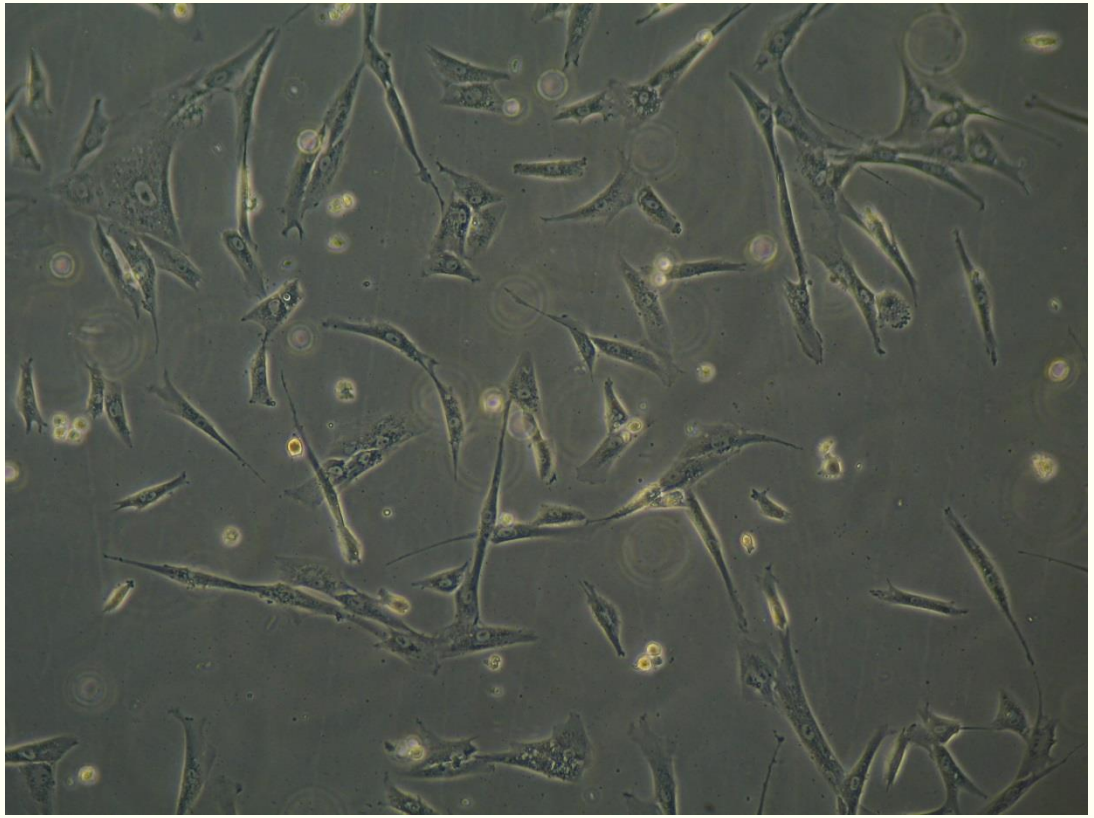


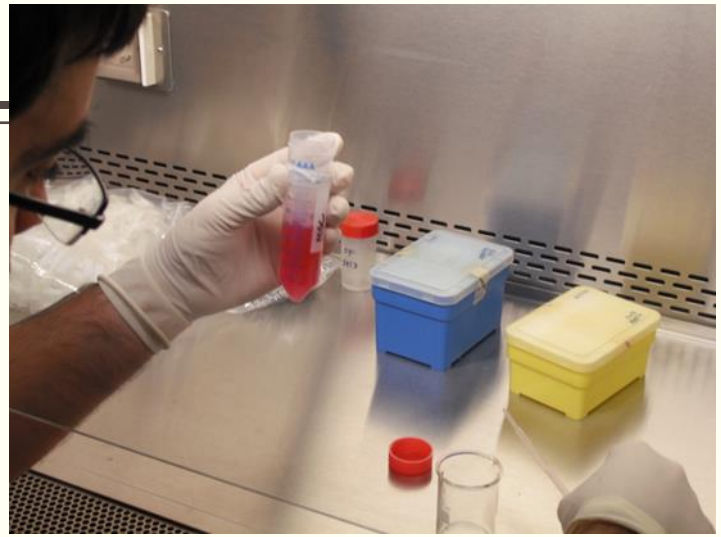
Methods – experimental model





Methods - experimental model





Perivascular injection of stem cells

Methods: functional evaluation

Day 1

- Functional evaluation: Doppler and B-mode
- Hematology
- Coagulation profile

Week 4

- Functional evaluation: Doppler and B-mode
- Hematology
- Coagulation profile
- Euthanasia: collection of samples

