Obiettivo Salute: Le nuove Tecnologie e le Ricerche del CNR

Tecnologie Emergenti su Biomateriali & Dispositivi per il Sistema Cardiovascolare

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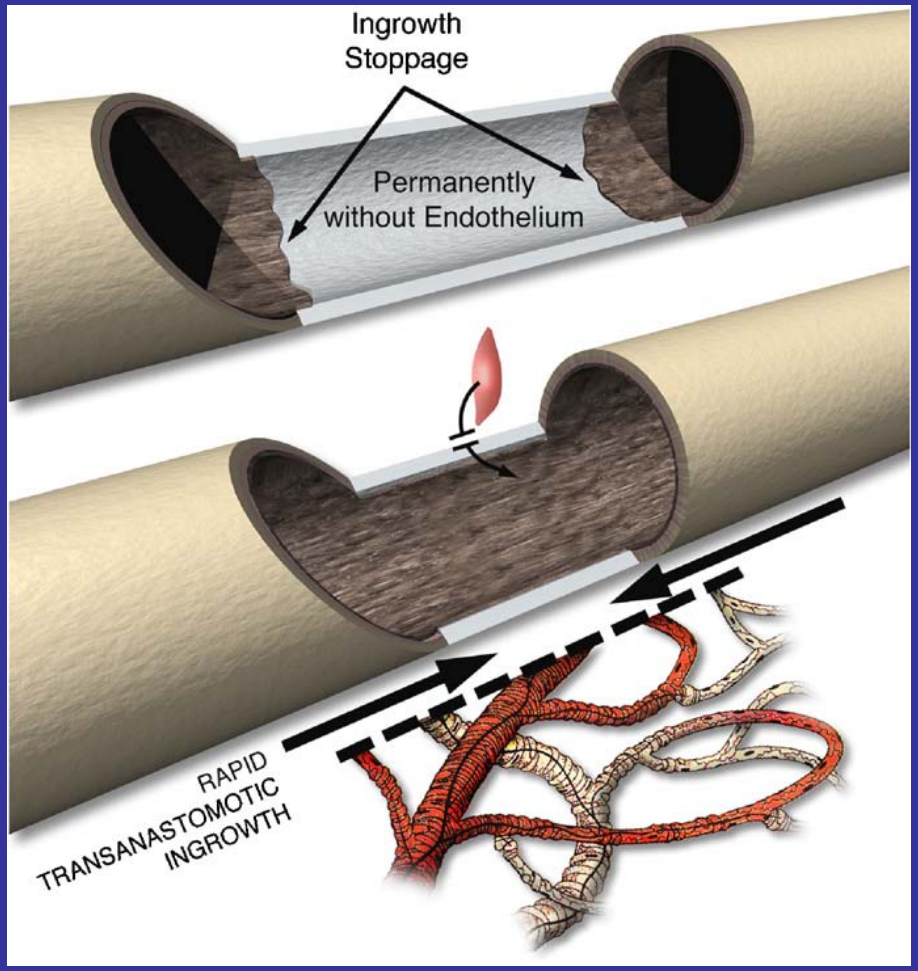

…..In spite of the enormous progress in surface optimization technologies……

……

……...the main problem of implanted devices in the vascular system
remains the poor ability
for self-endothelialization……

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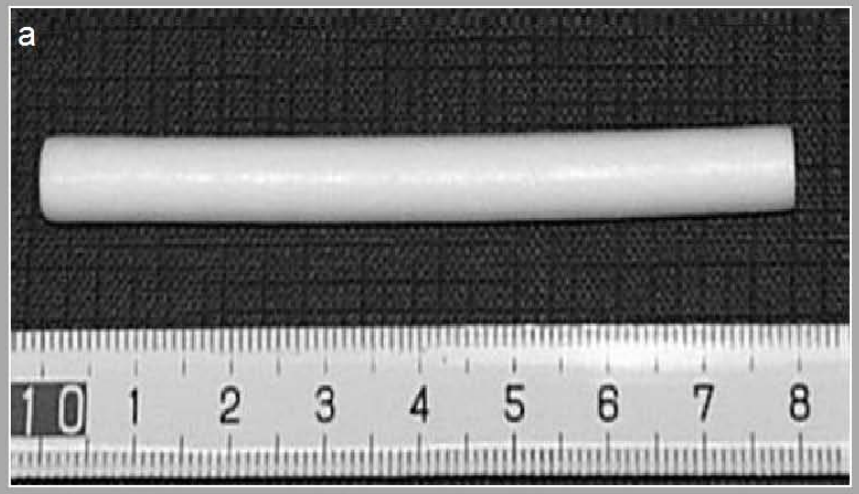
Leading Opinion

Prosthetic vascular grafts: Wrong models, wrong questions and no healing ☆

Peter Zilla*, Deon Bezuidenhout, Paul Human

Christian Barnard Department of Cardiothoracic Surgery/Cardiovascular Research Unit, University of Cape Town Medical School, Cape Town, South Africa


a
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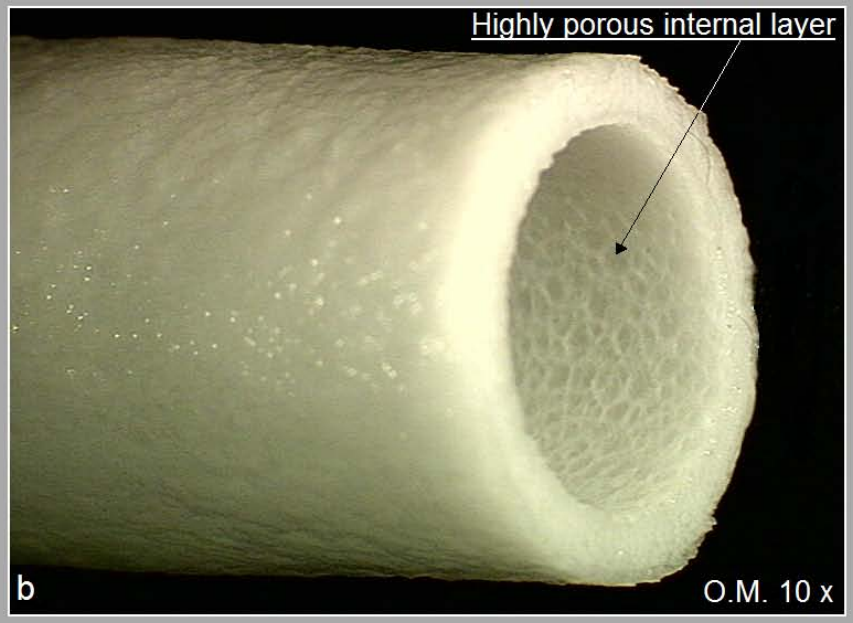
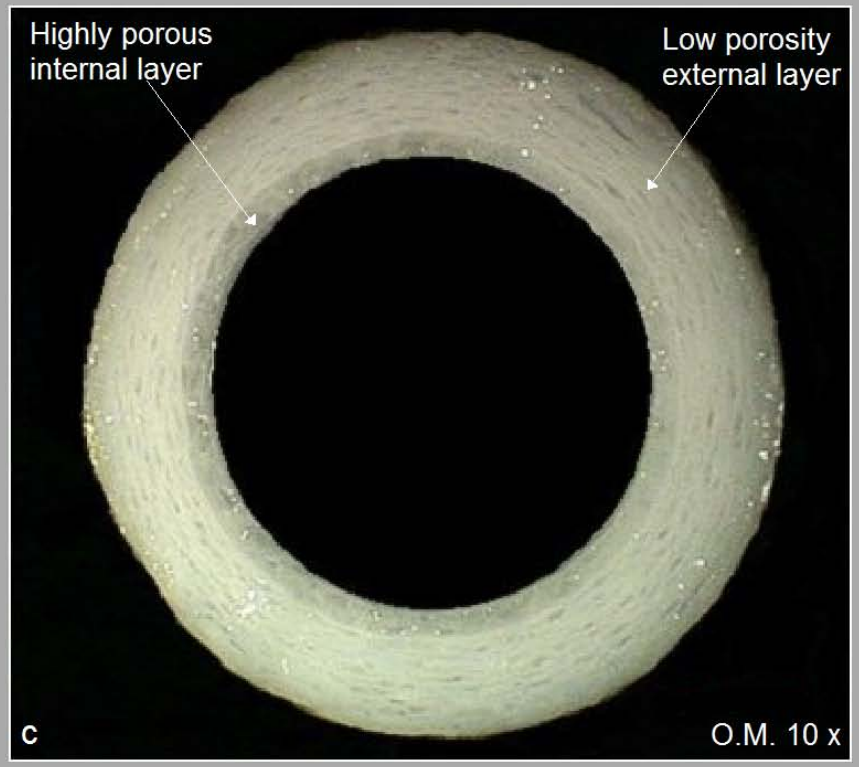
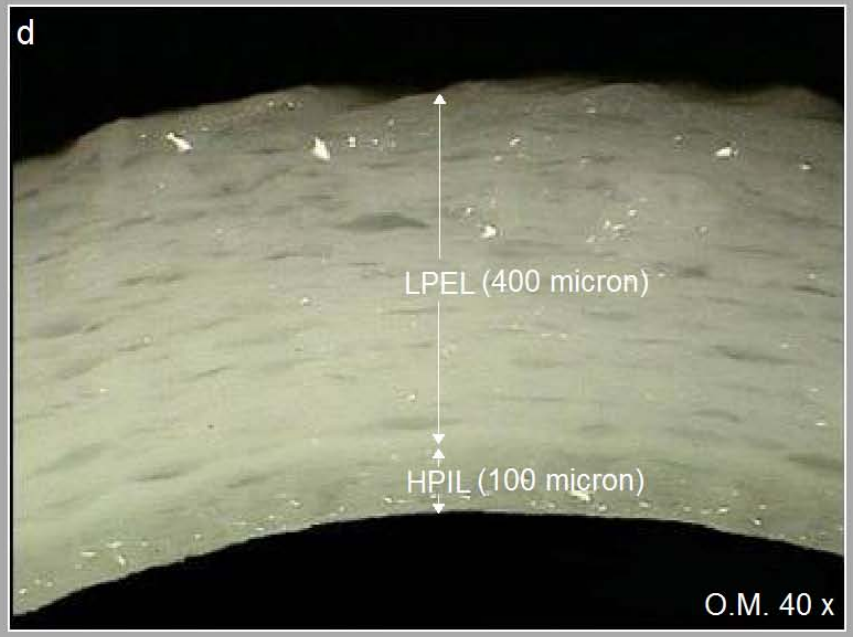
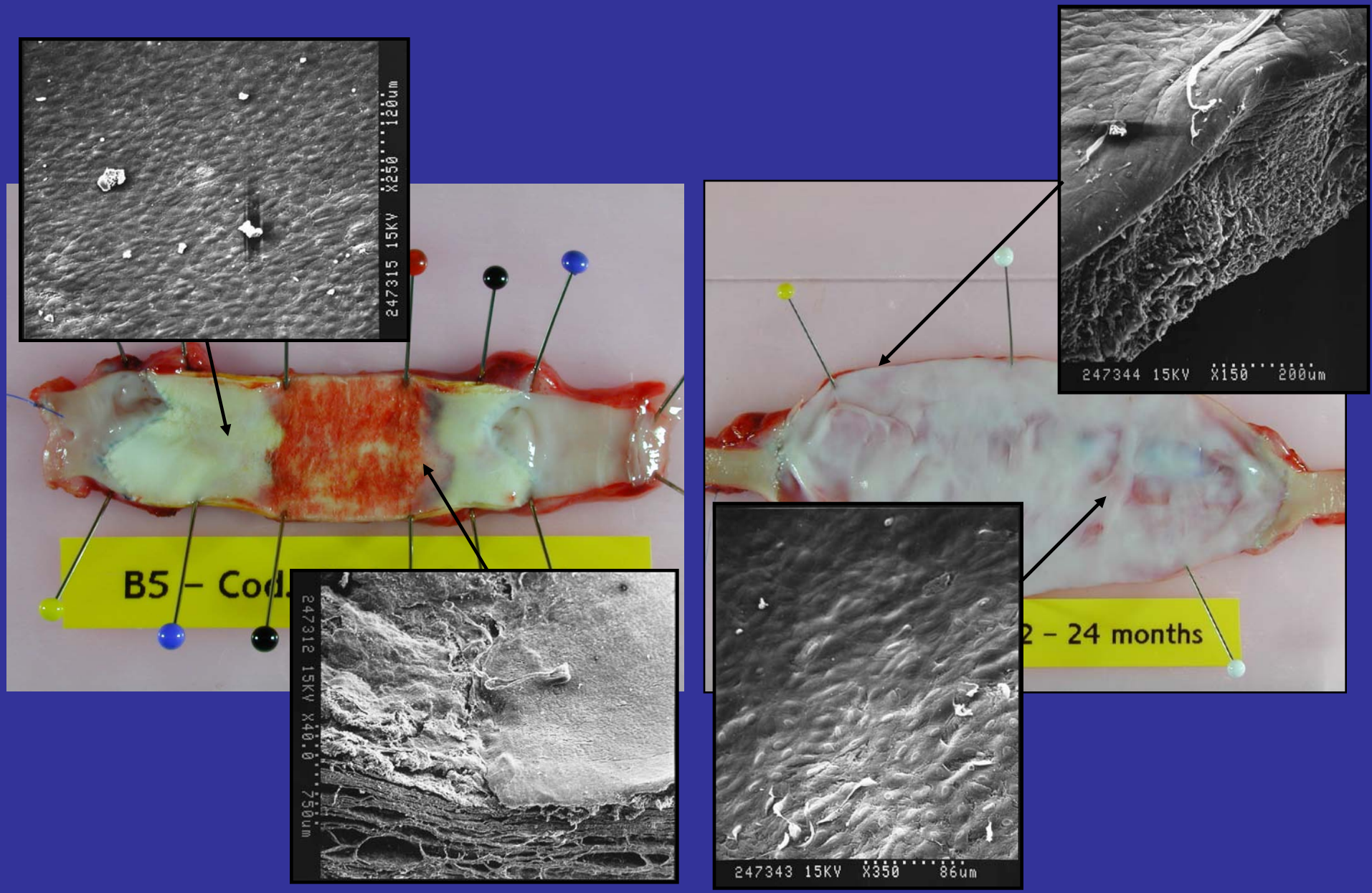