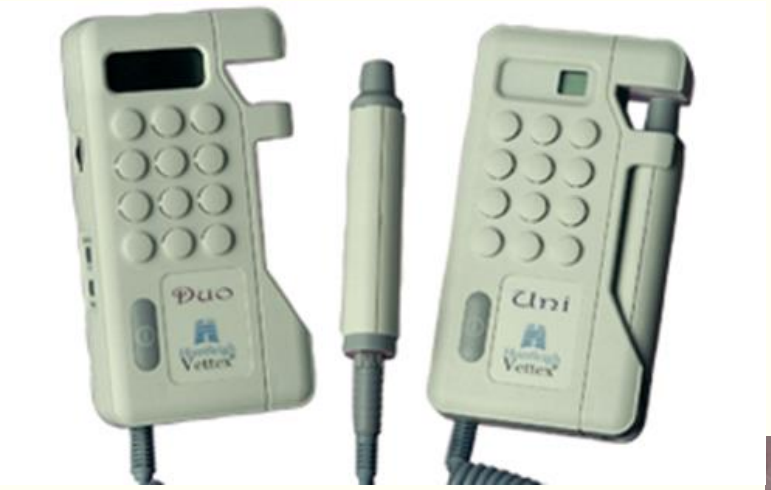
Week 8

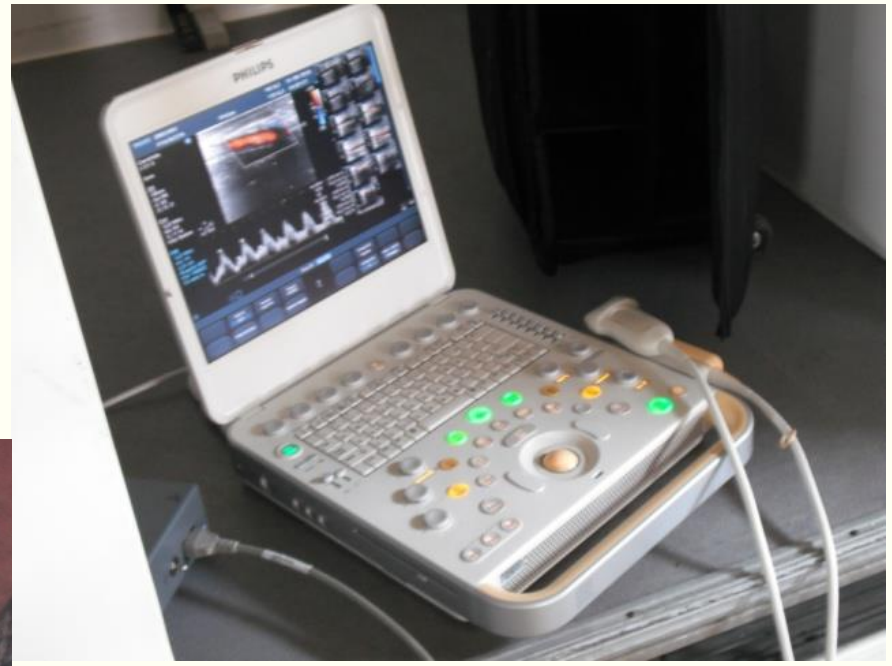
- Functional evaluation: Doppler and B-mode
- Hematology
- Coagulation profle
- Euthanasia: collection of samples

Week 12

- Functional evaluation: Doppler and B-mode
- Hematology
- Coagulation profile
- Euthanasia collection of samples

Methods: functional evaluation





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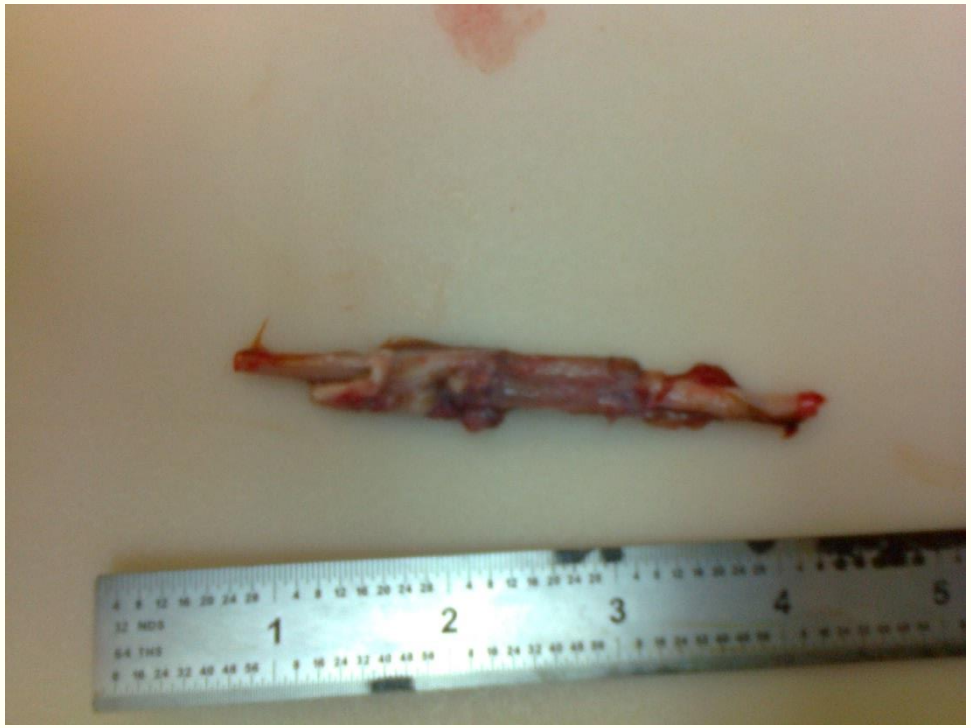
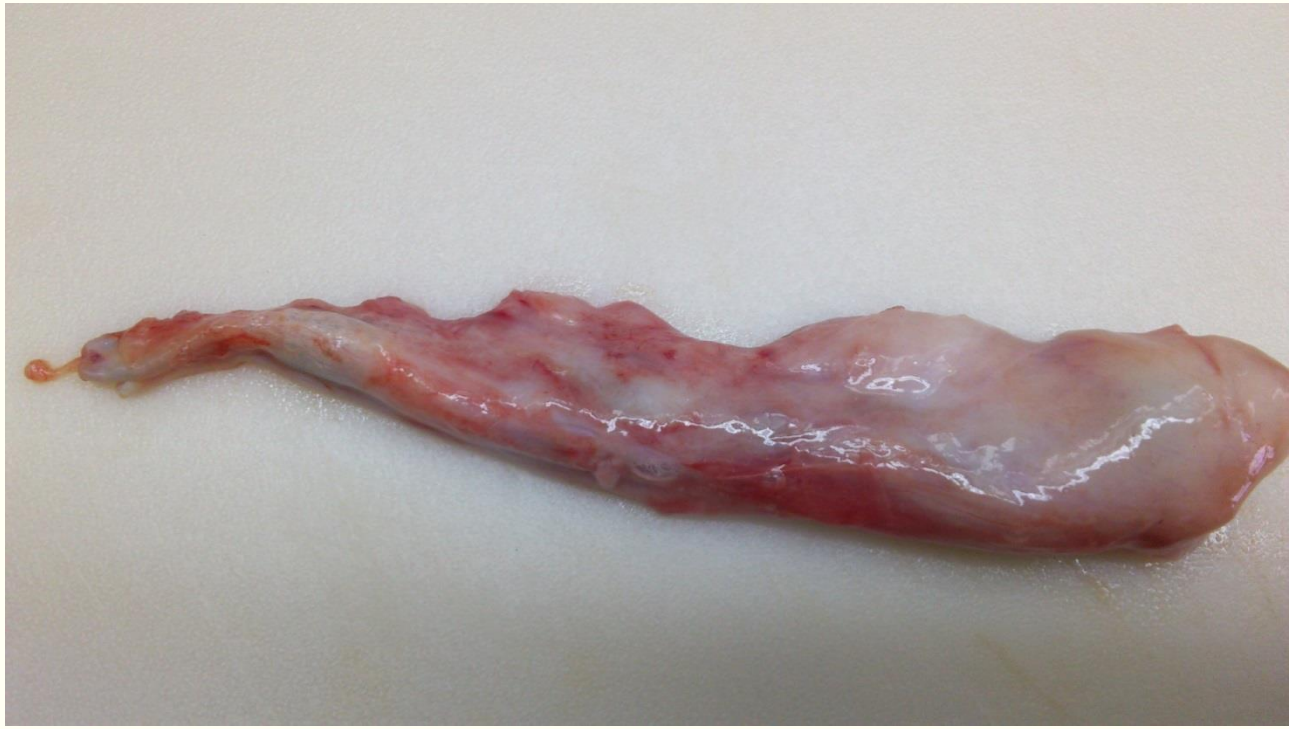


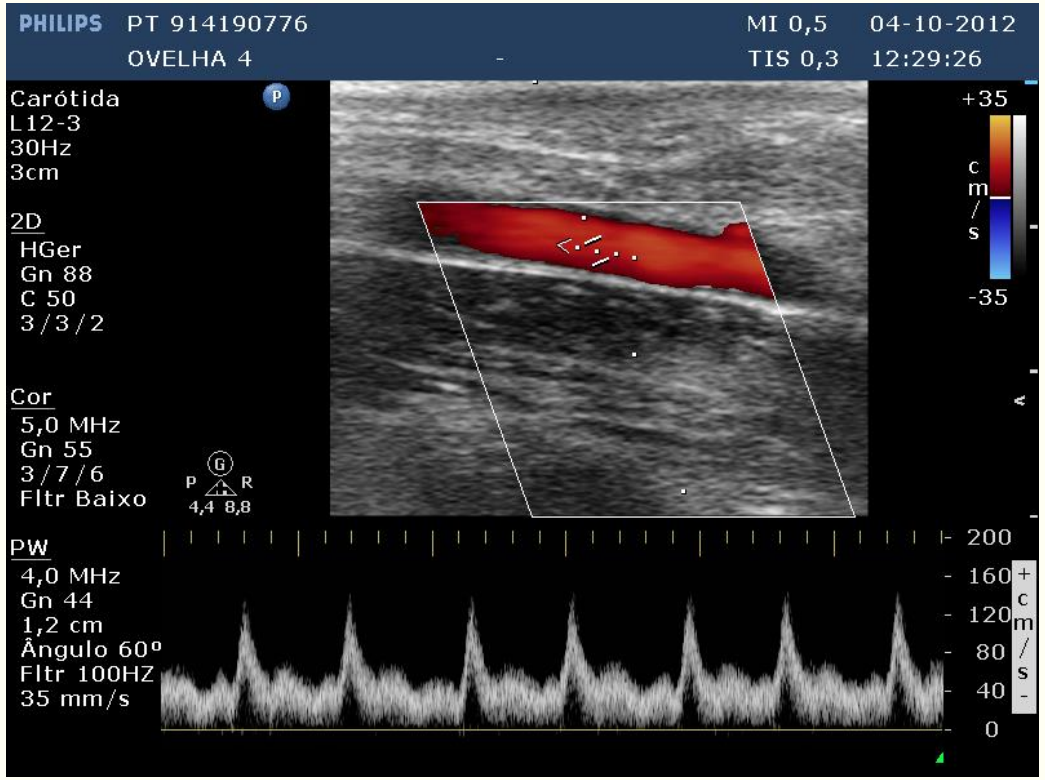


Results – patency rate (%)