Highly porous internal layer
b
O.M. 10 x


Highly porous internal layer
Low porosity external layer
c
O.M. 10 x


d
LPEL (400 micron)
HPIL (100 micron)
O.M. 40 x

PEtU-PDMS Grafts explanted at 6 and 24 months



Realization of bio-artificial vascular graft

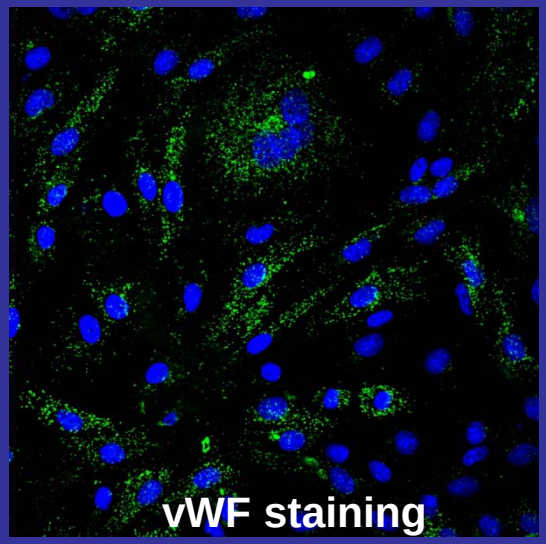
In vitro endothelialization

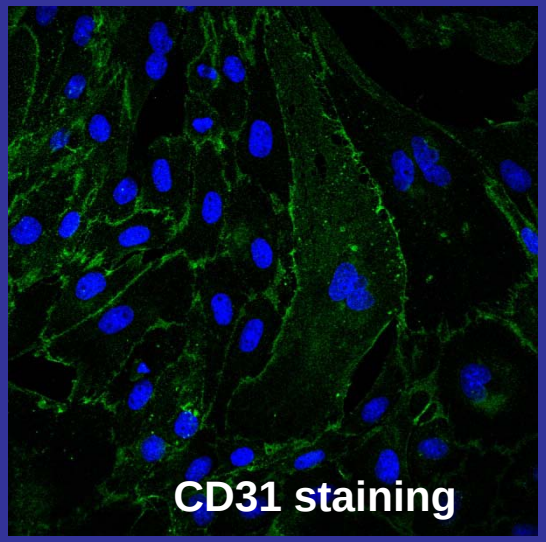
The tubular small-diameter PEtU-PDMS scaffold with compliance and mechanical properties similar to natural vessel.....

.....has to be covered on the internal surface by a confluent endothelial cells monolayer to improve the haemocompatibility of the synthetic material.

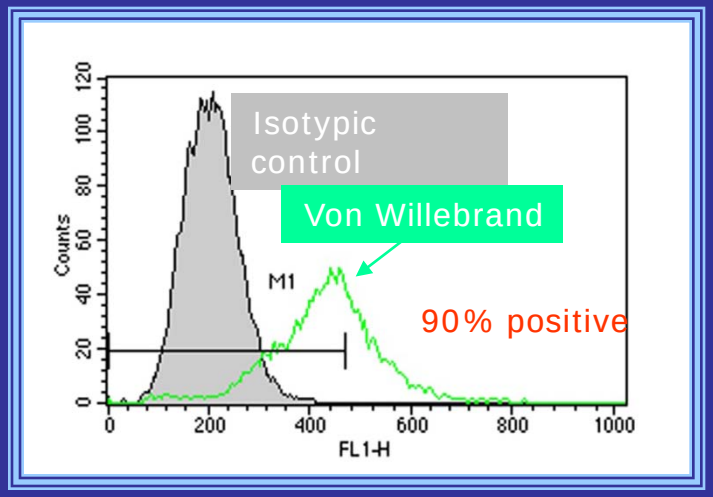
Swine endothelial progenitor cells from peripheral blood

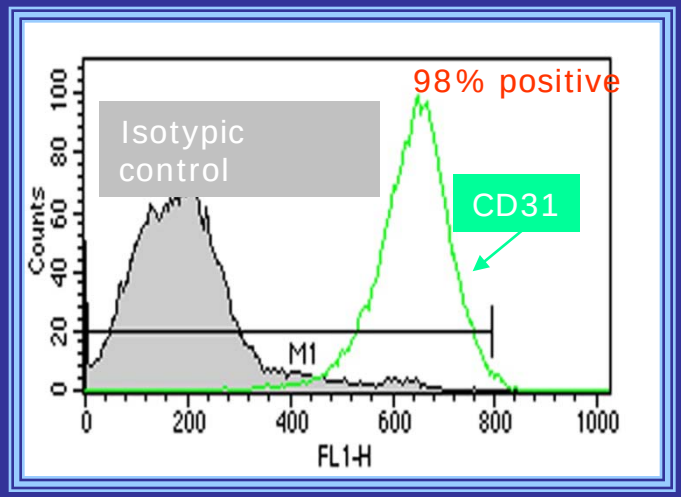
Endothelial phenotype study





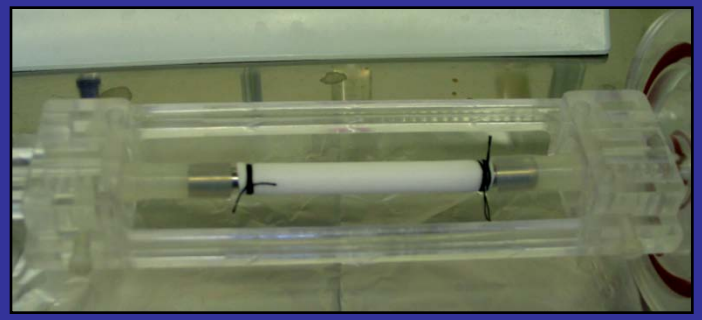
Flow cytometric analysis



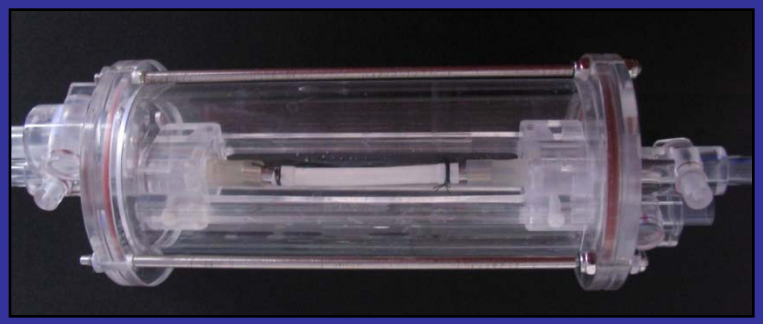


Realization of bio-artificial vascular graft

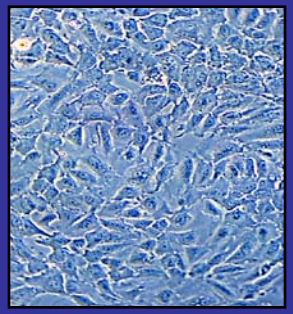
In vitro endothelialization



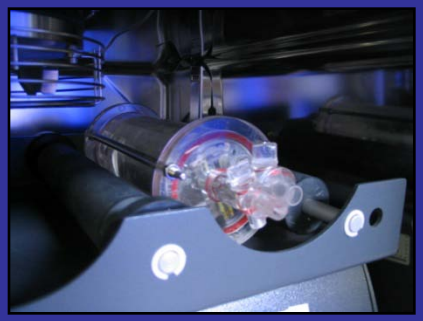
1. The vascular graft is cannulated and fitted in a Plexiglas support



2. The support is inserted into a cylindrical culture chamber, the graft is filled with fibronectin solution (20μg/ml) and incubated for 90 min. at 37°C



3. HUVECs are seeded into the lumen of fibronectin-coated graft at a density of $3x10^5$ cells/cm^2

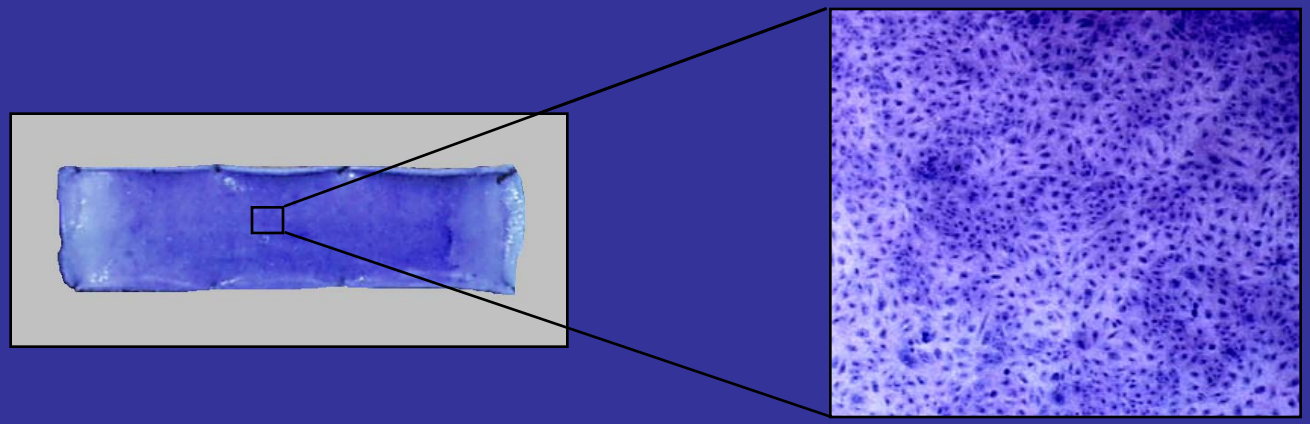


4. The seeded graft is rotated at 12 rph for 3 hours to guarantee an even distribution of cells on the luminal surface

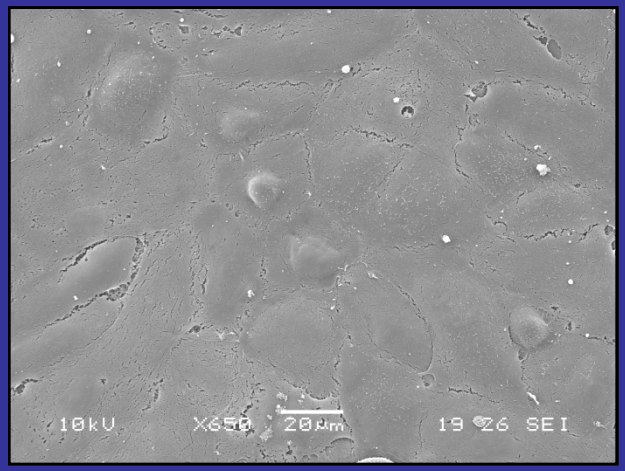
5. The culture chamber is connected with a close system of circulating culture medium