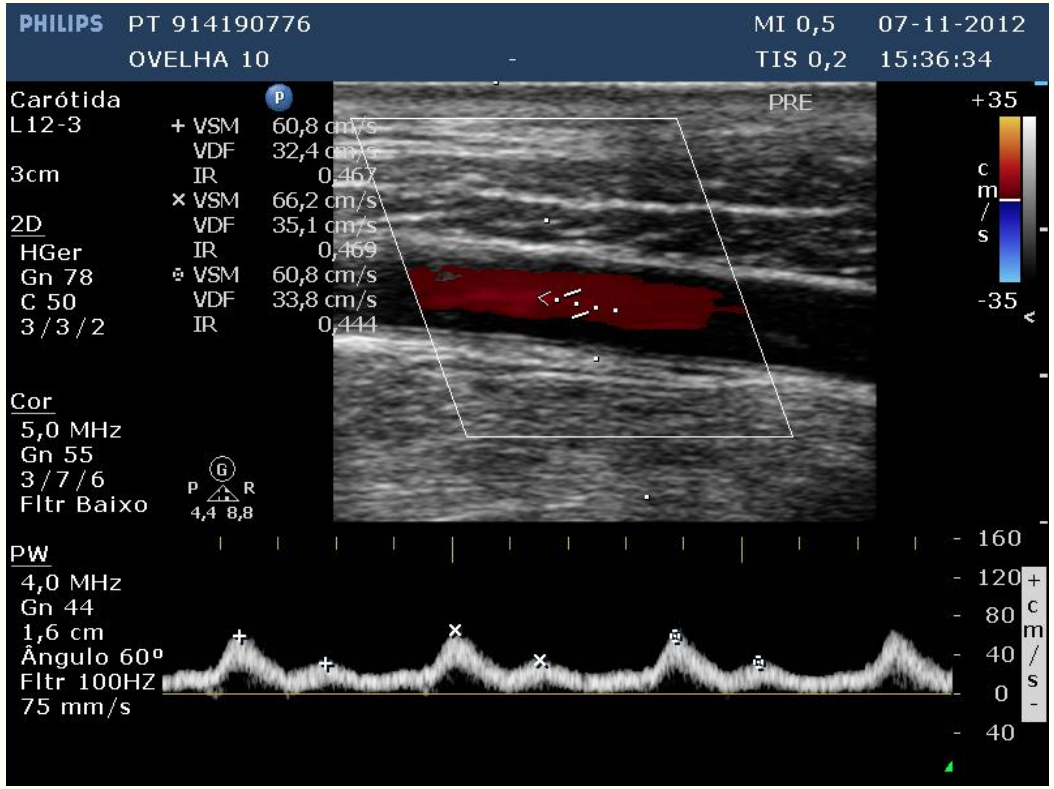
- **PVA prosthesis presented a patency rate (PR) of 100% at 24 hours.**
- **At 4 weeks the PR lowered for 50% and for 40%**
- **at 8 weeks post-surgery for 40%**
- **12 weeks post-surgery decreased further to 25%.**
- Cause of obstruction - thrombosis at implant - carotid artery transition
- Absence of signs of infection and adhesions at implant site
- No implant dilation or rupture was observed *in vivo* which supports the biomechanical properties observed *in vitro* has been published*

*N. Nunes. "PVA modificado para enxertos vasculares"MSc thesis. FE-UP, Porto (2012)

Results – patency rate (%)