Realization of bio-artificial vascular graft

In vitro endothelialization – Results



Observation by stereo-microscope of the luminal surface of a PEtU-PDMS endothelialized graft. Cells are stained with Giemsa (O.M. 60X)



SEM photographs of the luminal surface

of a PEtU-PDMS endothelialized graft

…….A fascinating alternative……

……..the in vivo endothelialization of synthetic materials after implantation Inside the body……

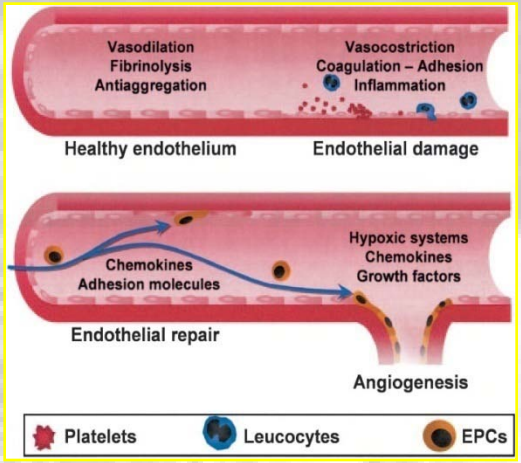
…….all patients should cover their implant with their own endothelial cells (ECs)……..

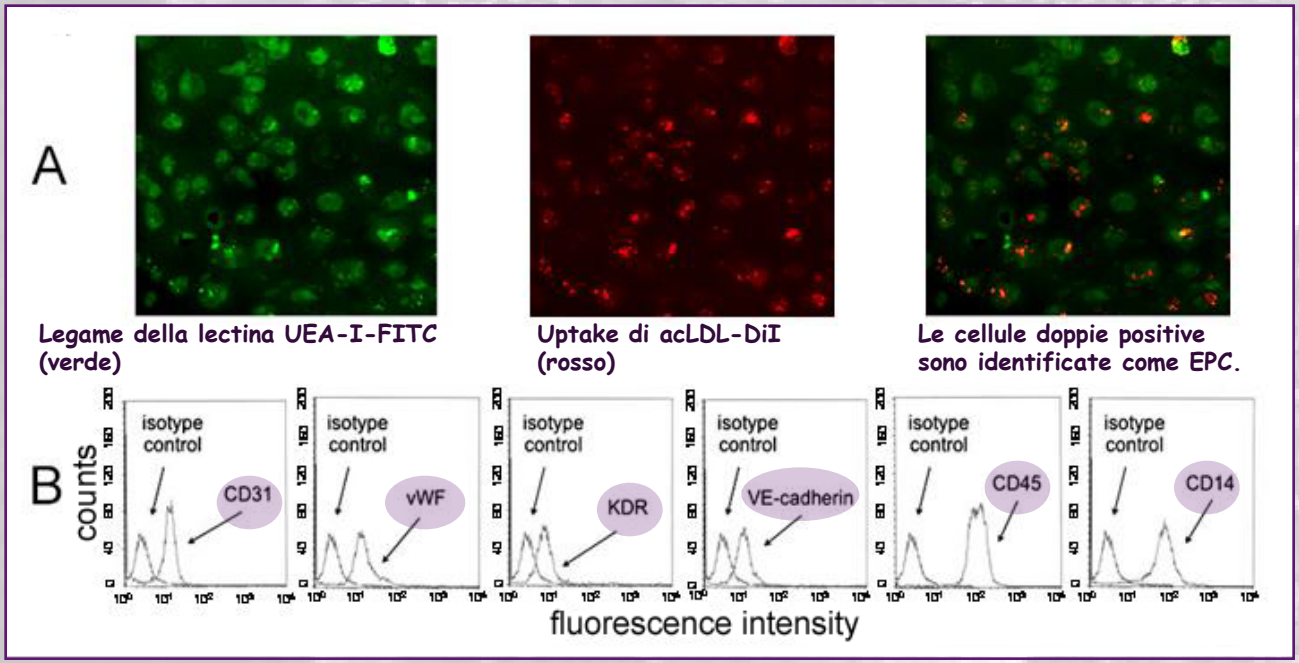
But what kind of ECs should be used and are available?

CIRCULATING ENDOTHELIAL PROGENITOR CELLS (EPCs)

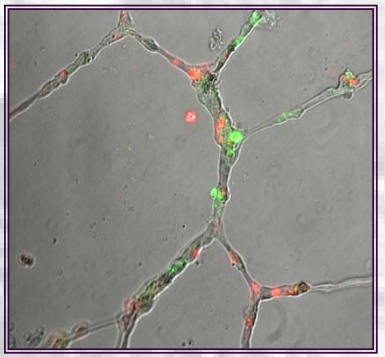
Courtesy of Dott.ssa Di Stefano - Laboratorio di Ricerca Cardiovascolare, DCTV, Università di Pisa

- First description in peripheral blood in 1997 (Asahara et al.1997).
- Circulating cells from bone marrow, can differentiate into mature endothelial cells (0,05-0,2% of peripheral blood cells).
- Homing and re-vascularizating at sites of vascular injury.
- Highly suitable for endothelialization of cardiovascular stents or tissue engineered implants.





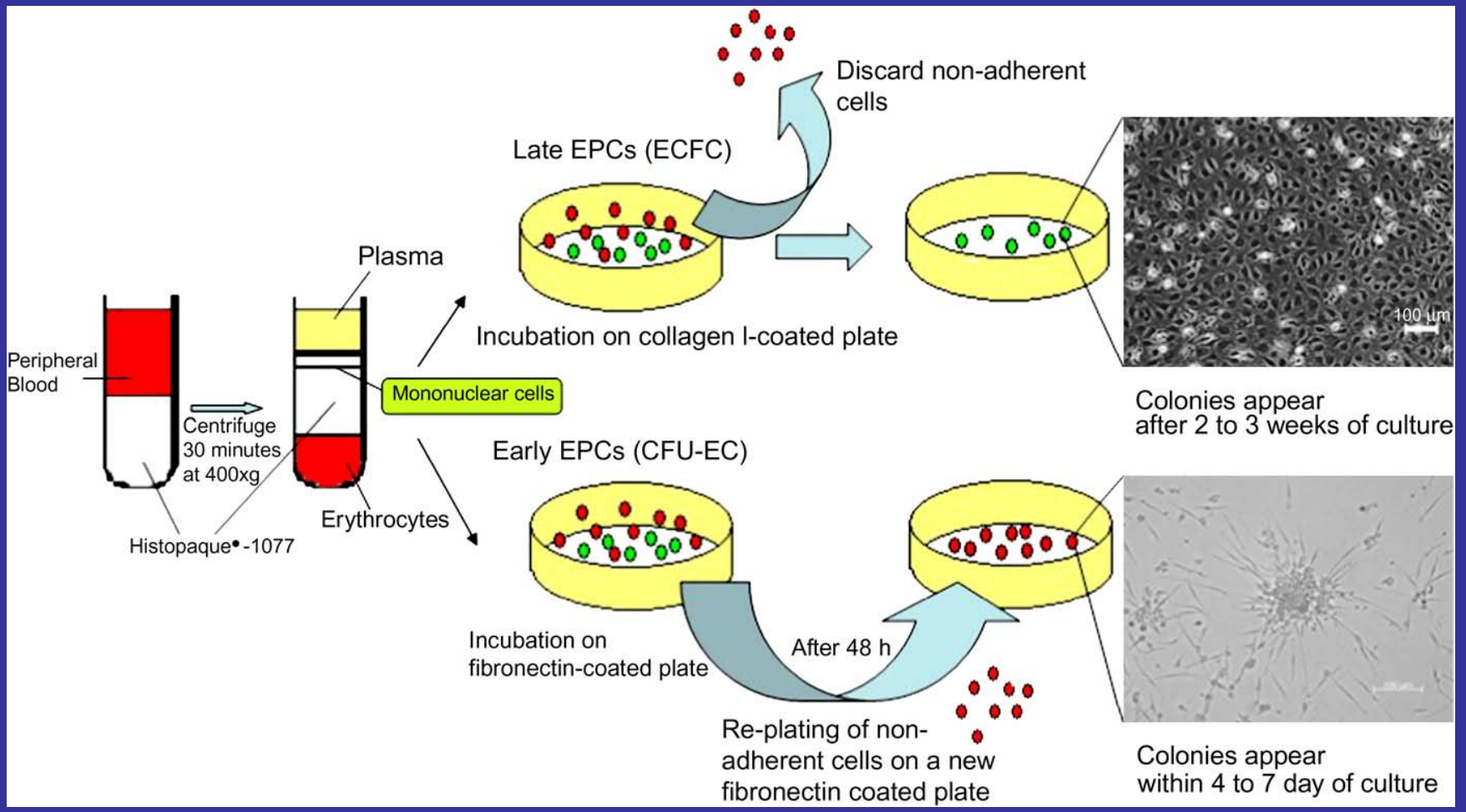
Monocyte and endothelial markers expression



In vitro angiogenesis:

EPC on matrigel

Two different methods for isolation and cultivation of EPCs



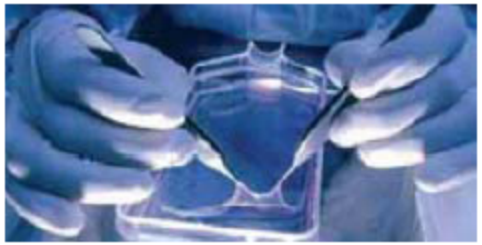
- "Early" EPCs are spindle-shaped cells with limited proliferation capability → minor vessel damage
- "Late " EPCs are cobblestone-shaped cells with rapid prolif. potential → extensive vascular injuries
- "Early" and "late" EPCs act synergistically to stilulate → neovascularization

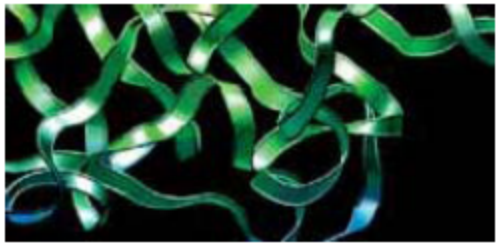
Fishing for endothelial progenitors cells (EPCs)

Nanomedicine

The term Nanomedicine refers to the application of nanotechnologies to diagnosis and treatment of diseases.

- ✓ It deals with the interactions of nanomaterials (surfaces, particles, etc.) or analytical nanodevices with "living" human material (cells, tissue, body fluids).
- ✓ It is an extremely large field ranging from in vivo and in vitro diagnostics to therapy including targeted delivery and regenerative medicine.

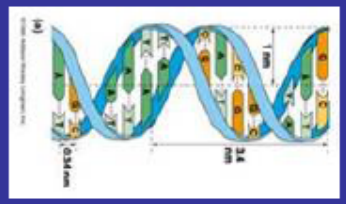


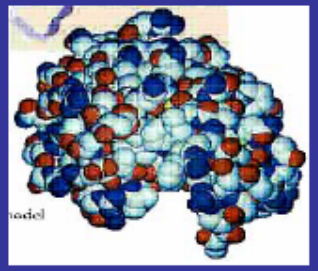


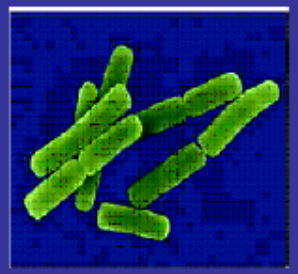
Why nano

NATURAL

Water molecule





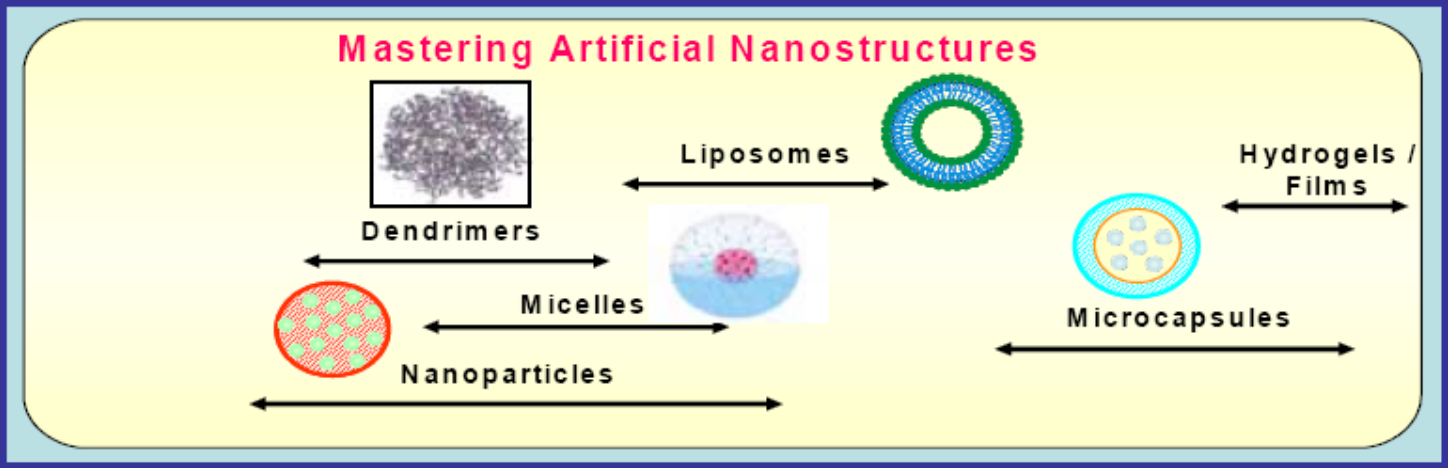


DNA base | DNA turn | Protein | Virus | Bacteria | Cells

0.1nm | 1nm | 10nm | 100nm | 1μm | 10μm

SYNTHETIC

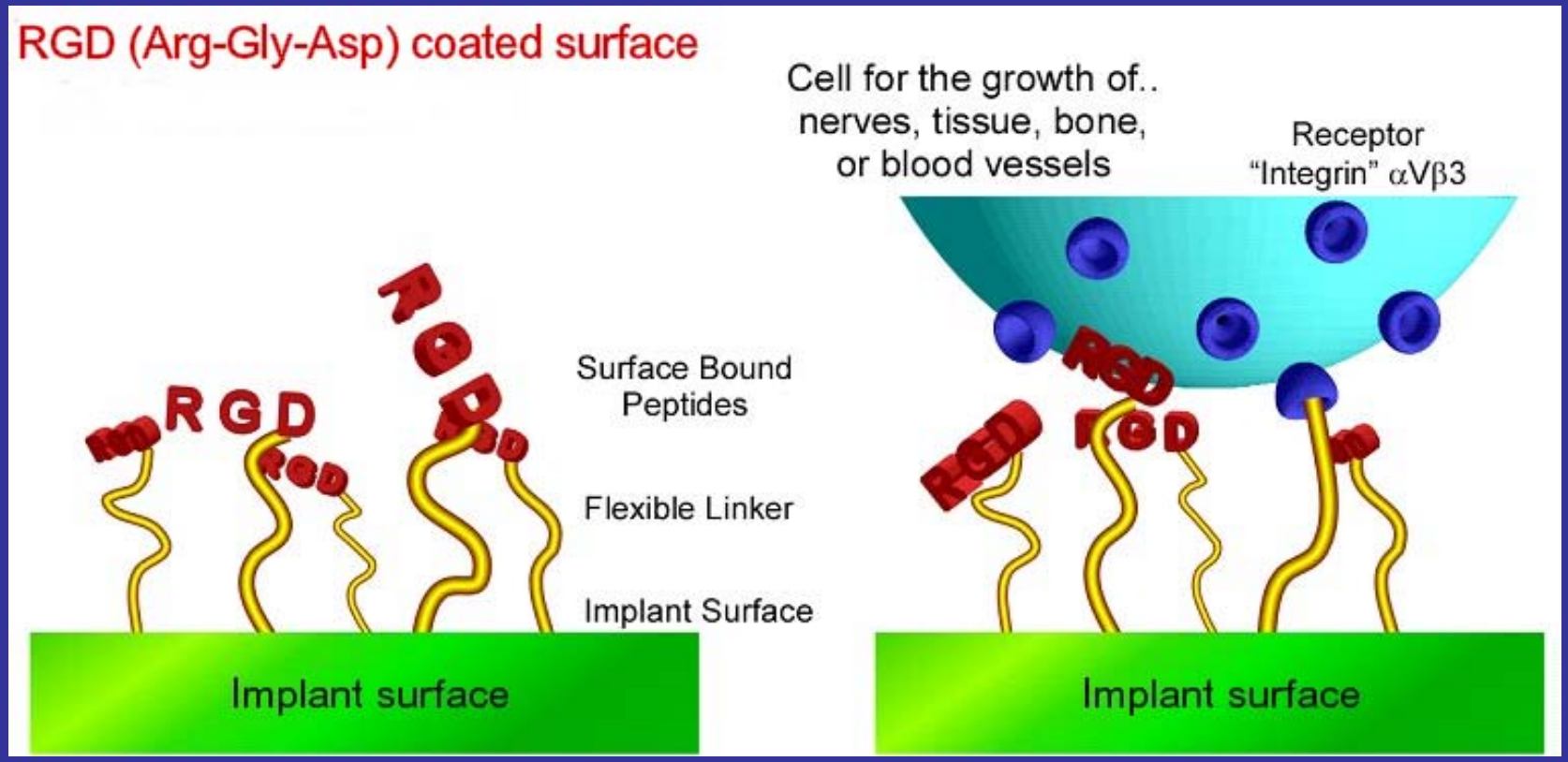
Water molecule



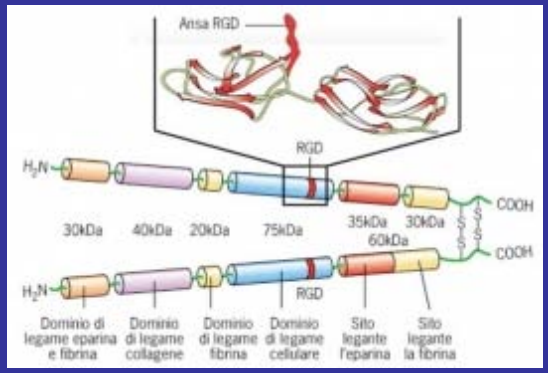
Strategies for EPCs homing onto implantable devices

To fish out EPCs directly from the blood stream 3 main classes of attractive substances can be used:

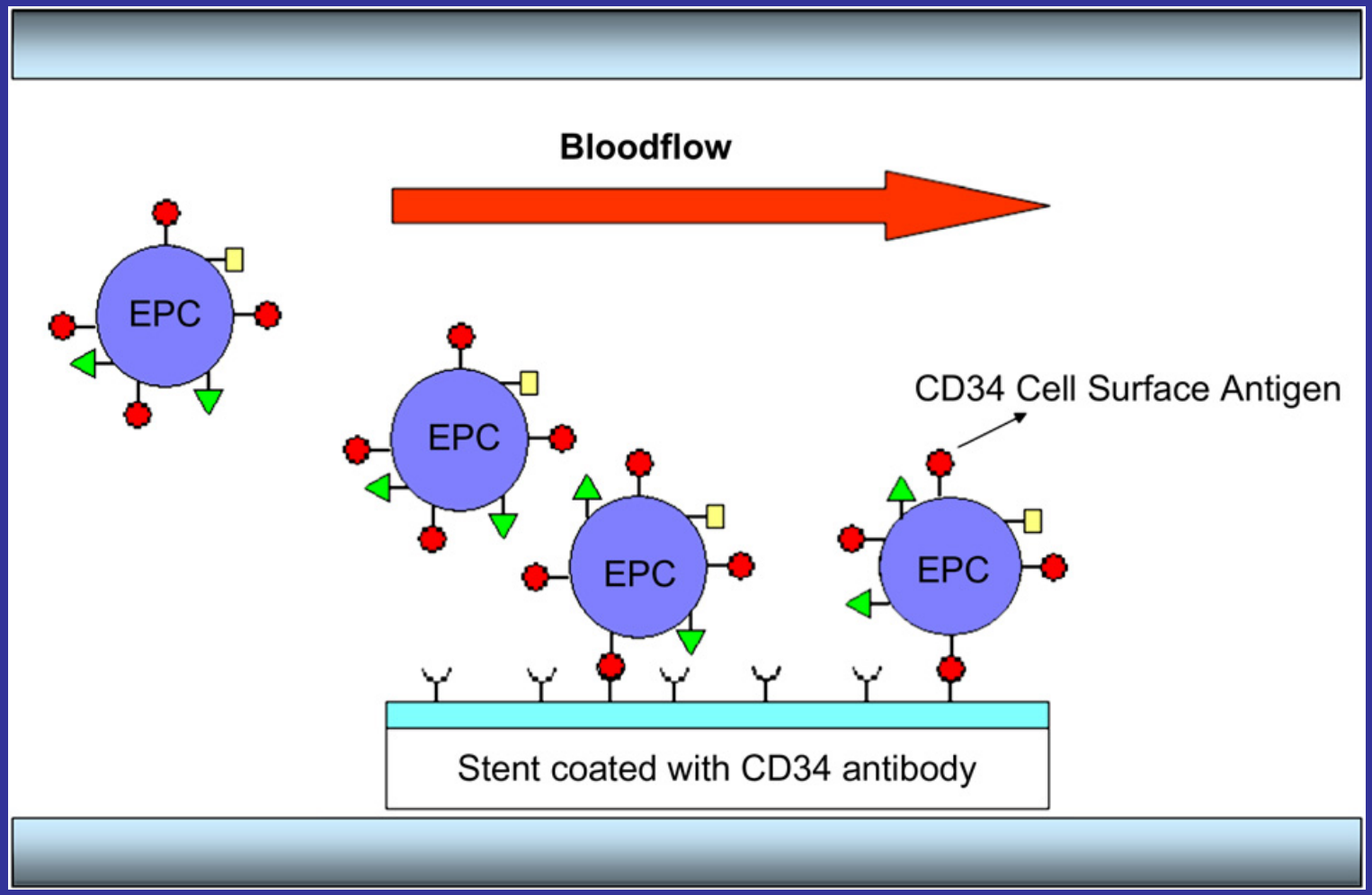
1. RGD peptides
2. CD34 antibodies
3. Aptamers



- Integrins are not exclusively found on EPCs.
- Extracelluar domains of integrins have binding sites also for RGD in fibronectin (fibroblasts), fibrinogen, von Willebrand factor (ECs and megakaryocytes), collagen, laminin (epithelial cells) or vitonectin (platelets).

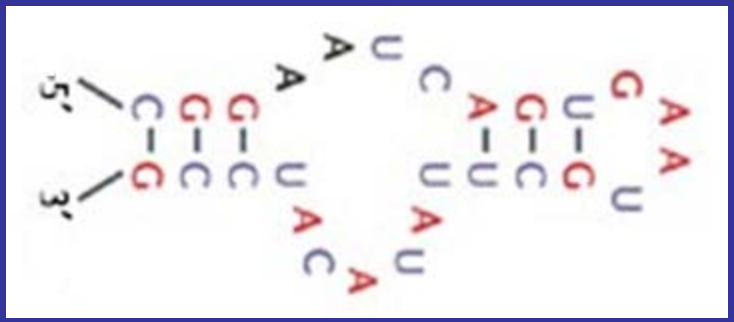


- Only von Willebrand factor is synthetized in great quantity on EPCs



- Developed by “ORBUS Medical technologies” - Good results in term of rapid endothelialization.
- However, endothelialization did not lead to a decrease in intimal hyperplasia, as expected – rapid endothelialization was associated with a significant increase of intimal hyperplasia.
- CD34 is a hematopoietic stem cell marker - antibodies to CD34 could have attracted CD34+ cells that still have the potential to differentiate into vascular smooth muscle cells.