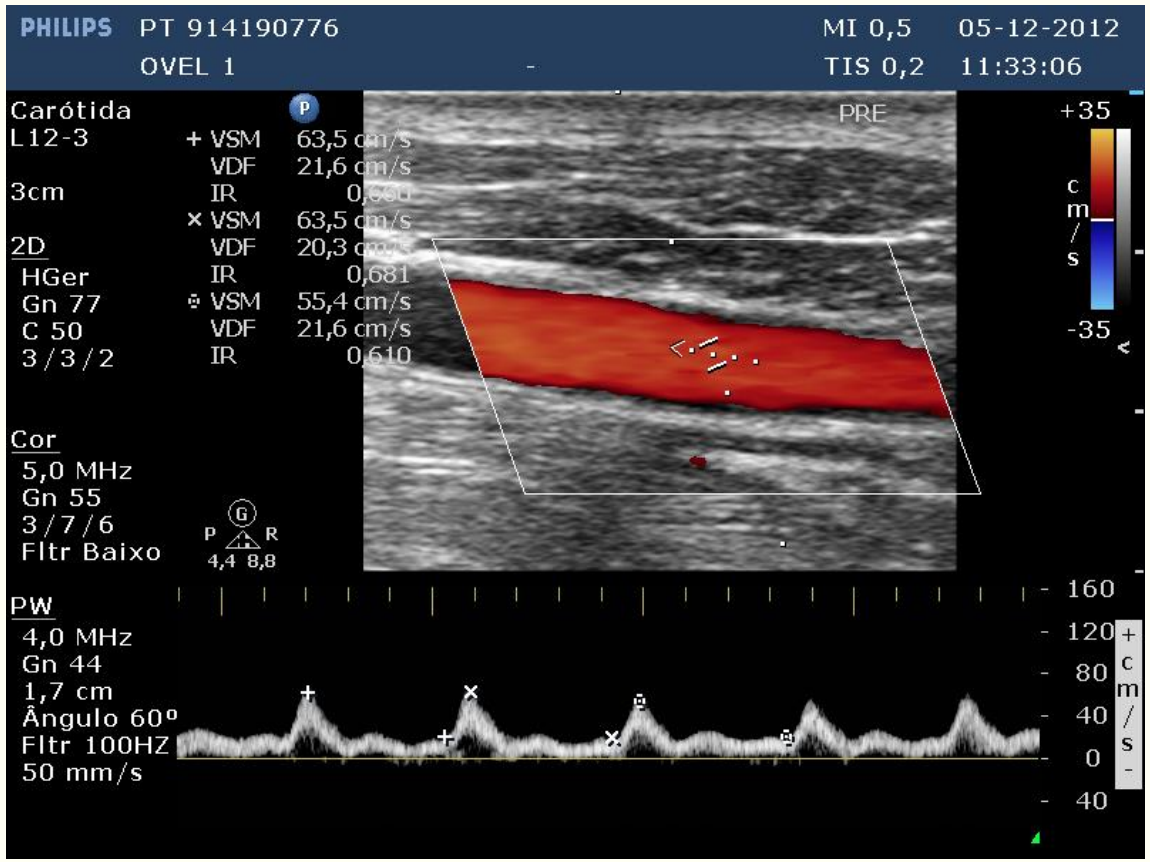


24 hours

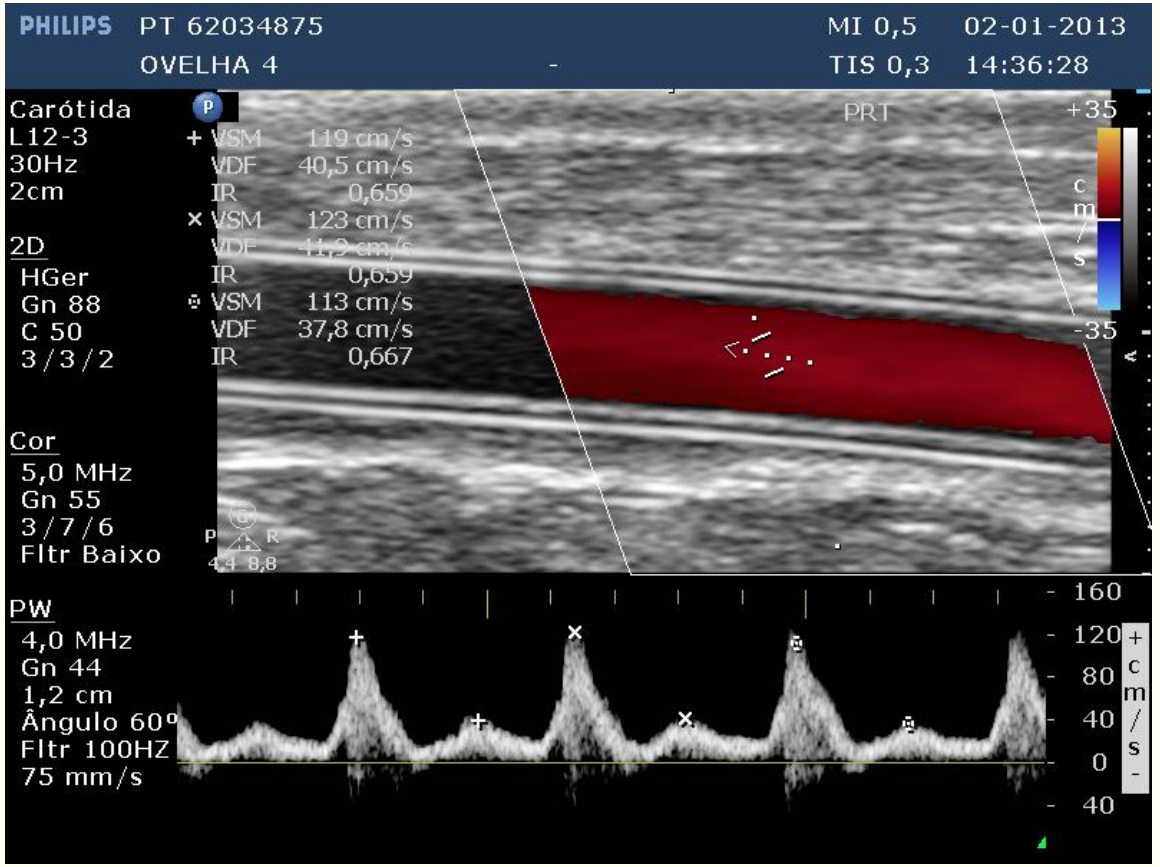


4 weeks

Results – patency rate (%)

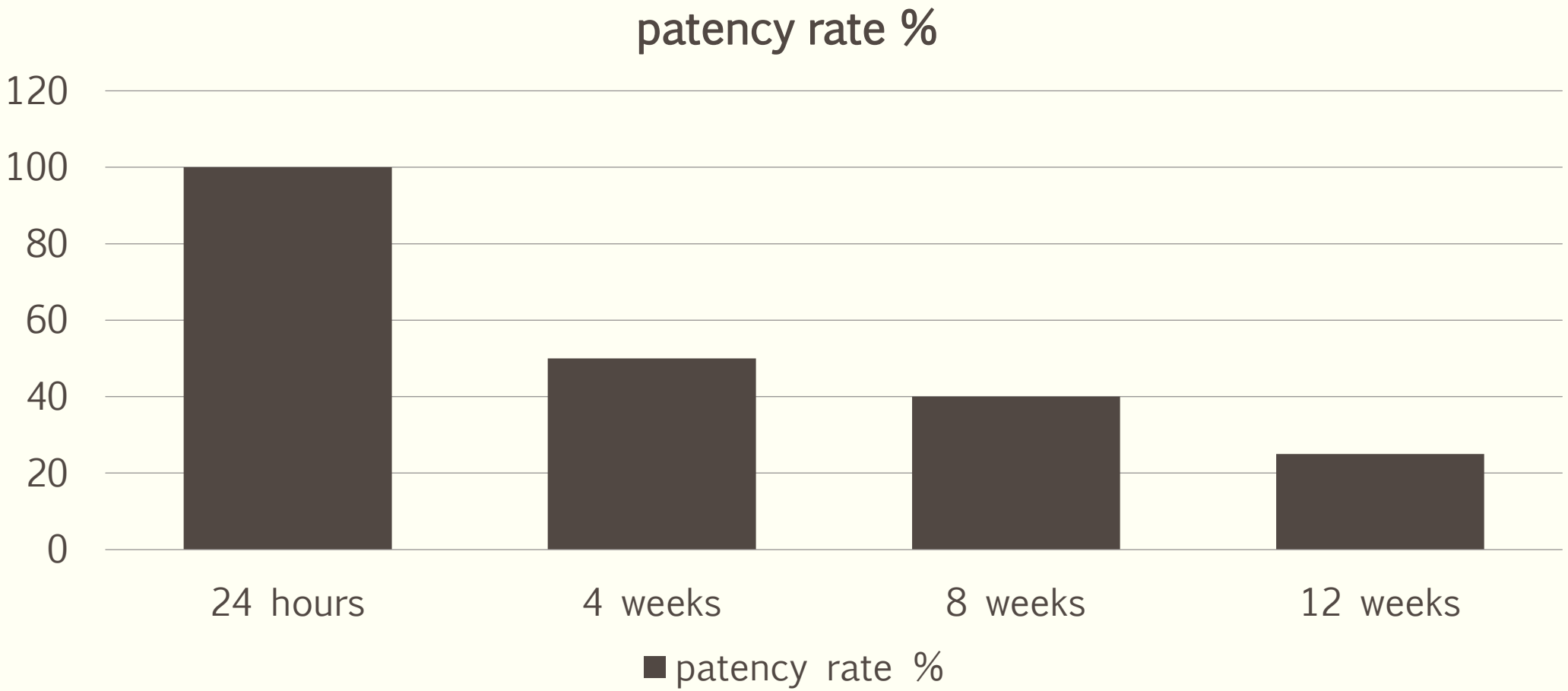


8 weeks

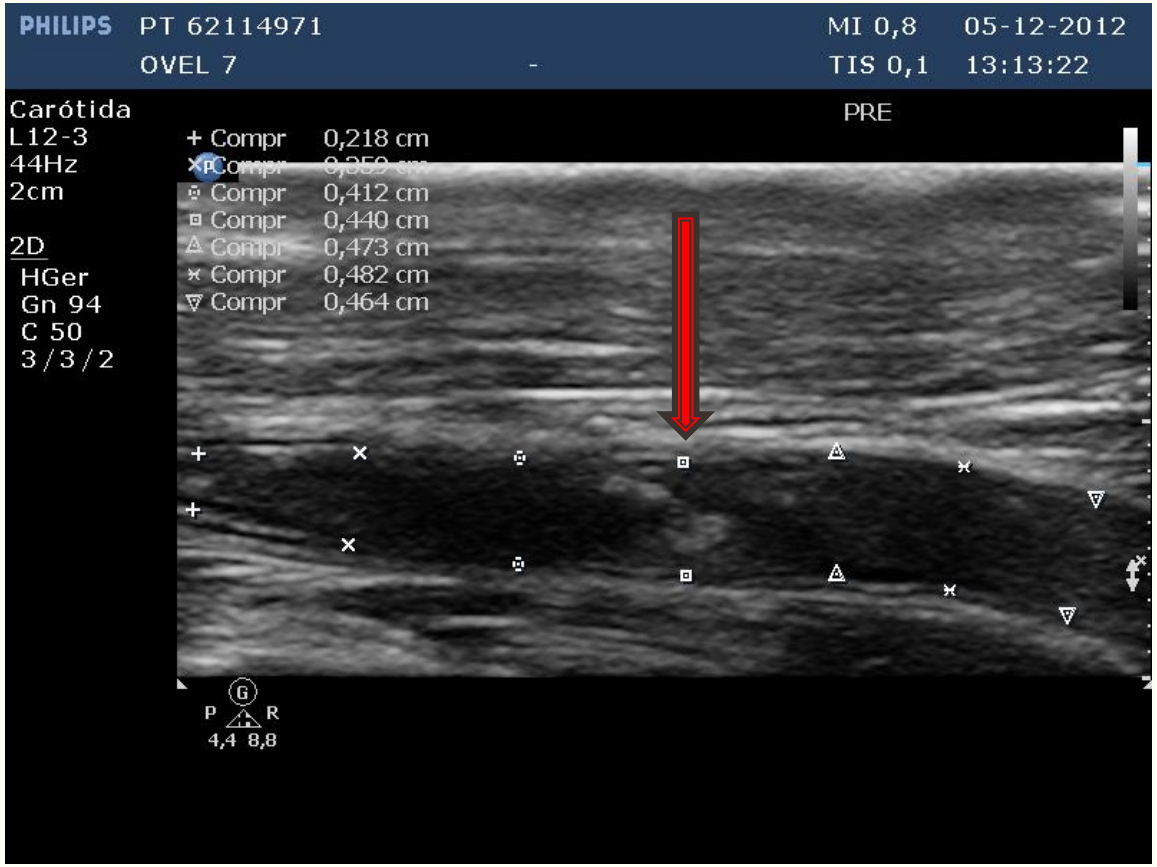


12 weeks

Patency rate evolution – PVA + dextran 1% grafts



Results –patency rate (%)