APTAMERS



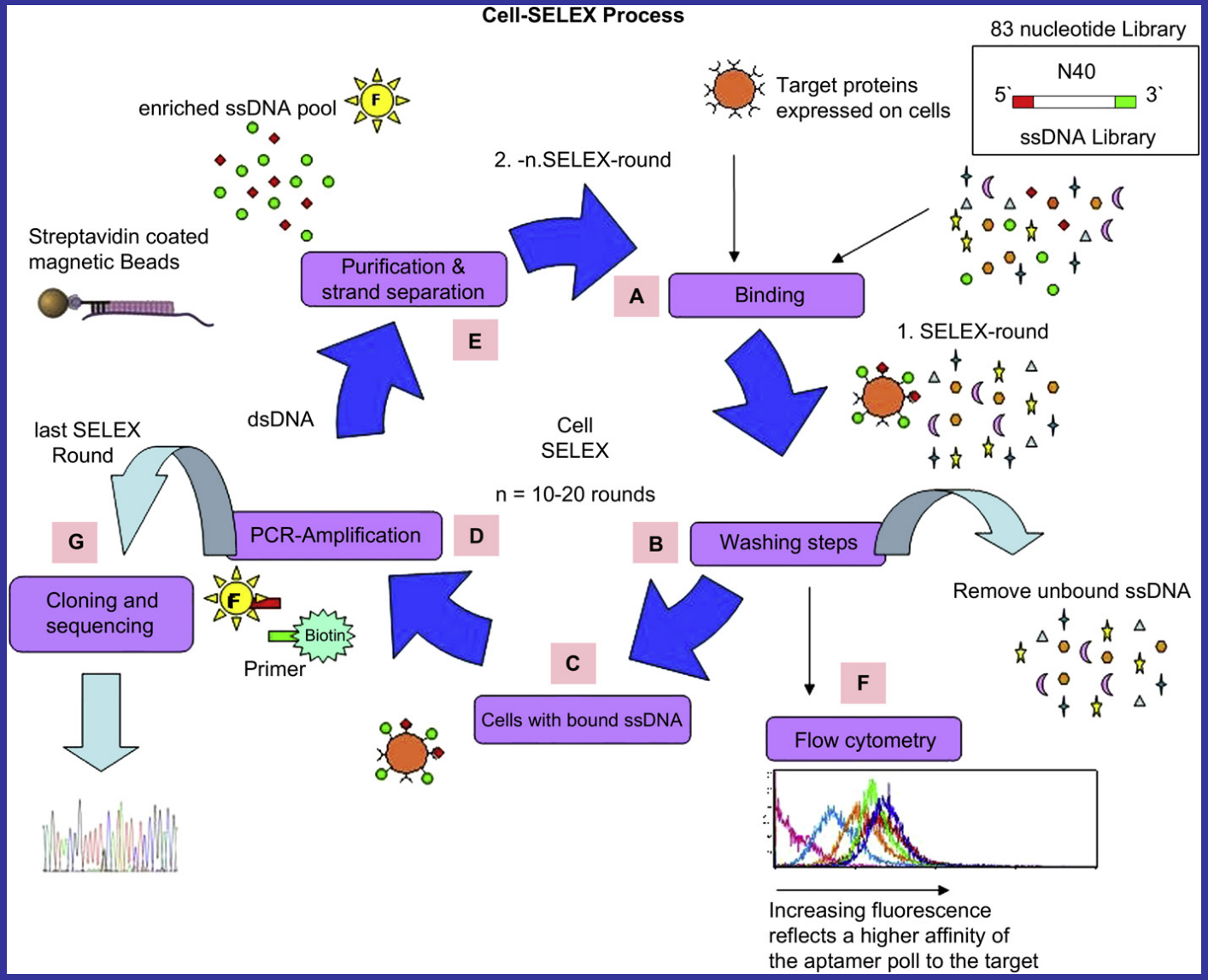
- Relatively short oligonucleotidic or peptidic seguences (ssDNA o ssRNA (12-30 nucleotides or aminoacids)

- In vivo undertake 3D specific structures able to link with hight affinity (range nM/pM) to target protein

APTAMERS vs. ANTIBODIES

- Higher stability
- Not immunogenic (animals or cell are not involved in aptamers identification)
- Are easily synthetised with high accuracy and reproducibility
- Can be chemically modified in a way to be protected and not to lose their activity in the biological environment (eg.: resistant to nucleases attack)
- Are stable for long time and undergoing a reversible denaturation (recover their native conformation in few minutes)

SELEX (systematic evolution of ligands by exponential enrichment)



In vitro procedure for isolating nucleic acid ligands with a high affinity binding capacity to a specific target

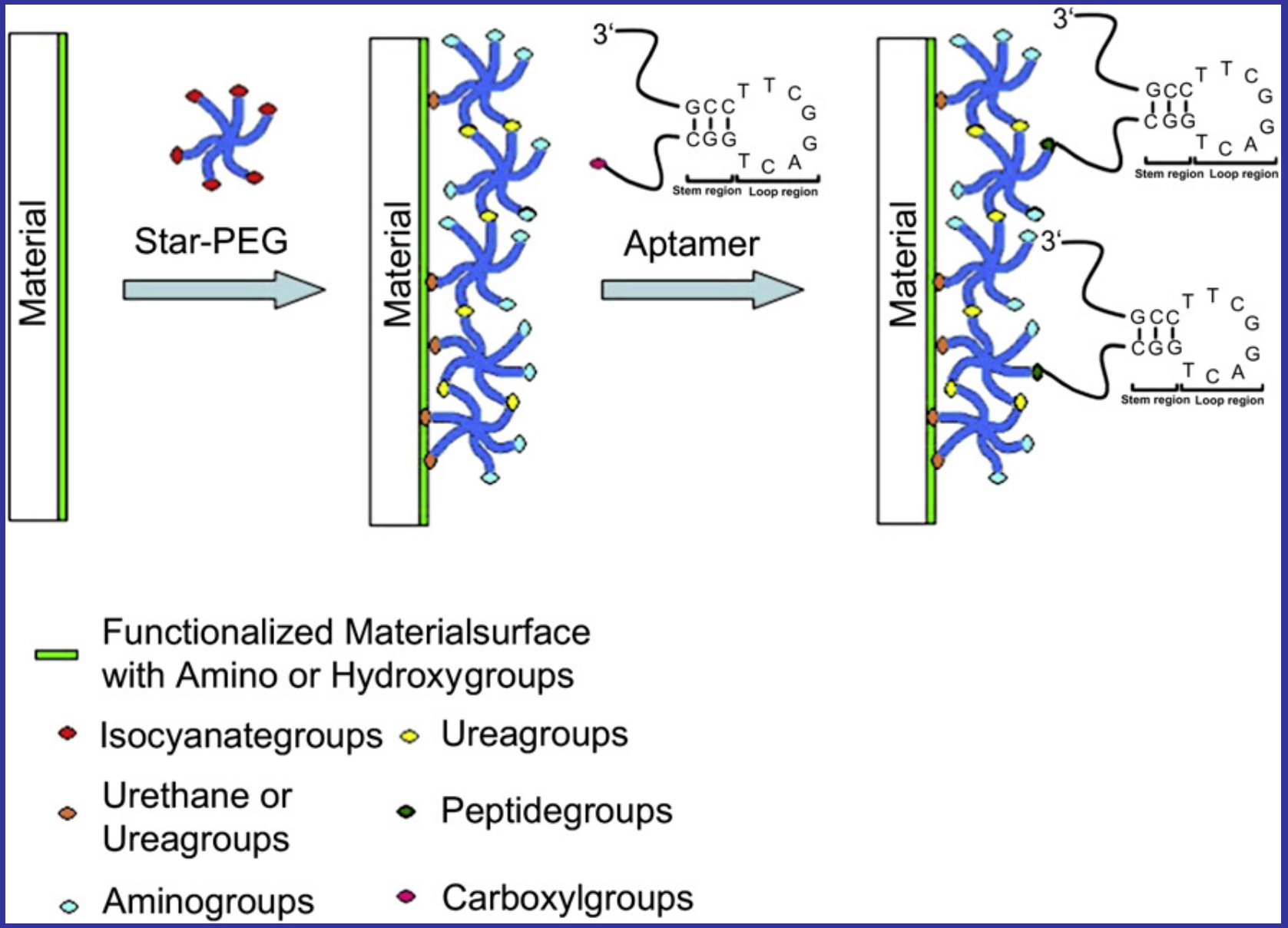


Fig. 4. Scheme for the immobilization of aptamers on graft material. Star-PEG contains isocyanate groups, which can react with amino or hydroxyl groups on functionalized material surface, thereby star-PEG can covalently couple onto the material surface through urethane or urea bonds. Accordingly, aptamers modified at 5'-end with a carboxyl group can covalently be coupled via a peptide bond to the aminogroups of star-PEG.

Immobilized DNA aptamers used as potent attractors for porcine endothelial precursor cells

Jan Hoffmann,[1] Angela Paul,[1] Marc Harwardt,[3] Jürgen Groll,[2,3] Thomas Reeswinkel,[3] Doris Klee,[3] Martin Moeller,[3] Heike Fischer,[4] Tobias Walker,[1] Tim Greiner,[5] Gerhard Ziemer,[1] Hans P. Wendel[1]

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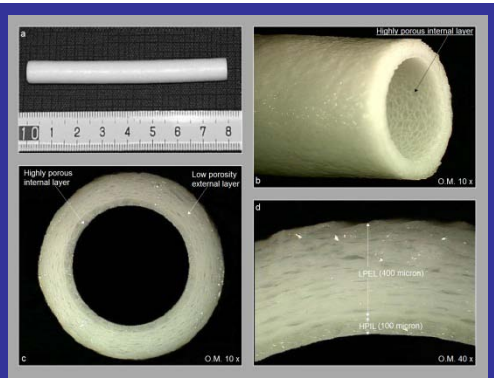
Abstract: Because of their insufficient biocompatibility and high thrombogenicity, small diameter artificial vascular prostheses still do not show a satisfactory patency rate. In vitro endothelialization of artificial grafts before implantation has been established experimentally years ago, but, this procedure is extremely time consuming and expensive. This study deals with the coating of graft surfaces with capture molecules (aptamers) for circulating endothelial progenitor cells (EPCs), mimicking a prohoming substrate to fish out EPCs from the bloodstream after implantation and to create an autologous functional endothelium. Using the SELEX technology, aptamers with a high affinity to EPCs were identified, isolated, and grafted onto polymeric discs using a blood compatible star-PEG coating. A porcine *in vitro* model that demonstrates the specific adhesion of EPCs and their differentiation into vital endothelial-like cells within 10 days in cell culture is presented. We suggest that the rapid adhesion of EPCs to aptamer-coated implants could be useful to promote endothelial wound healing and to prevent increased neointimal hyperplasia. We hypothesize that future *in vivo* self-endothelialization of blood contacting implants by homing factor mimetic capture molecules for EPCs may bring revolutionary new perspectives towards clinical applications of stem cell and tissue engineering strategies.
 J Biomed Mater Res 84A: 614–621, 2008

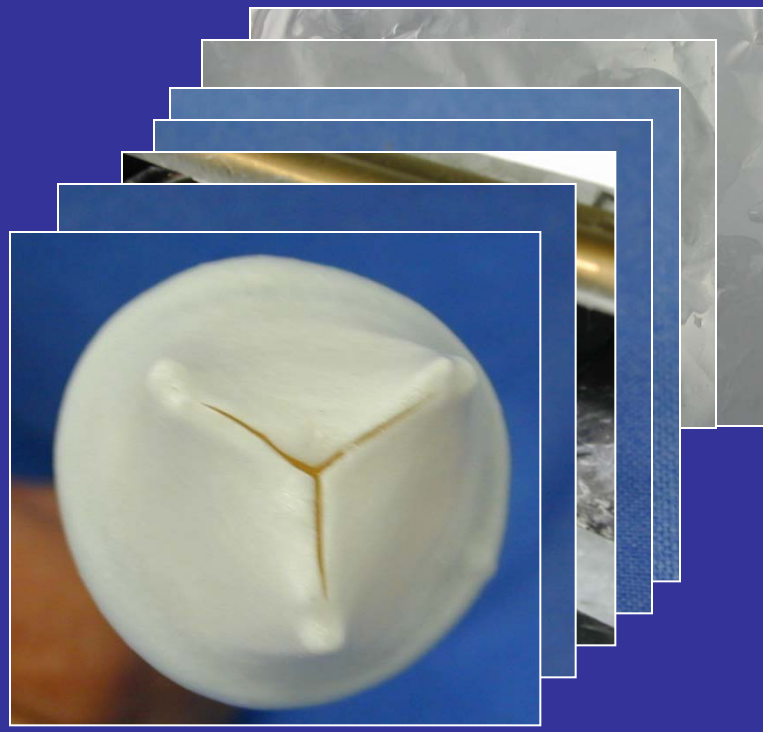
Key words: aptamer; whole-cell-SELEX; endothelial precursor cell; drug delivery



LA NUOVA VALVOLA POLIMERICA A CORPO UNICO
studio di fattibilità

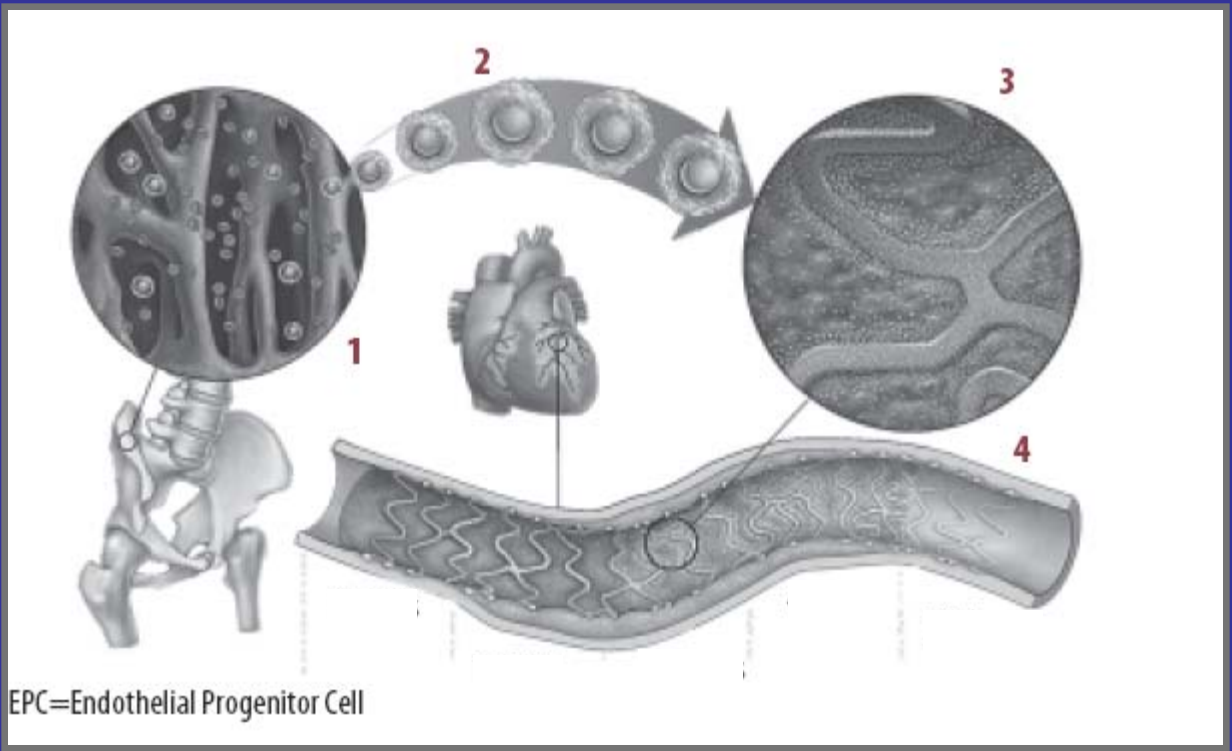
Basandosi sui risultati ottenuti nell'ambito del Progetto SILCROTHANE e considerando l'esperienza maturata negli anni sulle tecniche di processo dei materiali elastomerici, è stato deciso di eseguire uno studio di fattibilità con un materiale noto, per la realizzazione di un nuovo prototipo di valvola polimerica.

In particolare è stata valutata l'idoneità della tecnologia *spray* e della classe di materiali ottenuti mediante semi-IPN tra tra poliuretani e siliconi.



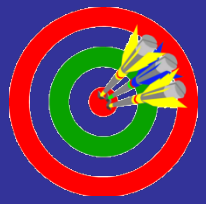
To reduce problems of in stent-restenosis and to turn away the spectre of late stent thrombosis that can affect coronary Drug Eluting Stents, a new bioactive coating for specific recruitment of circulating EPCs, is not so far to be obtained.

Arterial healing process with aptamer-coated stent capturing EPCs



1. EPCs originate from bone marrow
2. EPCs circulate through the blood stream
3. EPCs are attracted by aptamers attached to the stent surface
4. Lining is rapidly formed over and betwwen the stent surface in the diseased segment

Nanostructured 3D scaffold



Target of the study

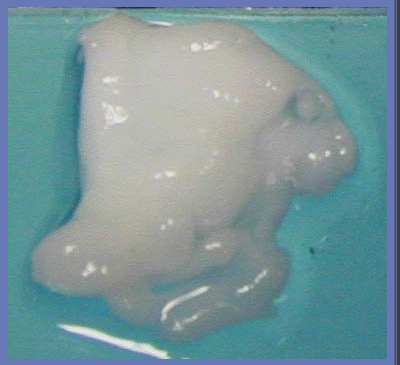
To develop a new implantable biosynthetic nanostructured 3D scaffold which:

- combine good mechanical properties with sustained release of bioactive pro-angiogenetic growth factors.
- can induce angiogenesis in organ/tissue in which is necessary to show resistance and mechanical strength

Synthetic and biological components of the composite scaffold:

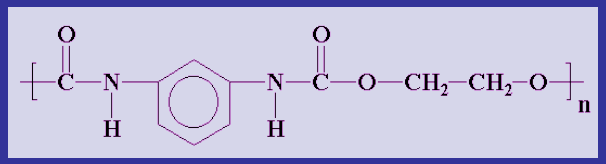
Polyurethane-polydimethylsiloxane material (PU/PDMS)

Natural fibrin gel



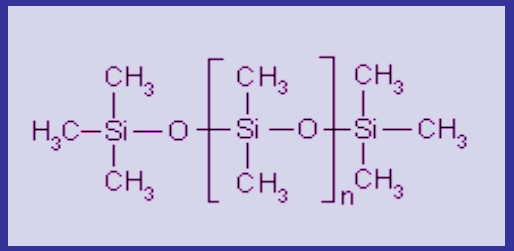
Il polimero sintetico PU–PDMS

Poliuretano (PU)



- buona emocompatibilità
- ottime proprietà elastiche
- eccellente resistenza alla fatica e all'abrasione

Polidimetilsilossano (PDMS)

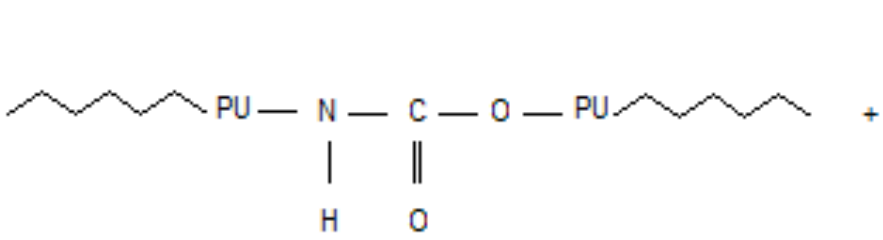


- ottima emocompatibilità
- biostabilità a lungo termine

Il polimero PU-PDMS utilizzato per la realizzazione degli scaffolds 3D è stato sintetizzato con il 30% di PDMS (p/v).

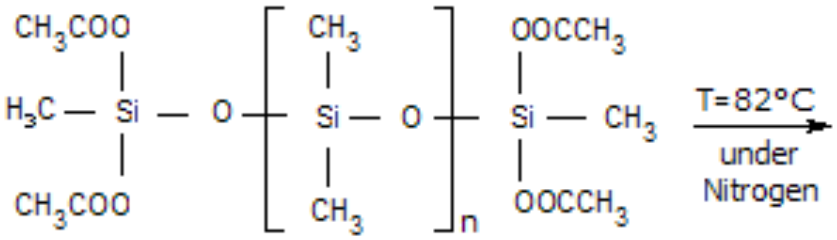
Reattore utilizzato per la sintesi del polimero PU-PDMS

Reaction between PEtU and PDMS

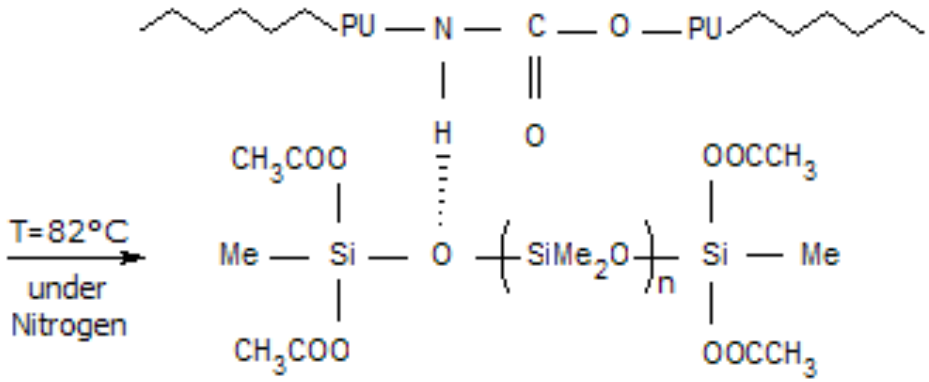


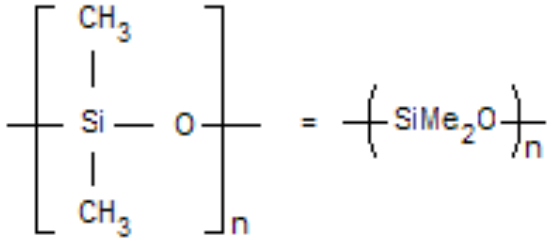
Repeating unit of the aromatic poly(ether)urethane (PEtU)

+



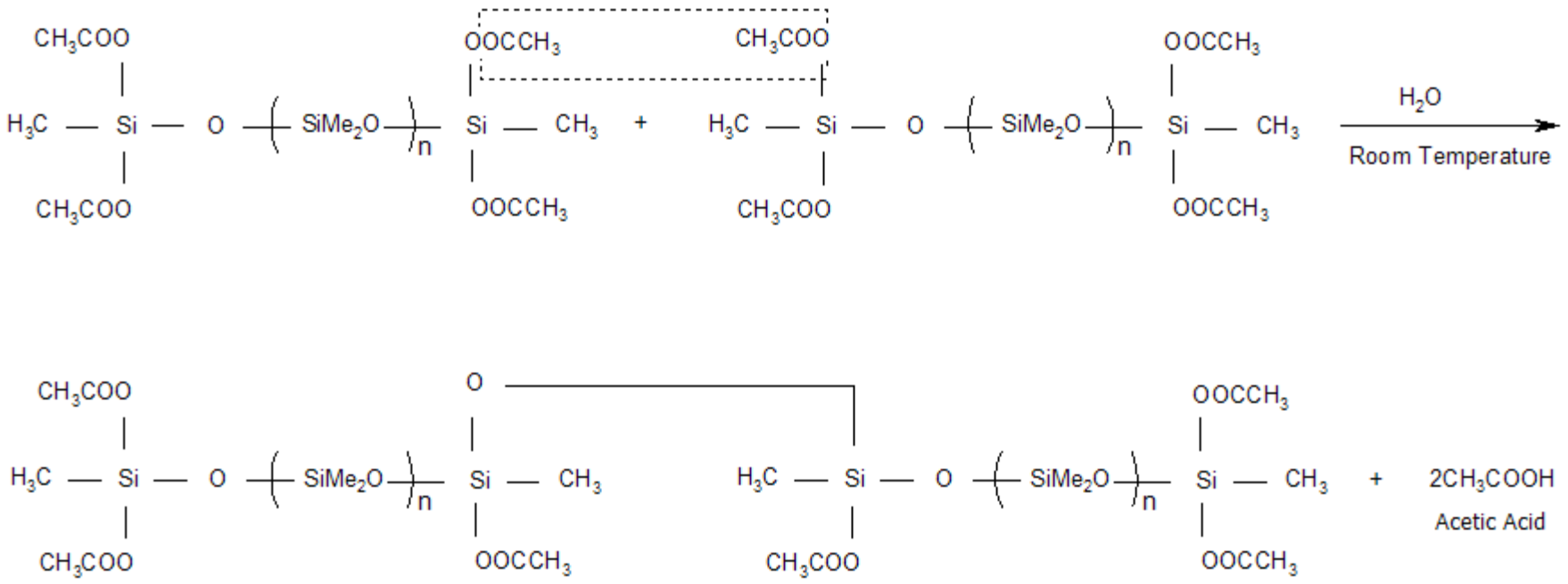
Diacetoxy silyl terminated PDMS
(tetraacetoxy functional)