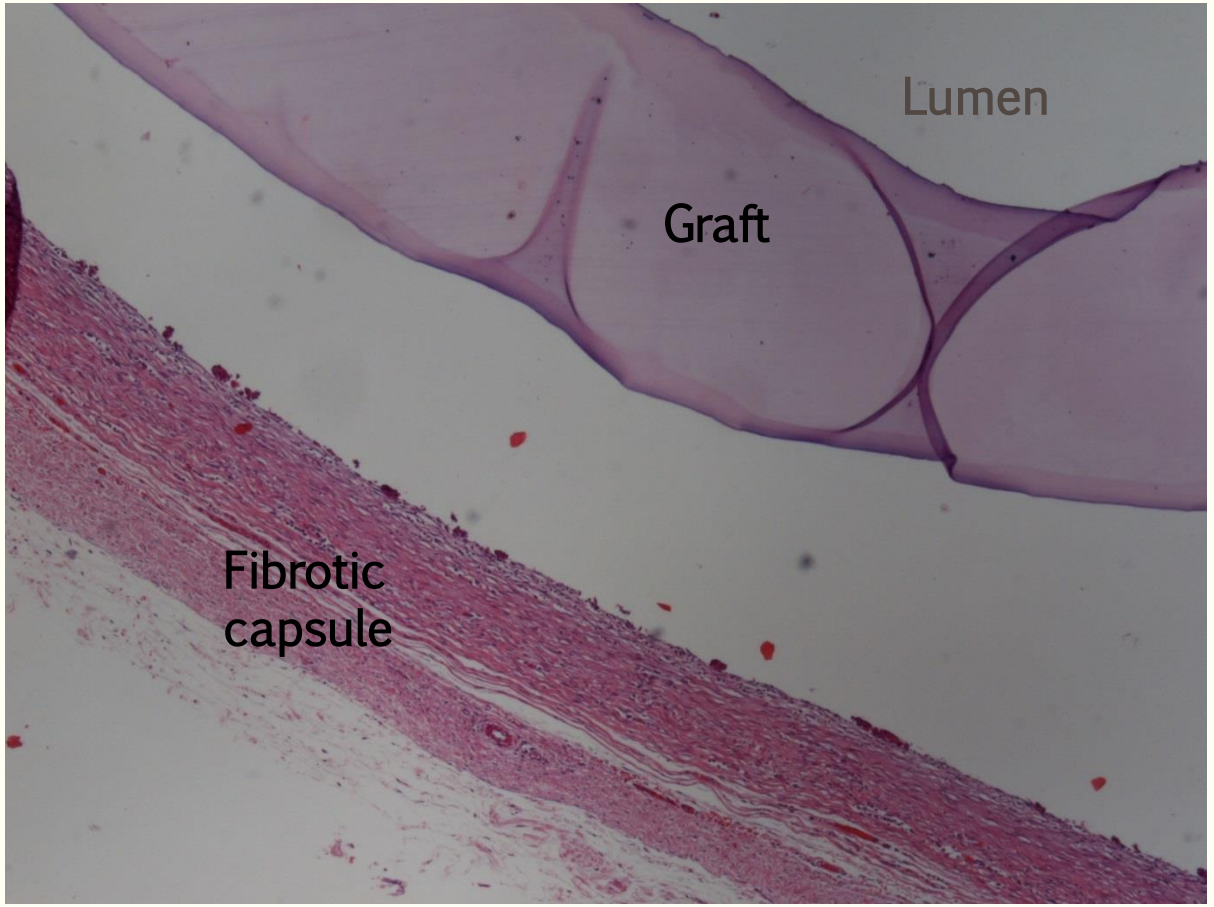
4 weeks

Results and discussion

- Histopathology results:
- Obstruction at graft-artery interface
  - confirmed to be non-organized thrombus rich
- At the lumen:
  - Endotelial cells were observed only in one animal, at graft lumen (to be confirmed with immunochemistry)
- Fibrotic capsule observed in all animals:
  - At graft – tissue interface
  - Without inflamatory infiltrate
  - Multinucleated giant cells present in one animal

Results –patency rate (%)

40 x – Hematoxylin – eosin
8 weeks post-surgery

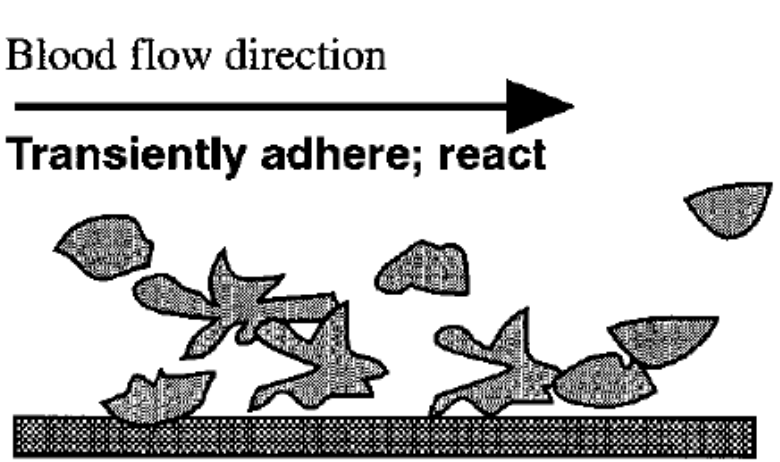


40 x - Hematoxylin – eosin
4 weeks post-surgery

Results and discussion

- Low prevalency of endotelial cells at graft lumen can be explained:
  - Hydrophilic scaffolds they are relatively resistant to protein adsorption and cell attachment*

- The patency rate is closely linked to thrombogenicity and hemocompatibility of the biomaterial:
  - Low lumen of the artificial conduit < 6 mm
  - Low activity of the fibrinolytic system of sheep
  - High levels of fibrinogen in ruminants
  - PVA interact with platelets, activated them



*Nuttelman CR, Henry SM, Anseth KS. Synthesis and characterization of photocrosslinkable, degradable poly(vinyl alcohol)-based tissue engineering scaffolds. Biomaterials. 2002;23:3617-26

Results and discussion

- In spite of PVA activation of platelets patency rate was better than expected

- Probably related to the anticoagulation properties* of the dextran component of the grafts

Coagulation

- inhibition of thrombin by both enhancing anti-thrombin and heparin cofactor II

Platelets

- various GAGs were reported to induce platelet dysfunction:

Complement

- enhance the
- inhibition of FXIa by C1 inhibitor

*Zeerleder, Sacha, et al. "Effect of low-molecular weight dextran sulfate on coagulation and platelet function tests." Thrombosis Research 105.5 (2002): 441-46.

Results and discussion

- Low grade inflamation at tissue – graft interface can be explained by

- Perivascular injection of mesenchymal stem cells (MSC) Derived from Wharton jelly

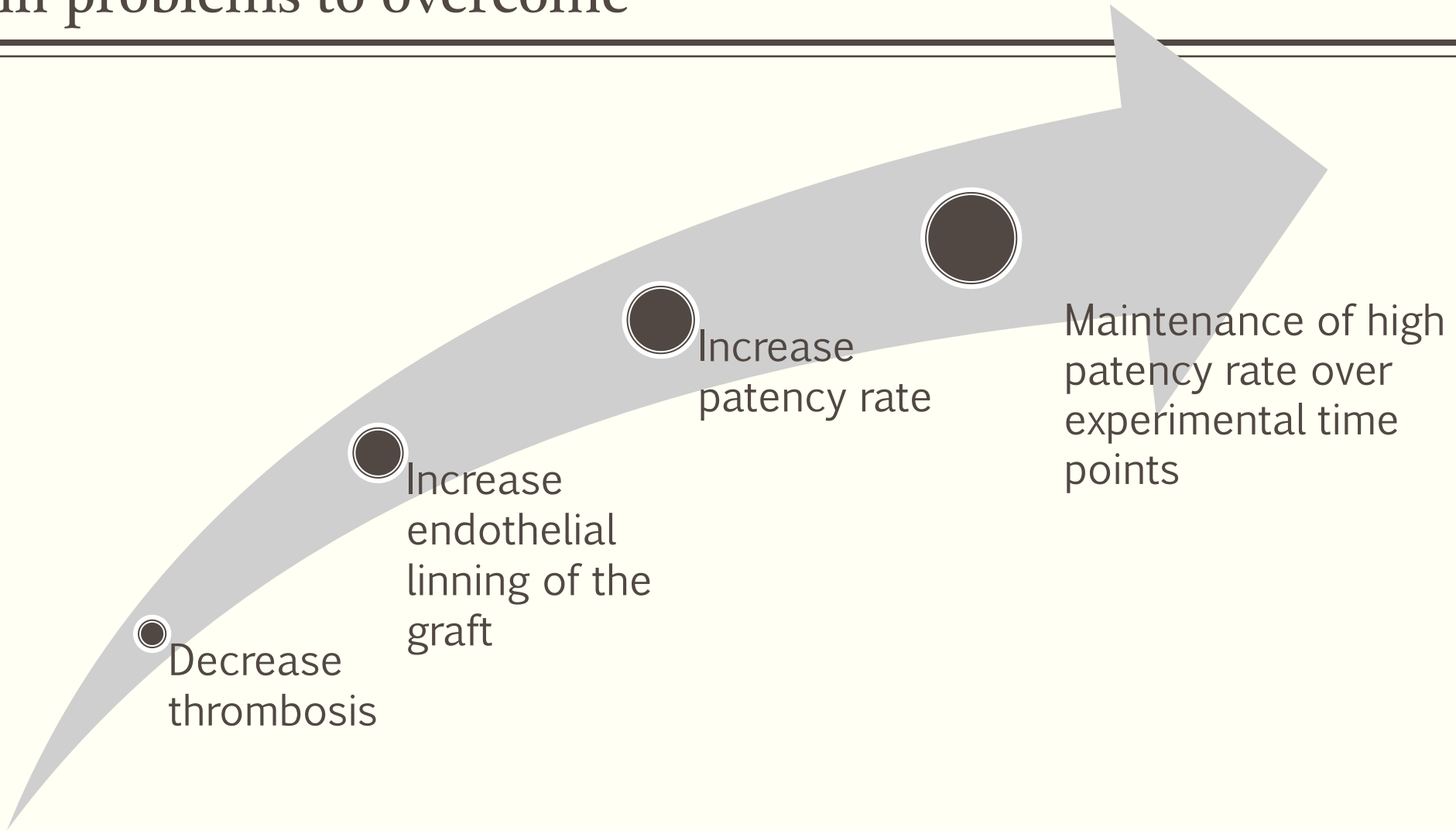
Capability of suppress T cells and antigen presenting cells

Can modify the biocompability through the immunomodulatory effects of these cell

Immunomodulatory changes linked to the suppression of inflammatory cytokines and to the induction of T cells with regulatory or suppressive phenotypes

Can induce faster biointegration avoiding an exuberant local inflammatory reaction

Main problems to overcome



Future perspectives

Improve cell adhesion

- RGD peptides
- Fibronectin/vitronectin
- Impregnation with growth factors for sustained release:
  - e.g.VEGF (Vascular Cell Growth Factor)
  - Binding to GAG's

Functionalization of graft surface

- Endothelialisation – previous to surgery
  - Seeding endothelial cells/endothelial progenitor cells/stem cells
- Adsorption of molecules like anticoagulants (heparin), antiplatelet factors (glycoprotein IIb/IIIa inhibitors), and antiproliferating agents (rapamycin)
- To minimise complications from blood – material interactions

Conclusions

- Was possible to demonstrate that PVA can be:
- Used as a functional vascular prosthesis
  - Without dilation or rupture of the graft
- Biocompatible material
- Can support patency of blood flow for several weeks (12) in a hipercoagulable animal model (sheep)

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THANK YOU FOR YOUR ATTENTION!