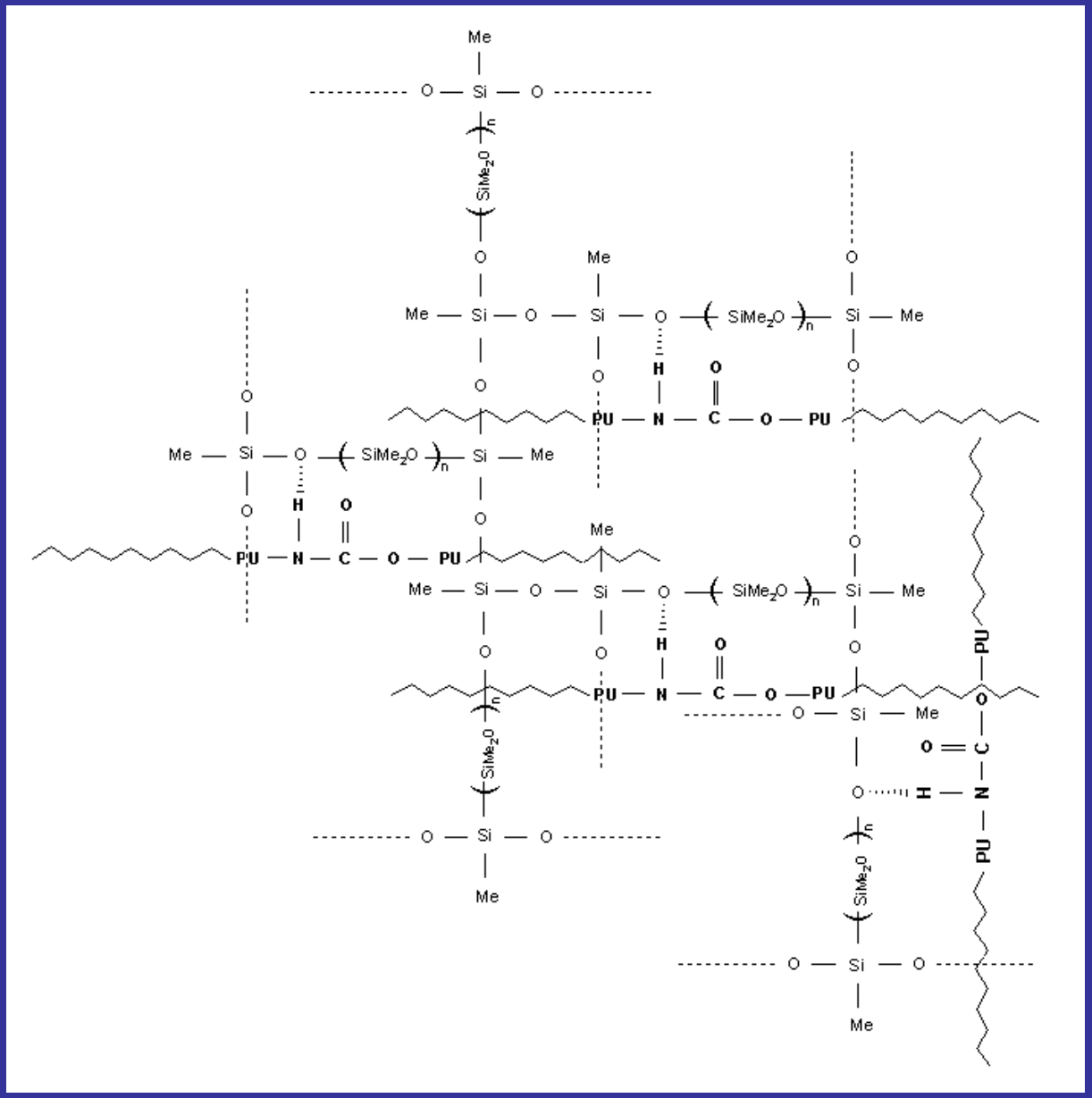


Repeating siloxane unit

Croslinking reaction of tetraacetoxy functional PDMS

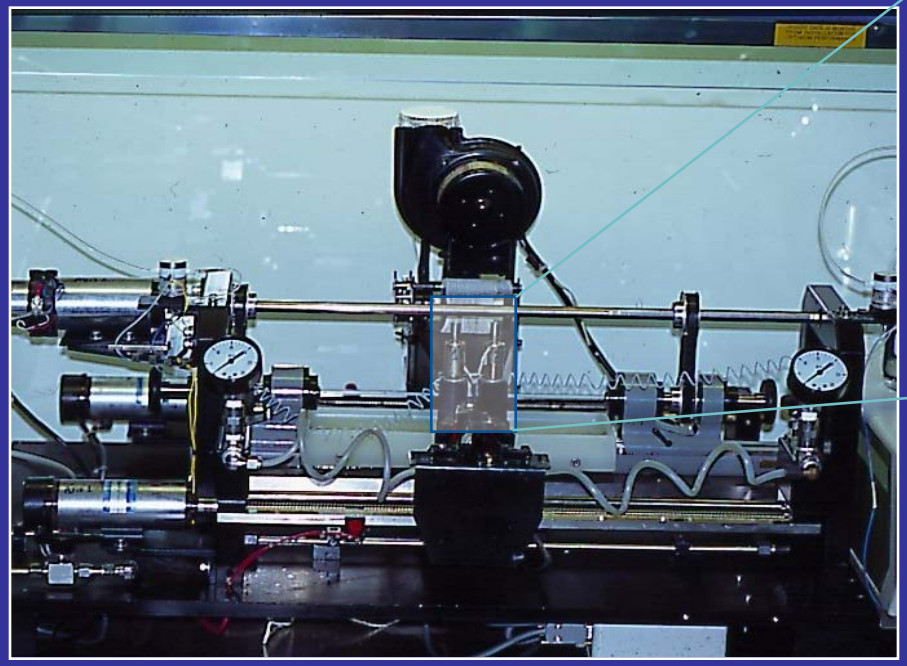


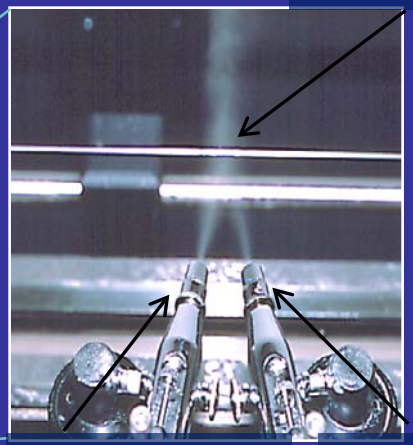
Semi-Interpenetrating Polymeric Network (semi-IPN)



Nanostructured scaffold fabrication

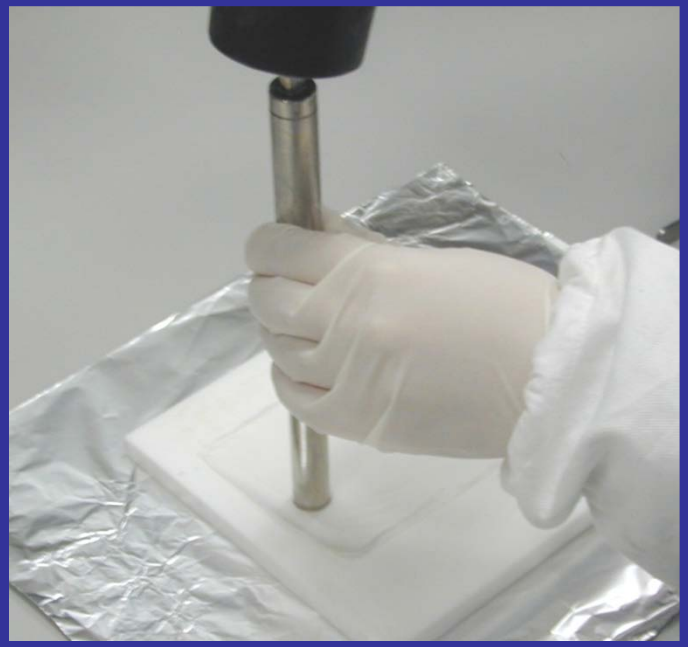
- the spray phase inversion technique -





The thrombin solution (25U/ml) was simultaneously sprayed with the PU-PDMS solution to impregnate the microporous layer of the synthetic scaffold with the enzyme

Nanostructured scaffold fabrication



The synthetic layer impregnated with thrombin was punched to obtain round samples (1 cm^2 of area)

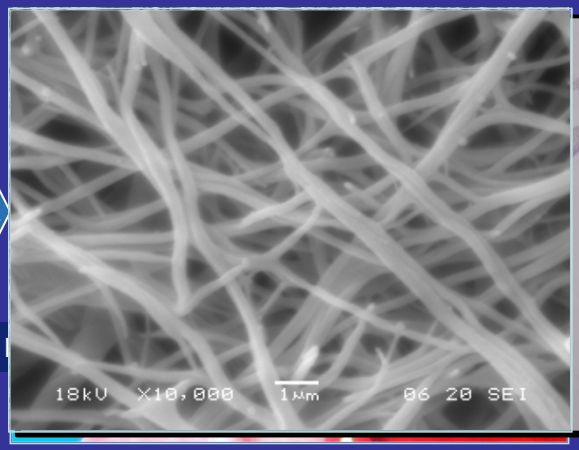
The samples were placed in a 24-well plate and fibrinogen solution was added to each well. The fibrin polymerization was completed after o/n incubation at 37°C

During the manufacturing process, Vascular Endothelial Growth Factor (VEGF), basic Fibroblast Growth Factor (bFGF), and heparin were incorporated in the fibrin layer of the scaffold

Morphological properties of the nanostructured scaffold

Cross-sectioned material (Histology)

Synthetic l



Synthetic layer

Layer of fibrin

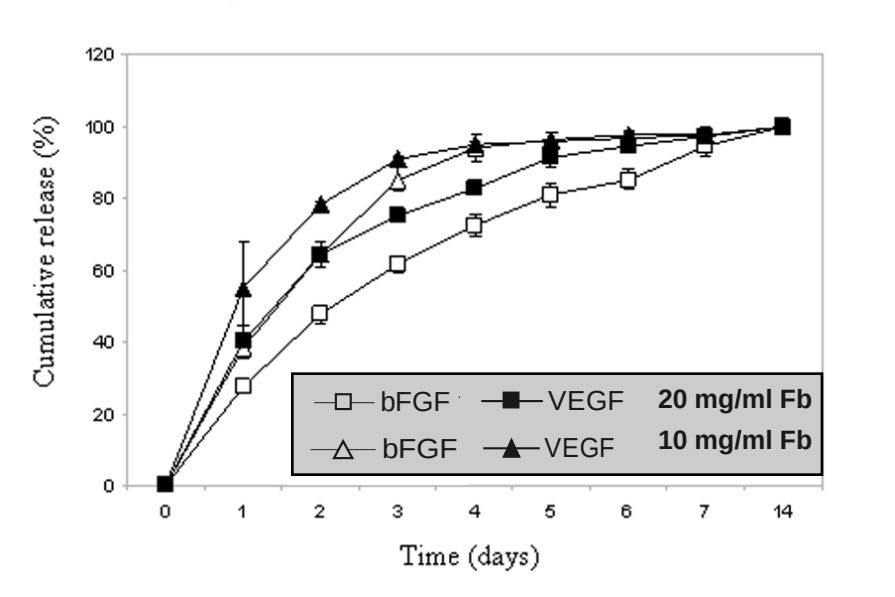
Fibrin layer

Fibrin layer

Nanostructured scaffold material surface

Growth factors release from nanostructured scaffold

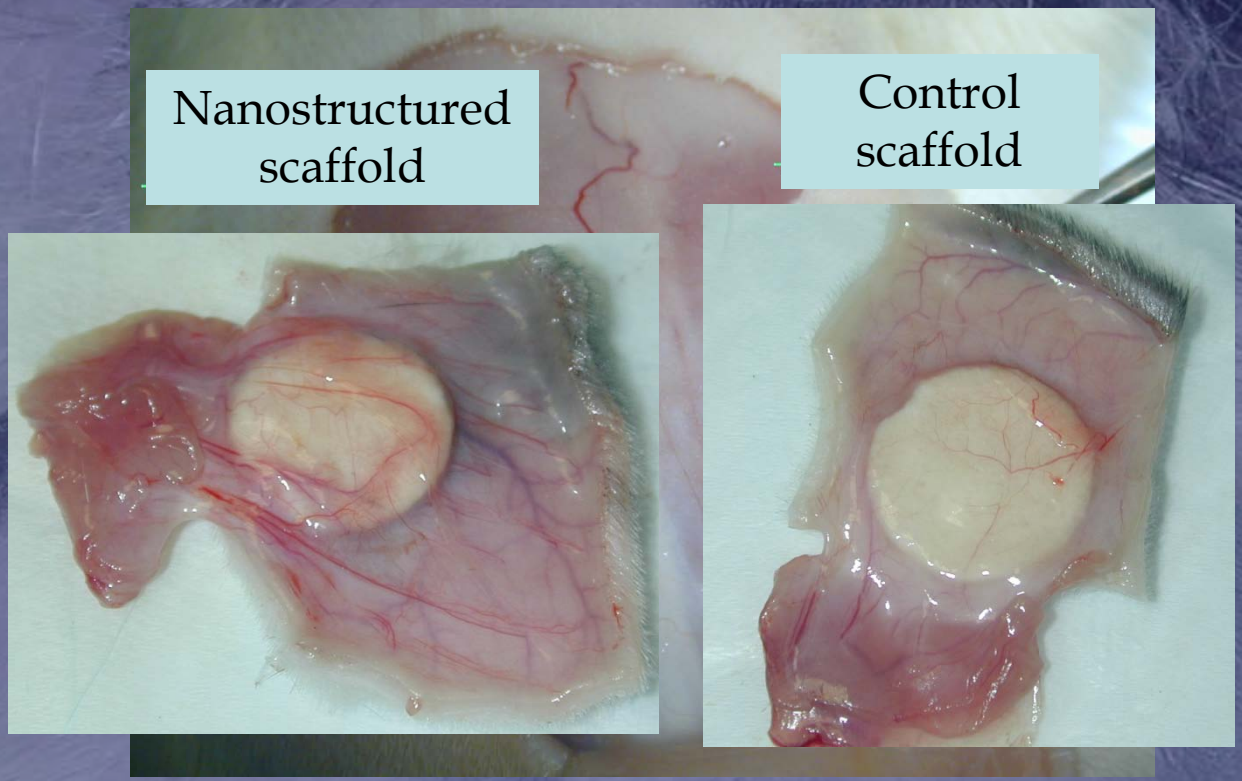
- in vitro release kinetics and bioactivity studies -



The release rate of VEGF and bFGF from the scaffold was controlled by fibrinogen concentration in the fibrin layer (20mg/ml determined slower release than 10mg/ml). Moreover, bFGF was retained for a longer time than VEGF and thus delivered more slowly

Induction of angiogenesis *in vivo*

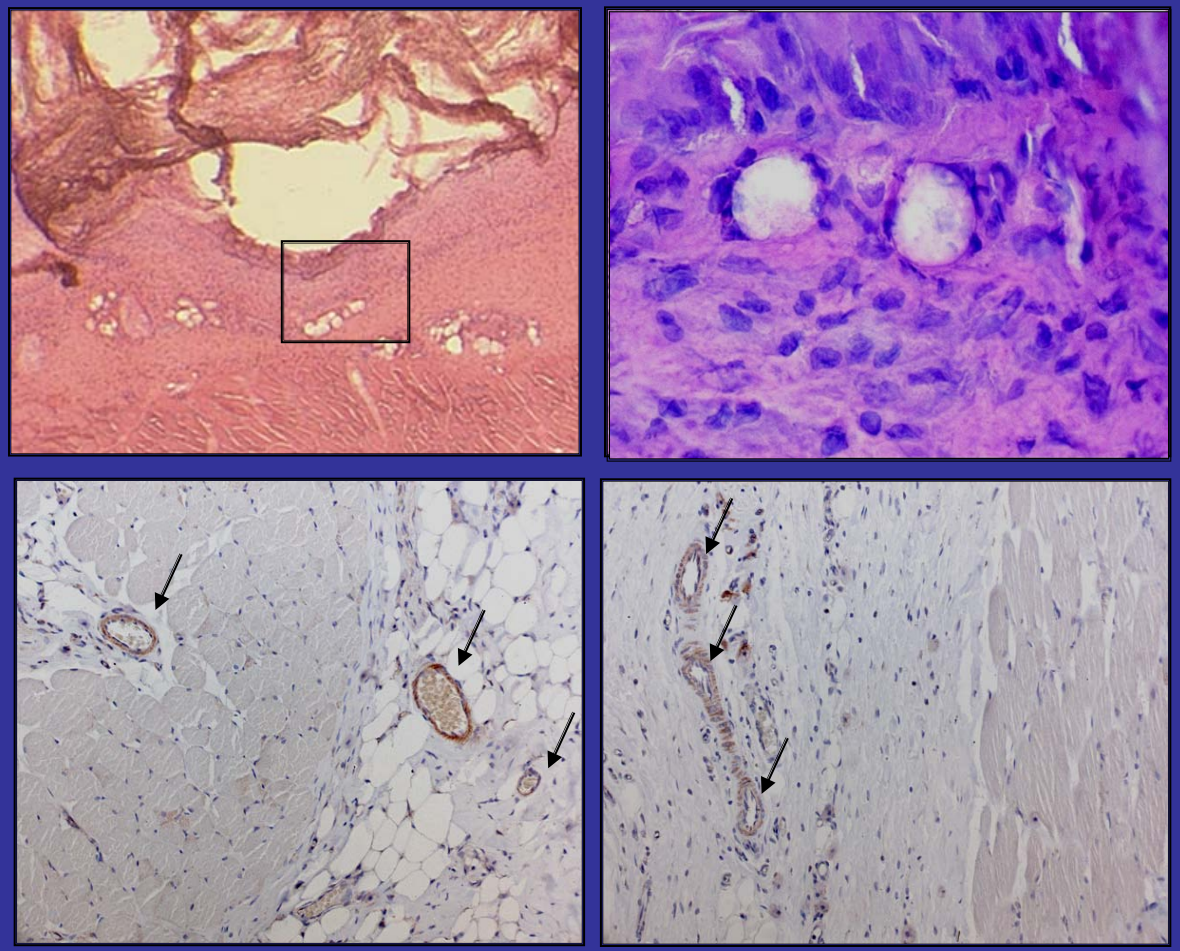
- subcutaneous implantation in the dorsal region of Wistar rat - macroscopical analysis after 14 days



Induction of angiogenesis *in vivo*

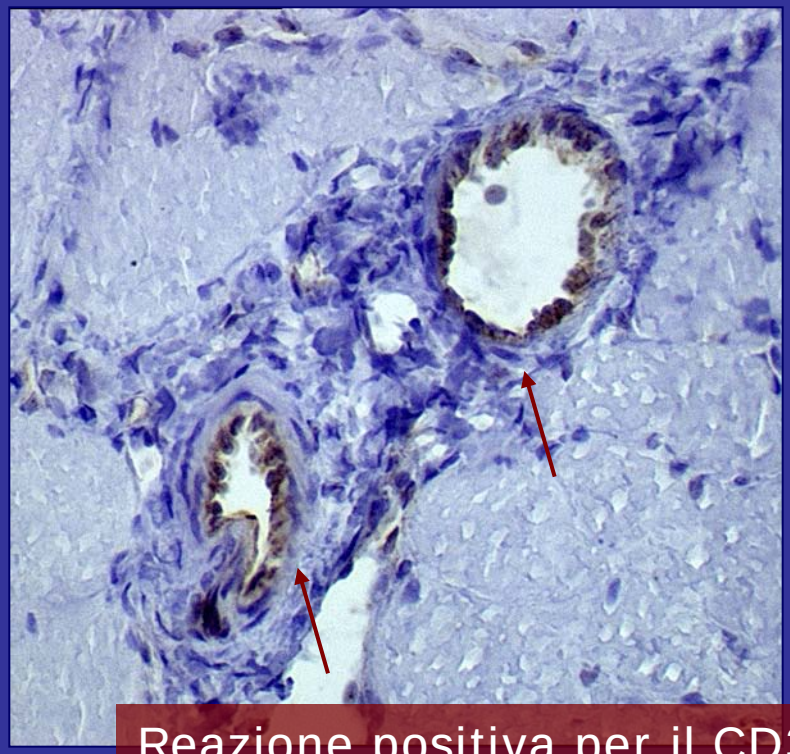
- histological and immunohistochemical analyses -

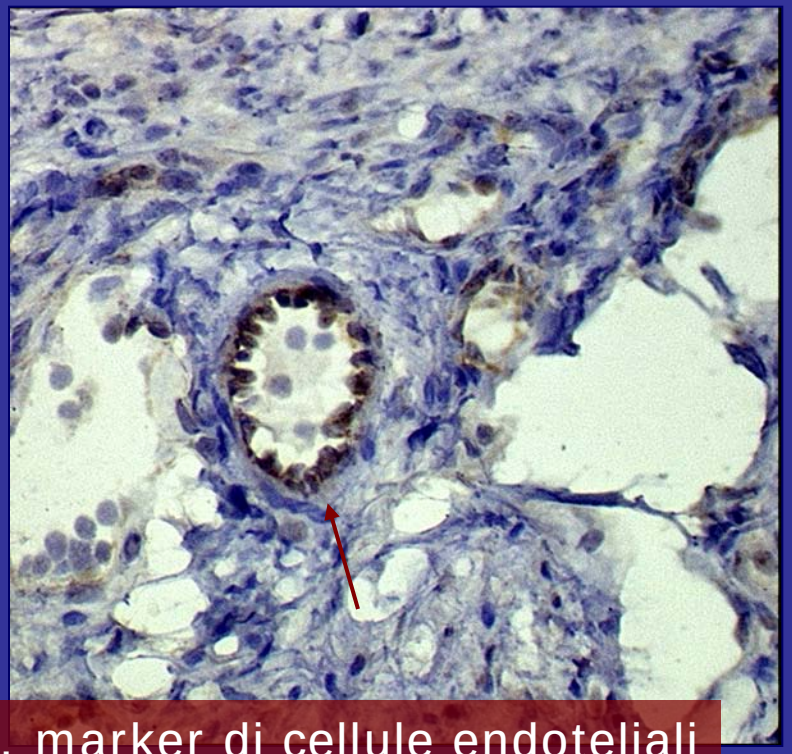
The histological (H&E staining) and immunohistochemical (PECAM-1 staining) analyses of 14 days-explants showed a significant increase of neovessels number around the scaffolds in comparison with control (scaffold without growth factors)



Scaffolds 3D nanostrutturati

- Analisi immunoistochimica dei campioni espiantati -





Reazione positiva per il CD31, marker di cellule endoteliali

Conclusions

We hypothesize that *in vivo* self-endothelialization of blood contacting materials by homing factor-mimetic capture molecules for EPCs and the development of nanostructured scaffold able to promote the formation of neo-vessels may bring revolutionary new perspectives towards future clinical application of stem cell and tissue engineering strategies.